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Cyanobacteria and their toxins in Guanting Reservoir of Beijing, China

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Abstract

The present study investigated the cyanobacteria and one family of their toxins-microcystins (MCs) in Guanting Reservoir of Beijing, China. The dominant species in the cyanobacteria found in August and September of 2006 was *Microcystis*, which accounted 99% of total algal cells. The specific species of the *Microcystis* in the cyanobacteria included *Microcystis ichthyobalbe*, *Microcystis novacekii*, *Microcystis botrys* and *Microcystis aeruginosa* which had different ratios in different sites. The qualitative analysis by HPLC showed that two microcystins were contained in cyanobacteria and one microcystin was in water of the reservoir. The major microcystins were microcystin-RR (MC-RR) and microcystin-LR (MC-LR), but only MC-LR was detected in water. The quantitative analysis by HPLC indicated that the maximum concentrations of MC-RR and MC-LR contained in cyanobacteria were 0.74 and 0.41 mg/g dry weight, respectively. The maximum microcystin concentration in water was 1.15 μg/L and others were below 1 μg/L.

Keywords: Guanting Reservoir; Investigation; *Microcystis*; Microcystins

1. Introduction

Guanting Reservoir, located in the northwest of Beijing (approximately 90 km northwest), was built in 1952, with a storage capacity of 410 million m^3 and is one of two main water resources (92.98% of total reservoir storage capacity in Beijing) for agriculture, industry and potable uses of Beijing. The reservoir dammed the Sanggan River, Yang River and Guishui River and converged into Yongding River [1]. Since Guanting Reservoir has suffered from extensive pollution over the last years (particularly in 1980s) due to runoff from non-point sources, direct dumping of wastes, unmanaged fishing, unrestricted shipping, mineral exploitation, and pollutants carried by rivers. Water from this reservoir was not used as potable water since 1997. As continuous droughts persist in north area in China and the population in Beijing keeps on quickly increasing, water supply deficiency is an urgent problem confronting Beijing. Thereby, it is determined that Guanting Reservoir will resume being an important water supply source.

Microcystins are the secondary metabolites of toxic cyanobacteria and are a family of extremely toxic compounds produced continuously by the species of freshwater cyanobacteria belonging to the genera of *Microcystis*, *Anabaena*, and *Oscillatoria*. The general structure of microcystin is cyclo (D-Ala-L-X-erythro-β-methyl-D-isoAsp-L-Y-Adda-isoGlu-*N*-methyldehydro-Ala) where X and Y represent the two variable amino acids and Adda is 3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyl-deca-4, 6-dienoic acid. So far, more than 70 microcystin variants have been detected. Microcystins inhibit eukaryotic serine/threonine protein phosphatases 1 and 2A, resulting in excessive phosphorylation of cytoskeletal filaments and causing serious liver damage. Long-term exposure to low level of MCs has also been implicated in liver tumor promotion. Their acute toxicity (given intraperitoneally to mice) varies between 50 and 800 μg/kg of body weight. Microcystins have caused several poisonings of domestic animals and wildlife around the world, and they also pose a hazard for humans' health through the use of water for drinking and recreation [2]. Due to the potential health effects of MCs including all kinds of MC, trace microcystins in the water body have attracted extensive interest from environmental scientists and the public.

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Compared to other contamination, such as heavy metals, organochlorine pesticides, there is limited information regarding microcystin pollution in the nearby regions. Beijing, whose annual water consumption was over 4 billion cubic meters, is one of the most populous regions in China. With economic development and continuous drought, Beijing is now facing a water shortage. The purposes of the study were to investigate the occurrence of MC and the composition, distribution and characterization in surface water of Guanting Reservoir in order to understand and access the status of contamination and to identify the factors that may control the MCs in the area. The information will be useful for the management and remediation of some water systems.

2. Materials and methods

2.1. Study area and sampling locations

Samples were collected from seven locations in Guanting Reservoir in August and September 2006. The locations of the seven sampling sites were shown in Fig. 1. The sites were chosen based on hotspots of pollution around Guanting Reservoir such as industrial regions, domestic wastewater discharge areas or entrances of rivers. G_1 is in front of the dam of reservoir; G_2 represents the condition of Yongding River; G_3 is the convergence of Sanggan River and Yang River; G_4 is the estuary; G_5 is under of the Hui Bridge; G_6 and G_7 are in the north of reservoir and represent the Guishui River. Sanggan River and Yang River, which contained 80% pollutants, pour into Guanting reservoir and directly influenced G_2, G_3 and G_4.

Throughout the survey, a global positioning system (GPS, *HOLUX*GM-101) was used to identify the sampling locations precisely. Table 1 listed the longitude and latitude of the seven sites.

Surface water samples with volumes of 15–20 L were collected at the seven sites. Temperature was measured in the field. Samples were loaded in polyethylene plastic barrels and stored in the dark at 4 °C before laboratory analysis. The concentrations of microcystins, total nitrogen (TN), total phosphorus (TP) and the pH value of the water samples were measured within 48 h.

Phytoplankton species were collected by 25# plankton net and stored in polyethylene plastic barrels. They were washed and concentrated after returning to the laboratory. Then 50 mL plankton culture in polyethylene bottle was sent to Institute of Hydrobiology, Chinese Academy of Science to identify the phytoplankton species. Other 50 mL phytoplankton culture was filtered through the dried and constant weight filter papers. Then the filter papers were dried in desiccators for 24 h at 110 °C until constant weights. The dry weight was enregistered. The residual about 100 mL plankton culture was stored at −20 °C until further analysis.

2.2. Extracting microcystins in water samples

Water samples collected from Guanting Reservoir were pre-filtered using 0.45 μm fiber glass filters (Whatman) under vacuum after returning to the laboratory within 1 or 2 days. Two liters filtered water was extracted by SPE as the following procedures. Each of C18 cartridges was conditioned with 10 mL of methanol and 10 mL ultra-pure water and slowly aspirated. Water samples were passed through the cartridges at a flow rate of 5 mL/min under vacuum. The column was washed with 3 times × 3 mL of methanol/water (10/90, v/v), followed by vacuum drying for 10 min. Subsequently, the elution was taken place with 4 times × 3 mL of methanol. The extract was concentrated to 0.5 mL under a gentle stream of nitrogen in a water bath and measured for MC on HPLC.

2.3. Extracting MC within the cells

Hundred milliliters methanol was added to 100 mL cyanobacterial culture to extract MC within cyanobacterial cells. The extraction solution was staved using ultrasonic equipment (JY92-IID, SCIENTZ, China) for 30 min and then was put

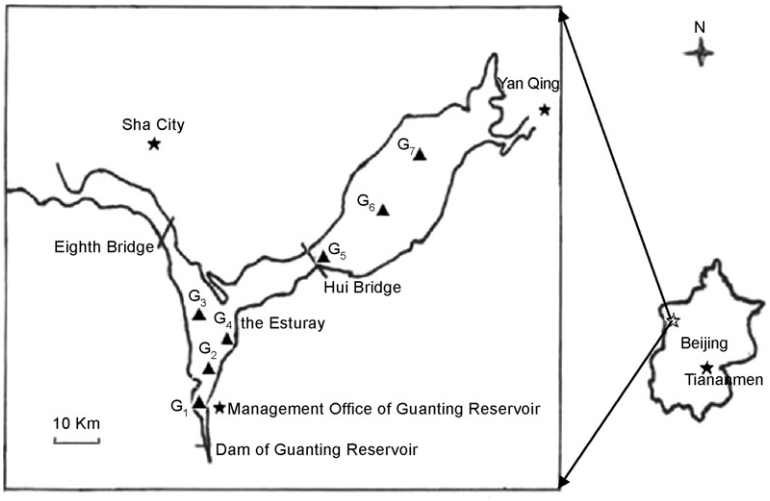

Fig. 1. Sampling sites in Guanting Reservoir and its geographical location (1–7 denote seven sampling sites in Beijing Guanting Reservoir: G_1, front of the dam of reservoir; G_2, Yongding River; G_3, the convergence of Sanggan River and Yang River; G_4, estuary; G_5, Hui Bridge; G_6 and G_7, Guishui River).

Table 1
Limnological characteristics of the seven sampling sites in Guanting Reservoir

Sampling sites	Longitude E	Latitude N	August 14, 2006		August 28, 2006		September 13, 2006	
			pH	Temperature (°C)	pH	Temperature (°C)	pH	Temperature (°C)
G_1	115°36.83′E	40°15.067′N	8.66	26.3	9.09	27.3	8.94	21.6
G_2	115°35.167′E	40°16.183′N	8.74	26.3	9.11	26.8	9.03	21.2
G_3	115°37.617′E	40°18.033′N	8.57	25.3	9.08	26.5	9.11	21.6
G_4	115°37.65′E	40°18.683′N	8.79	25.6	8.94	26.8	8.92	22.9
G_5	115°42.867′E	40°20.683′N	8.78	25.5	9.00	26.8	9.10	22.7
G_6	115°45.65′E	40°21.733′N	8.80	26.2	9.09	27.8	9.05	22.0
G_7	115°46.8′E	40°22.583′N	8.73	26.6	9.19	27.1	8.94	22.2

in boiling water for 20 min to extract MC. The solution was centrifuged at 8000 r/m for 10 min and the supernatant was filtered through 0.22 μm membrane. The filtration was enriched to 0.5 mL by mild nitrogen gas and measured for MC on HPLC. The MC content in the cells was calculated from the ratio between the concentration of MC in the extraction solution (mg/L) and the dry weight (g/L).

2.4. Determination of MCs

Standard MC-RR and MC-LR bought from Sigma (98% purity) and samples of MC-RR and MC-LR were analyzed by HPLC (Hitachi L-2000) with a reverse ZORBAX SB-C18 column (4.6 mm × 250 mm, Agilent Co., USA) and a Diode Array Detector at 238 nm. The mobile phase was 35% (v/v) acetonitrile–water solution containing 0.05% (v/v) of frozen acetic acid. The flow rate was 1.0 mL/min and the injection amount was 20 μL. Microcystins were identified by their characteristic absorption spectra and retention times.

2.5. Measurement of cell number

After straining with Lugol's Iodine solution, the population of *Microcystis aeruginosa* cells in the water samples was counted under a microscope using a hemocytometer to calculate the density of cyanobacterial cells. Cell counts were performed to a minimum precision of 20%.

2.6. Analysis nitrogen and phosphorus

Total nitrogen in water was determined by the alkaline potassium persulphate oxidation-UV spectrophotometric method [3]. Total phosphorus was determined by the molybdenum-Antimony Anti-Spectrophotometric method after potassium persulphate digestion [3].

3. Results and discussions

3.1. Basic limnological character

Some basic limnological characteristics of the Guanting Reservoir were shown in Table 1 and Fig. 2. The physical conditions of the reservoir were characterized by eutrophic state because of the high concentration of both phosphorus and nitrogen. The water temperature decreased slightly from 27.8 to 21.2 °C throughout the sampling period. The pH values ranged between 8.57 and 9.19 during the study period at all stations. Fig. 2 showed TN and TP concentrations ranged between 0.40–5.59 mg/L and 0.016–0.178 mg/L, respectively. The maximum TN concentration (5.59 mg/L) was recorded in August directly after the rainy season.

The higher values of TP inshore as compared to the open water could be due to the contamination of the water by phosphate rich detergents since activities such as bathing and washing around the lakeshores were observed during the period of investigation. The highest nitrogen concentrations that were found during the months of August immediately after the rainy season may be attributed to terrestrial runoff as has been suggested by Ochumba and Kibaara [4]. During the study period, the amount of TN in water significantly decreased, whereas TP concentration remained stable. This might be due to the reason that nitrogen was consumed by algae and microbes while phosphorus was released from the sediment in eutrophic and hypereutrophic lakes [5]. TN/TP ratios ranged from 5 to 212 and were significantly higher on August 14 than on August 28 and September 13. According to the TN/TP ratio criteria of Forsberg and Ryding [6], most sites were potentially phosphorus limited (TN/TP > 17) on August 14 and 28 of the reservoir and only G_1 site was nitrogen limited (TN/TP < 10) on September 13.

3.2. Plankton biovolume and composition

In order to keep the transparency and control water bloom, WHO referred the guideline value of 100,000 cells/mL for a moderate health alert in waters [7]. As shown in Fig. 3, cyanobacterial biovolume was low over the entire study period (292–21079 cells/mL). It was lower at the beginning of the sampling period in August, and increased in September. The maximum biovolume was 21079 cells/mL in G_3 site on September 13. However, none of sample was in excess of the guideline value of WHO [7]. Among all sampling sites, there were the higher biomass level in G_2, G_3, G_4 and G_5 site.

Dominant algae species and their ratios of G_2 and G_5 were listed in Table 2. Both samples were composed of several codominant species and additional subdominant species, and no large accumulation of cyanobacteria was observed in the surface layers. However, there was significant difference in phytoplankton composition between G_2 and G_5. Phytoplankton was

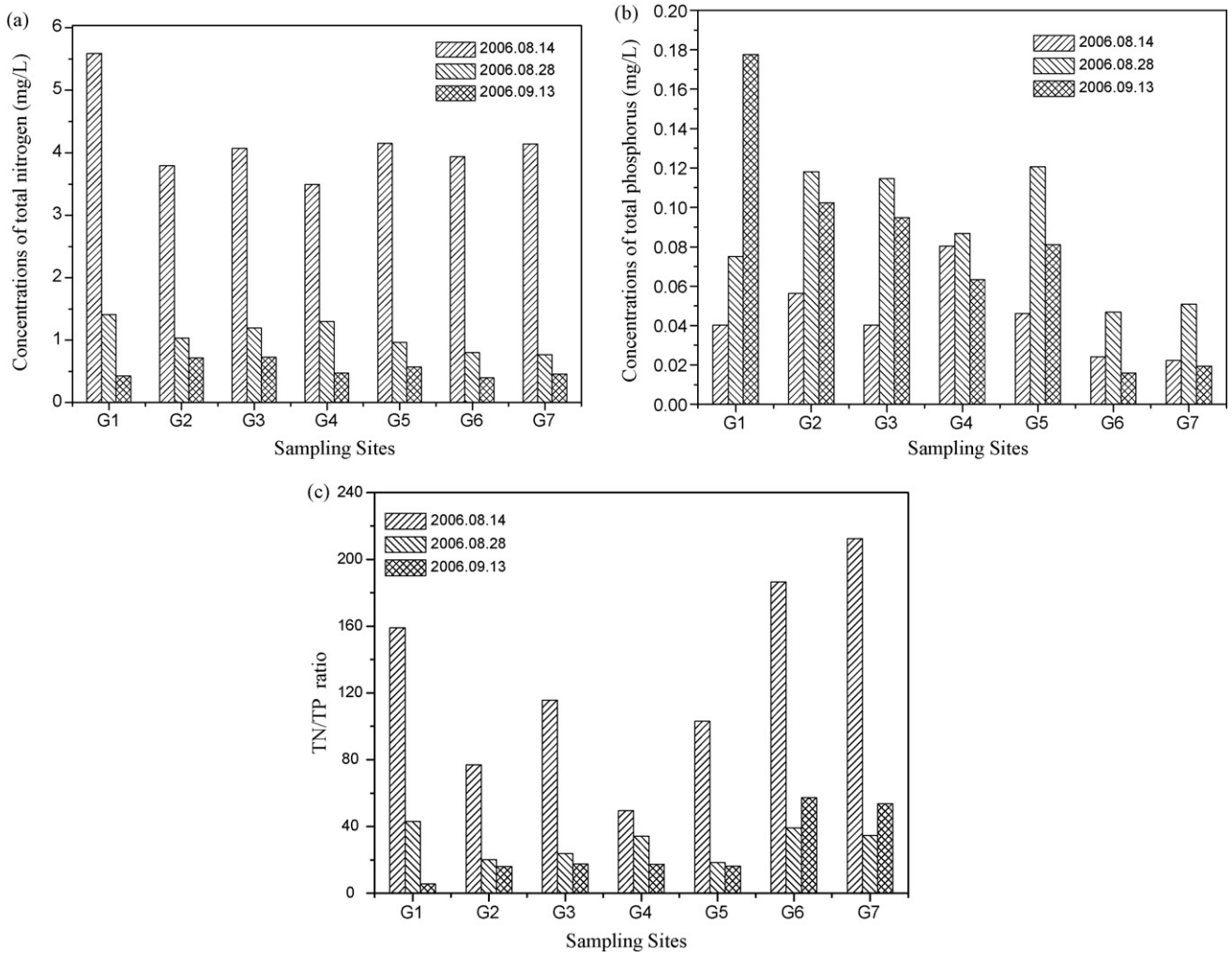

Fig. 2. Physicochemical parameters of Guanting Reservoir during sample period (a) Concentrations of total nitrogen; (b) concentrations of total phosphorus; (c) TN/TP ratio.

composed of *Microcystis*, *Oscillatoria agardh*i and *Melosira* which occurred in different proportions in different sites. *Microcystis* were the most abundant among phytoplankton, when their prevalence and biovolume grew markedly. *Microcystis* spp. prevailed in samples and contributed to 99% of the total cyanobacterial biovolume, with only a few species coexisting. The most abound species was *Microcystis novacekii* in G_2 and *Microcystis ichthyoblade* in G_5. The proportions of the genera known to produce microcystins *M. aeruginosa* were 3.6 and 13% in G_2 and G_5, respectively.

In contrast to the dominance of *Microcystis ichthyoblabe* and *M. novacekii*, Shi et al. [8] found *M. aeruginosa* and *Microcystis wesenbergii* much more abundant in the Guanting Reservoir in September 2004. These results confirmed the general species change in the phytoplankton composition in Guanting Reservoir. Within cyanobacteria, *M. aeruginosa* and *M. wesenbergii* not only have been found in this study but also have been reported from earlier investigation [8].

Notably the cyanobacterial cell numbers in September were rather higher than that of August. However, this increase in

Table 2
Dominant cyanobacterial genera and their ratios in G_2 and G_5 site of Guanting Reservoir

Cyanobacterial genera	*Microcystis ichthyoblabe*	*Microcystis novacekii*	*Microcystis botrys*	*Microcystis aeruginosa*	*Microcystis wesenbergii*	Microcystis peseudofilamentosa
G_2	16.07%	60.71%	17.86%	3.6%	1.5%	ND
G_5	60.56%	5.59%	20.78%	13%	ND	ND
G_2 [8]	ND	ND	ND	52.7%	36.2%	8.3%

ND, not detected.

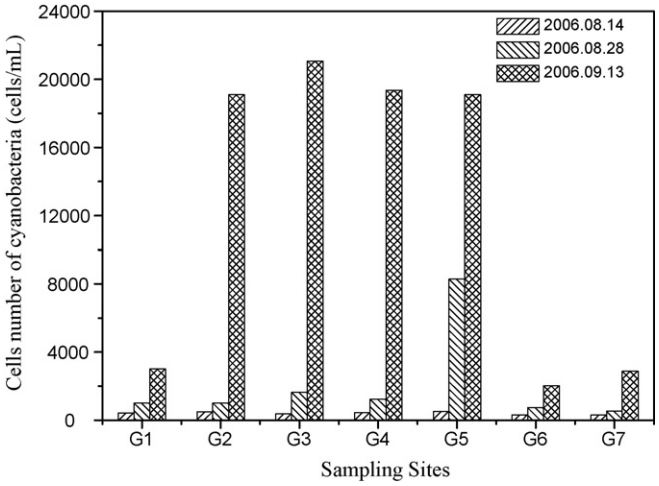

Fig. 3. Cells number of cyanobacteria on different time at all sites of Guanting Reservoir during sample period.

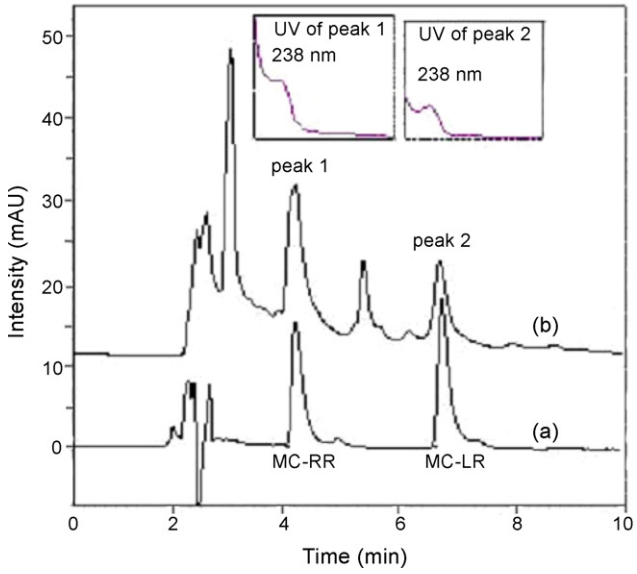

Fig. 4. The HPLC profiles for (a) standard MC-RR (RT = 4.33 min) and MC-LR (RT = 6.76 min) and the profiles for (b) species of MC within the cells of algae in Guanting Reservoir. Concentrations of standard MC-RR and LR are 5 mg/L, respectively.

cyanobacterial biomass could not be related to increasing nutrient concentrations, as no such increase was observed in TP concentration, and the amount of nitrogen in the water significantly decreased in September. Although nutrient concentrations were considered fundamental for the development of cyanobacterial blooms, many other variables were involved in their ecological success [9]. This indicated that the algal growths was probably limited by other factors apart from phosphorus and nitrogen, which was temperature, light, and trace metal and so on.

According to Johnson and Jacoby [10], who studied the migration of *Microcystis* in mesotrophic Lake Sammamish (WA), *Microcystis* occurrence was associated with a stable water column, increased total phosphorus concentration, surface temperature greater than 22 °C, high total nitrogen-to-phosphorus ratios, and increased water transparency. Water temperature appeared to be the most important factor influencing bloom development in a eutrophic pond and little growth was seen at temperature below 22 °C. On September 13, 2006, the concentrations of nitrogen in G_2 and G_5 were 0.72 and 0.57 mg/L, and that of phosphorus were 0.1 and 0.08 mg/L. These ratios of TN/TP were 15.98 and 16.11. According to nutrients and TN/TP ratio, they were suitable in favor of the growth of *Microcystis*. However, temperature decreased below 22 °C which inhibited the remarkable growth of *Microcystis*. Although there were some cyanobacteria in the surface water, heavy water bloom formation could not emerge.

3.3. Contents and species of microcystin within cells of algae

Microcystis spp. has been reported as a dominant MC producer worldwide; especially *M. aeruginosa* was the major MC-producing cyanobacteria [11]. Fig. 4(a) was the HPLC profile of standard solution of 5 mg/L MC-RR and MC-LR. Peaks of MC-RR and MC-LR appeared at 4.33 and 6.76 min, respectively. The wavelength of 238 nm was the maximum absorbance of MC-RR and MC-LR and was used for the detection of MC on HPLC in this experiment. Good linear relationships between the peak area and the standard concentrations of MC-RR and MC-LR were obtained at the wavelength of 238 nm ($R^2 = 0.998$ and 0.999). Fig. 4(b) was the HPLC profile for species of MC within the cells of algae. It was obvious that the algae in Guanting Reservoir produced MC-LR and MC-RR. Pronounced prevalence of both MCs is often reported to be characteristic of *Microcystis*-dominated algae. However, it is difficult to attribute MC production to one particular *Microcystis* because the cyanobacterial samples are composed of several codominant *Microcystis* and additional subdominant species.

Microcystin content in cells was highly variable in different time and space. As shown in Table 3, on August 28, 2006, algae

Table 3
MCs concentrations and species within the cells of cyanobacteria in G_2, G_3, G_4 and G_5 on August 28 and September 13, 2006

	G_2 (mg/g dry weight)	G_3 (mg/g dry weight)	G_4 (mg/g dry weight)	G_5 (mg/g dry weight)
August 28, 2006	ND	ND	ND	MCRR: 0.74 MCLR: 0.41
September 13, 2006	MCLR: 0.006	MCLR: 0.004	MCLR: 0.009	MCRR: 0.094 MCLR: 0.062

ND, not determined.

in G_5 produced high content of MC-LR and MC-RR. In contrast, on September 13, 2006, *Microcystis* spp. produced less microcystins, especially in G_2, G_3 and G_4 which produced MC-LR only but no MC-RR. Even at the same time of August 28, in G_2, G_3 and G_4, there was no any microcystins detected. Such phenomenon indicated a pronounced prevalence of toxic strains at different time. MC content in cells is affected both directly by MC-producing cyanobacteria and indirectly by environmental factors, and these factors vary temporally and spatially. Therefore, MC content in Guanting Reservoir was different in different time and space. If all conditions for MC production and MC-producing cyanobacteria were optimal, MC content would be up to a potential maximum. However, MC content often fell below the potential maxima because other abiotic and biotic factors were sub-optimal.

A weak correlation between *Microcystis* biovolume and MC content in cells reflected remarkable differences in microcystin contents of respective algae. These differences were caused by the numerous morphologically identical but genetically different strains of the same species [12,13]. These strains might or might not produce various microcystins according to the presence or absence of microcystin-encoding genes. Some strains did not produce microcystins, even though they contained genes for their biosynthesis [13]. G_2 contained *Microcystis ichthyoblabe*, *M. novacekii*, *Microcystis botrys*, *M. aeruginosa* and *M. wesenbergii* and dominant algae was *M. novacekii* (60.17%). The production of MCs by *M. novacekii* had not been reported in China. Thus, MCs content in G_2 was less than that of G_5. *Microcystis ichthyoblabe* (60.56%), which produced the heptatotoxin similar with *anabaena flos-aquae* toxin, dominated in G_5. This toxin was different from microcystin and was prone to be decomposed. Therefore, MC concentrations in G_5 were rather low, although it was higher than G_2.

Among the environmental factors, temperature was an important variable closely related with MC, suggesting that high temperature in summer could promote MC production [14]. On August 28, temperature was 26.8 °C and higher than that of September 13 (22.7 °C) at G_5. In this condition, the dominant MC-producer, *M. aeruginosa*, might be prevalent and gradually dominated among cyanobacteria. Thus, MCs concentrations were higher than that of September 13. If the temperature kept over 26 °C for several days, water blooms would occur and cyanobacteria in blooms would produce much more MCs.

3.4. Contents and species of microcystin in water samples

To date, more than 70 structural variants of microcystins have been isolated and characterized from cyanobacterial blooms and cultures. Pronounced prevalence of these variants was often reported to be characteristic of *Microcystis*-dominated blooms, although the variants did not always concur and their relative ratios were variable [15]. Changes in MC composition during sample period were both quantitative and qualitative. The present study indicated that MC-LR and MC-RR were the primary microcystins within the cells in the reservoir of the Guanting, while only MC-LR was detected in water. In the present study, *Microcystis* was the main cyanobacteria in the

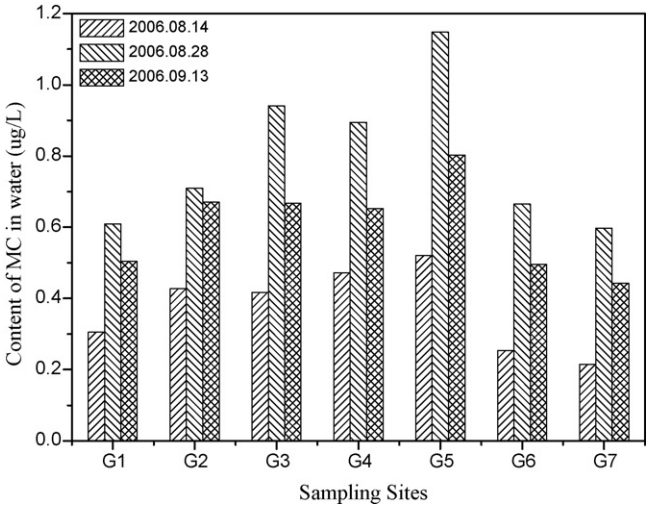

Fig. 5. Distributions of MC-LR in water sample on different time at all sites of Guanting Reservoir during sample period.

Guanting Reservoir, which was in accordance with the results of regional studies in American and Finnish lakes [16,17]. Fig. 5 showed the content of MC in the water samples of Guanting Reservoir. Microcystin was detected at all sites, although MC concentrations were low. The maximum MC value (1.15 μg/L) was in G_5 on August 28, 2006 and the minimum (0.21 μg/L) was in G_7 on August 14, 2006. Fig. 5 demonstrated that MC-LR was the dominant toxin in all sites and most of concentrations were below 1 μg/L, a level that the World Health Organization has deemed unsafe for long-term consumption [18]. MC-LR is the most commonly reported microcystin worldwide. It possesses higher hepatotoxicity amongst the identified microcystins and acts as a potent tumor promoter. The data presented demonstrated that the concentrations of main MCs (MC-LR) varied considerably with time and space. This was likely a consequence of different cyanobacterial species and strain compositions during sampling periods.

As show in Fig. 5, G_2, G_3, G_4 and G_5 sites had higher microcystin content comparing with other sites. Water quality of G_2, G_3 and G_4 was influenced by Yang River and Sanggan River which carried over 80% pollutants of Guanting Reservoir. Therefore, the contents of nitrogen and phosphorus were higher in these locations which offered favorable condition for cyanobacteria growth and toxin production. G_5 site was under the Hui Bridge and its water quality was affected by Guishui River. In addition to physical factors, we believed that geographical location and landform character were also correlated to higher microcystin or higher biovolume of cyanobacteria.

The data in the present study indicated cell numbers did not reflect the toxin concentrations in water. On September 13, 2006, cell numbers were much more than that of on August 28, but the maximum MC value occurred on August 28. Heresztyn and Nicholson [19] reported the toxin concentration followed the general trend of cyanobacterial cell numbers, whereas other studies found no relationship between Microcystis cell numbers and MC concentrations with changes in MC concentrations related to the dominant species of Microcystis [20]. However,

in the present study, the MC content within the cyanobacteria and in water sample was consistent. For example, cyanobacteria contained considerably more MCs on August 28 than that of on September 13. That is, the concentration of MCs in water followed the same trend as MCs content in cells.

Two biotic factors affect microcystins concentration in the field: cellular microcystin production and content [21], and the species composition of cyanobacteria in reservoirs [22]. Environmental conditions also indirectly influenced microcystins through their effects on these two factors. Numerous studies and investigations in specific lakes had examined the effects of various environmental factors (e.g. nutrients, light, and temperature) on microcystin production [23,24]. In addition to these, MC concentrations in water could vary substantially among reservoirs because of buoyancy and windblown cell accumulation along shorelines. Obviously, these factors varied temporally and spatially; it was not surprising that the relationships between MC and environmental factors were not always consistent. For example, MC in plankton was strongly correlated with TN of the lake water in America [25], but not in Canada [26], Germany [22] and in the present study.

Microcystins were supposed to be retained mainly in cyanobacterial cells and not released in huge amounts into ambient water except for cytolysis [27]. In many cases, no extracellular MCs were detected, although the total amount of MCs reached levels of several hundred micrograms per liter [28]. However, high concentration of extracellular MCs might occur during the senescence and decomposition of the cyanobacterial bloom at the end of summer. Accordingly, the total concentrations of both dissolved and intracellular MCs might be even higher than values reported in this survey.

MC concentrations were usually below 1 μg/L in Guanting Reservoir. However, MC concentrations in water occasionally exceeded the derived World Health Organization guideline of 1 μg/L [18]. A threat to public health in potable and recreational reservoir occurred primarily through the formation of scum and the accumulation of biomass. This was repeatedly observed along the shores, where public baths and campsites were located. As a consequence of the high incidence of toxic cyanobacteria, it was highly desirable to regularly monitor the reservoir studied in order to minimize potential health risks to the human population.

4. Conclusions

This study showed high prevalence of MC-producing cyanobacteria in Guanting Reservoir. The most common cyanobacteria, *Microcystis*, which tended to dominate the phytoplankton principally in August and September, were presumably the major producer of MCs. The specific species of the *Microcystis* in the cyanobacteria included *Microcystis ichthyobalbe*, *M. novacekii*, *M. botrys* and *M. aeruginosa* which had different ratios in different sites. The major microcystins were MC-RR and MC-LR within cyanobacteria, but only MC-LR was detected in water. The quantitative analysis by HPLC indicated that the maximum concentrations of MC-RR and MC-LR contained in cyanobacteria were 0.74 and 0.41 mg/g dry weight, respectively. The maximum microcystin concentration in water was 1.15 μg/L and others were below 1 μg/L. Although microcystin values of water in most sites were below levels likely to cause acute toxicity, microcystin posed a potential chronic health risk in Guanting Reservoir.

Natural systems are characterized by a vast of biotic and abiotic gradients coupled with multiple species interactions. In the Guanting Reservoir variation of MC concentration along environmental gradients proved similarly complex. Further investigation of the influence of environmental factors on MC production are critical to assist effective lake management and minimization of risks to human health risks from exposure to MC, especially in the seasonally river-connected lakes in the Guanting Reservoir.

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