Cyanobacterial population and harmful metabolites dynamics during a bloom in Yanghe Reservoir, North China

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ABSTRACT

A significant outbreak of odorous and toxic cyanobacteria occurred in Yanghe Reservoir, North China, in the summer of 2007. The dominant species was *Anabaena spiroides* and it was accompanied with the occurrence of very high concentrations of the odor metabolite geosmin. The event included two cycles of growth of *A. spiroides*: the first one of approximately 16 days between 21 June and 8 July, with the peak density 70,000 cells mL⁻¹ on 2 July, and the second smaller bloom of over 50 days between 9 July and the end of August. The bloom also included a range of species of *Microcystis*, predominantly *M. aeruginosa*, as the second largest population, which varied from 5800 cells mL⁻¹ to 28,000 cells mL⁻¹ in the first cycle and from 2000 cells mL⁻¹ to 11,000 cells mL⁻¹ in the second. Geosmin reached the peak value of 7100 ng L⁻¹ on 3 July, which was the highest value ever reported. The average geosmin production potential for *A. spiroides* cells was approximately 0.1 pg cell⁻¹, which was also very high and 85–97% of geosmin was found within the cells and in addition the dissolved geosmin did not increase even during the cyanobacterial decay period. In addition to odor metabolites, three microcystin variants MC-LR, MC-RR, MC-YR and anatoxin-a, most of which were intracellular, were detected, with MC-RR being the dominant cyanotoxin. The highest cyanotoxin concentrations were: dissolved: MC-RR 1.56 µg L⁻¹, MC-YR 0.066 µg L⁻¹, MC-LR 0.544 µg L⁻¹, anatoxin-a 0.106 µg L⁻¹; intracellular: MC-RR 70.1 µg L⁻¹, MC-YR 3.76 µg L⁻¹, MC-LR 24.6 µg L⁻¹, anatoxin-a 0.184 µg L⁻¹, and these occurred on 2 July. The correlation between geosmin and *A. spiroides* was excellent ($R^2 = 0.912$), however the correlation between anatoxin-a concentrations and *Anabaena* densities and between MCs concentrations and *Microcystis* densities was not as strong. This study demonstrated the relatively complex requirement for monitoring cyanobacterial blooms in this lake in China, where both the range of odors and cyanotoxins can be produced in concentrations which can change rapidly over a short time along with the bloom composition. It also demonstrated that both odor compounds and cyanotoxins should be considered in terms of the potential hazard to public water supply when a bloom was dominated by *Anabaena* and *Microcystis* occurs.

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1. Introduction

Cyanobacterial blooms have become more intensive worldwide during the last two decades, due to the increasing anthropogenic inputs of nitrogen and phosphorus to water environments (Paerl et

al., 2001; Khan and Ansari, 2005). In addition to the aesthetic and ecological problems, cyanobacterial blooms can have serious consequences for drinking water supply by physically blocking the filtration processes in water treatment plants, and producing a range of metabolites such as harmful cyanotoxins and taste and odor (T/O) compounds (Watson, 2004; van Apeldoorn et al., 2007; Waton et al., 2007).

Microcystis, which is the most frequently reported cyanobacterial genus responsible for freshwater cyanobacterial blooms worldwide (Haider et al., 2003), has long been a primary focus of attention because of its potential to produce microcystins (MCs), a group of cyclic heptapeptide hepatotoxins with more than 60

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variants (Duy et al., 2000). The *Microcystis*-dominated cyanobacterial blooms frequently occur in shallow lakes in tropical and subtropical regions with adequate nutrient supply, high temperature and high water stability usually associated with stratified conditions (Neilan et al., 1994; Sedmak and Kosi, 1998; Dokulil and Teubner, 2000; Xie et al., 2003; Yoshinaga et al., 2006; Xu Y. et al., 2008). *Anabaena*, which is another significant bloom-forming cyanobacterial genus, is well known for its ability to produce geosmin, a taste/odor (T/O) compound with earthy or moldy smell (Izaguirre et al., 1982; Hayes and Burch, 1989; Saadoun et al., 2001; Tsujimura and Okubo, 2003; Wang et al., 2005). In addition, *Anabaena* has recently been identified as a producer of anatoxin-a, a potent postsynaptic depolarizing neuromuscular blocking cyanotoxin (Beltran and Neilan, 2000; Pawlik-Skowronska et al., 2004; Osswald et al., 2007) as well as MCs (Sotero-Santos et al., 2008). Although *Anabaena* is a common bloom-forming cyanobacterium with a worldwide distribution, there are few reported studies of the occurrence of *Anabaena* and the associated metabolites geosmin and cyanotoxins worldwide.

There have however been several studies of the production of odorous and toxic metabolites by cyanobacteria in well-known eutrophic lakes in southern China. These have included the dynamics of odorous metabolites in Dianchi Lake (Li L. et al., 2007), and seasonal variation of microcystin in Taihu Lake (Shen et al., 2003; Xu Q. et al., 2008). All of these studies have monitored the lakes at monthly intervals and did not capture the dynamic variability in the cyanobacterial population growth cycle of individual species.

In addition there have been no studies of cyanobacterial blooms in China with mixed or co-dominant species that concurrently produce both cyanotoxins and odorous metabolites. The production of both cyanotoxins and odorous metabolites at the same time will present an interesting challenge for the management of blooms and the selection of treatment options to properly ensure the safety of drinking water supplies.

Yanghe Reservoir is an important water source for Qinhuangdao City in north China, and has been known for the occurrence of major cyanobacterial blooms in early summer in recent years (Li W. et al., 2007). Significant earthy geosmin-like odors in water supply have been reported to be associated with these blooms. The current study reports an intensive 2-month investigation from late June 2007 to track the cyanobacterial population and the metabolites in Yanghe Reservoir at short-time intervals to investigate the bloom dynamics in a mixed odor and toxin-producing population over the summer bloom season. The study was initiated to assess the relative production and dynamic variation of the range of compounds and assess the threat to drinking water treatment for the treatment works associated with Yanghe Reservoir.

2. Materials and methods

2.1. Sampling

Yanghe Reservoir has a capacity of $3.58 \times 10^8 \text{ m}^3$, an average depth of 5.7 m, average water area of 13 km^2 , and volume of $0.75 \times 10^8 \text{ m}^3$ during the period of the investigation. Source water was taken from the water intake as shown in Fig. 1, and supplied to four waterworks in Qinhuangdao City through a 30 km long pipeline. Samples were taken daily from the water intake area ($119^\circ 12' 51'' \text{E}$, $39^\circ 58' 51'' \text{N}$) from 25 June to 24 August 2007. Surface layer samples were collected in polypropylene bottles from a depth of 0.1–0.5 m, and transported immediately to the laboratory in the city for analysis or pretreatment. Cyanobacterial cells were counted under the microscope within 1 h of arrival at the laboratory, and samples for cyanotoxin analysis were

Fig. 1. Schematic map of Yanghe Reservoir and its geographical position in China, main sample site was the water intake.

pretreated within 2 h. If the samples could not be analyzed immediately, they were stored under refrigeration at 4°C . The samples for odor compounds determination were stored in brown glass bottles with airtight stopper at 4°C and analyzed as soon as possible, after $10 \text{ mg L}^{-1} \text{ HgCl}_2$ was added to prevent biodegradation (American Public Health Association, 2001).

2.2. Chemicals and reagents

All chemicals and solvents were of analytical or HPLC grade. Geosmin, MIB, 2-isobutyl-3-methoxy-pyrazine (IBMP) and 2-isopropyl-3-methoxy-pyrazine (IPMP) standards were obtained from Acros Organics, Belgium. Anatoxin-a, microcystin LR, RR, YR standards were bought from Alexis Company, Switzerland. HPLC grade methanol, acetonitrile (ACN) and formic acid were products of Fisher, USA. NaCl, KI, I_2 , HgCl_2 of analytical grade were from Beijing Chemicals Ltd.

2.3. Cyanobacterial cell counting

Cyanobacterial cell number was counted with a 0.1 mL phytoplankton counter chamber (HL-JS 0 Wuhan Hengling Ltd., China) using a microscope (Zeiss Axioskop 2 Mot Plus, Germany). For the samples with a high cell density, cell numbers were counted without concentration. For other samples, cell numbers were counted after preconcentration of $10\times$ (from 100 mL to 10 mL) by settling in Lugol's solution.

The samples were dominated mainly by *Anabaena spiroides* and several species of *Microcystis*. The cell number of the *Microcystis* species, which is a colonial aggregation of individual cells, was

Table 1
Mass spectrometry parameters.

Toxin	Retention time (min)	Precursor ion m/z	Product ion m/z	Product ion m/z	Cone voltage (V)	Collision energy (V)
Anatoxin-a	1.480	166.1	148.8	130.6	35	15
MC-RR	2.525	520.1	135.2	887.7	45	20
MC-YR	2.660	1045.8	213.1	375.5	75	55
MC-LR	2.750	995.8	135	213.4	75	55

estimated by judging the individual colony volume and dividing by the average single-cell volume for the different species (Hötzel and Croome, 1999). For *A. spiroides*, the coiled filamentous cyanobacteria, the average cell number of individual spiral coils was obtained under 40 $\times$ objective lens, and then the coil number was counted under 10 $\times$ objective lens, and the cell density was determined by multiplying the coil number with the average cell number of one coil. Cyanobacterial microscopic photos were taken under the bright field (BF) mode or the differential interference contrast (DIC) mode.

2.4. Analysis of odor compounds

2.4.1. SPME procedure

The headspace solid phase micro-extraction (HS-SPME) method (Liang et al., 2005) was employed for the extraction of odor compounds with a DVB/CAR/PDMS fiber (57348-U, Seplco, America). Briefly, after 12.5 g NaCl and 20 μ L internal standard solution containing IBMP 50 μ g L⁻¹ and IPMP 50 μ g L⁻¹ were added in a 75 mL vial containing 50 mL water samples, the vial was then sealed with a silicone-teflon septum cap inserted with the extraction fiber, and put into a 65 °C water bath shaken at 800 rpm. After a 30 min headspace extraction, the fiber was taken out from cap and immediately inserted into the GC injection port to desorb for 3 min at 240 °C. Dissolved odor compounds were determined by using the same procedure after the samples were first filtered through GF/C (Whatman, England, 1.2 μ m) filters to remove cyanobacterial cells.

2.4.2. GC-MS analysis

The GC-MS analysis was based on the method developed by Liang et al. (2005). Analysis was carried out in an Agilent 6890/5975 GC-MS system with a 30 m HP-5 ms capillary column (i.d. 0.25 mm, coating film thickness 0.25 μ m). In scan mode, oven temperature was held at 40 °C for 3 min, raised to 200 °C at 4 °C min⁻¹ and maintained at this temperature for 2 min, then raised to 260 °C at 15 °C min⁻¹, and kept at 260 °C for 2 min. The scan ion range m/z was 46–260. In quantitative select ion mode (SIM), oven temperature was raised from 40 °C to 240 °C at 8 °C min⁻¹ and maintained at this temperature for 5 min. The transfer-line temperature was 280 °C and ion-trap temperature was 230 °C. The ion-trap mass spectrometer was operated in the EI positive mode (70 eV). The quantitative ions (m/z) selected for geosmin, IBMP and IPMP were 112, 124 and 137, respectively. Geosmin concentrations were determined by calibrating with the relative response factor (RRF) of IBMP and IPMP.

2.5. Cyanotoxins analyses

2.5.1. Sample pretreatment

Water samples (2 L) were filtered through a GF/C filter to remove cyanobacteria after the pH was adjusted to 5.0 with 1 mol L⁻¹ HCl. The filtrates were used for the determination of dissolved toxins, and the cyanobacterial cells remaining on the GF/C filters were used for measurement of intracellular cyanotoxins. A solid phase extraction (SPE) procedure (Aranda-Rodriguez et al., 2003) was used for concentration of toxins with a little

modification, which involved activation of the cartridge (Oasis[®] HLB 500 mg, Waters, America) with 10 mL of methanol, conditioning with 10 mL of distilled water, and percolation of the sample at a flow rate of 10 mL min⁻¹. After a drying procedure of successive centrifugation and evaporation under a gentle stream of nitrogen, the cartridges were washed with 10 mL 40% methanol and eluted with 10 mL of 80% methanol slowly (Zhang et al., 2005). The elution was concentrated to a final volume of 1 mL by evaporation under a gentle stream of nitrogen. The extracted samples were filtered with 0.2 μ m disposable nylon membrane before injection.

For the determination of intracellular cyanotoxins, the above GF/C filters were put into a 22 mL glass vial containing 10 mL of 80% methanol. Cell lysis was conducted in an ultrasonic cell crusher for 10 min (10 s–10 s pulse, 500 W). The solutions were then treated using the same procedure as for the dissolved toxins after centrifugation (8000 $\times$ g, 10 min) and filtration with GF/C filters.

2.5.2. HPLC-MS/MS parameters

The HPLC-MS/MS system consists of Waters Acquity UPLC[™] equipment (Waters, Milford, USA), an Acquity UPLC[™] BEH C18 column (100 mm $\times$ 2.1 mm, 1.7 μ m) and a Quattro Ultima[™] Pt Mass system. The column oven was set at 40 °C with a mobile phase flow of 0.3 mL min⁻¹. The mobile phase consisted of ACN and water with 0.1% (v/v) formic acid. The injection volume was 5 μ L. The mass spectrometer used was operated in ESI positive mode with a capillary voltage of 3.0 kV and multiplier voltage of 650 V. The cone voltages are listed in Table 1. The nebulizing gas was adjusted to the maximum and desolvation gas at a flow rate of 450 L h⁻¹. Cone gas was not used. The source and desolvation temperatures were held at 100 °C and 350 °C, respectively. UHP argon was used as the collision gas, and the pressure of the collision chamber was held at 3.3×10^3 mbar. Conditions for mass spectrometry are shown in Table 1.

2.6. Water quality and other environmental parameters

All of the water quality analyses were performed according to standard examination methods for drinking water (GB/T 5750-2006). The pH, ammonia nitrogen and permanganate index were analyzed daily, and other parameters determined occasionally. The temperature and rainfall information was kindly provided by local weather bureau (Fig. 2).

3. Results and discussion

3.1. Cyanobacterial bloom outbreak

The cyanobacterial bloom first appeared and was associated with strong earthy smell in Yanghe Reservoir from 20 June. Cyanobacterial scum appeared in the water surface layer near the bank and was concentrated by wind and waves. The cyanobacterial population consisted mainly of several *Microcystis* species and *A. spiroides* as dominants (Fig. 3), while the phytoplankton community had sporadic occurrence of a range of other cyanobacterial and algal types including *Oscillatoria* sp., *Melosira varians*, *Asterionella formosa*, *Cyclotella* sp., *Fragilaria crotonensis*,

Fig. 2. Temperature and rainfall at Yanghe Reservoir from June to August 2007.

Synedra acus, *Pediastrum simplex*, *Chlorella* sp., *Ceratium hirundinella* and *Volvox* sp. The *Microcystis* population in Yanghe Reservoir was comprised predominantly of *Microcystis aeruginosa*, which is the most common microcystin producer and bloom-forming cyanobacterium (Moore et al., 1993; Codd, 1995). Other *Microcystis* species (10–30%) present included *M. wesenbergii*, *M. novacekii*, and *M. botrys*. *A. spiroides*, which has been shown to produce geosmin, anatoxin-a and MCs (Van der Ploeg et al., 1992; Velzeboer et al., 2000; Pawlik-Skowronska et al., 2004) was the only *Anabaena* species present in the reservoir.

As shown in Fig. 2, it had been fine weather with a temperature varying between 22 °C and 26 °C for 20 days with no rainfall before the occurrence of the cyanobacterial bloom. Other conditions were as follows: total nitrogen (TN): 3–7 mg L⁻¹, total phosphorus (TP): 0.04–0.08 mg L⁻¹, pH: 8–9, and dissolved oxygen: 3–9 mg L⁻¹, Permanganate index: 3.3–12.2 mg L⁻¹.

3.2. Cyanobacterial population dynamics

The variation in cell densities of *Microcystis* spp. and *A. spiroides* at the water intake during the investigation period is shown in

Fig. 4. It is clear that the growth dynamics of the cyanobacterial population could be divided into two cycles over the summer. The first cycle of rapid growth and decline was of approximately 20 days from 20 June to 9 July, and the second of approximately 40 days started from 10 July. The *A. spiroides* population showed very rapid growth and decay rates in the first cycle: the cell density increased from 500 cells mL⁻¹ on 20 June to the maximum value of 70,000 cells mL⁻¹ on 2 July, replacing *Microcystis* spp. as the dominant cyanobacterium, and then declined rapidly to the minimum level of 1000 cells mL⁻¹ on 9 July. The cell density of *Microcystis* spp., on the other hand, did not change as much, only varying in the range of 1800–28,000 cells mL⁻¹.

A slow increase and decrease of *A. spiroides* cell density with a much lower peak value (19,300 cells mL⁻¹) were observed in the second cycle. The cell densities of *Microcystis* spp. were relatively stable and *A. spiroides* was again the dominant cyanobacteria throughout most of this period. From 10 August *A. spiroides* began to decline and *Microcystis* spp. became the dominant species for a short period. Also at this same time, the algal population diversity increased with the appearance of diatoms including *F. crotonensis* and *S. acus*.

Fig. 3. Photomicrographs of the dominant cyanobacteria in Yanghe Reservoir June–August, 2007. *Anabaena spiroides* was the dominant species and the other dominants were a mixed population of *Microcystis* spp. which consisted primarily of *M. aeruginosa*, with smaller contributions from *M. wesenbergii*, *M. novacekii*, and *M. botrys*.

Fig. 4. Cyanobacterial population dynamics of the dominant genera in the water intake.

The possible reasons for lower peak cyanobacteria biomass during the second cycle as opposed to the first is not known, however it may relate to nutrient availability as much of the nutrition assimilated by cyanobacterial cells during the first cycle may have sedimented with the collapse of the population and had not remineralised from the sediments to become bioavailable for the latter period of growth.

Gray foam and scum appeared on the water surface between 6 July and 10 July, which was associated with the appearance of *Volvox* sp. (3300 cells mL⁻¹ on 6 July). This scum vanished suddenly together with *Volvox* sp. The organic matter might be responsible for the growth of *Volvox* sp. (Gladyshev et al., 1998). It was observed that the permanganate index increased from 3.18–4.60 mg L⁻¹ (June 25–1 July, average 3.78 mg L⁻¹) to 5.4–12.2 mg L⁻¹ (July 2–July 8, average 8.54 mg L⁻¹), and then decreased to approximately 4 mg L⁻¹. The organic matter increase may be as a result of decomposition of massive dead cyanobacterial cells after the peak.

The rapid growth and then sharp decline of the cyanobacterial population in Yanghe Reservoir are intriguing. The numbers of cyanobacteria in particular collapsed from the maximum value of 81,000 cells mL⁻¹ to 28,000 cells mL⁻¹ in 2 days then continued to decrease gradually to reach the lowest value of 3600 cells mL⁻¹ on 13 July. Although the trigger for this sharp decline is unclear, it is possible to speculate about possible causes as follows: (1) bioavailable nutrients had been depleted; (2) rain event (approximately 90 mm) in June 30 and July 1 (Fig. 2) resulted a major local run-off which flowed into Yanghe Reservoir and caused water turbulence. Cyanobacteria are favored by a stable water column and turbulence may impact the relatively fragile cell wall of cells such as *Anabaena*; (3) aging of the cyanobacterial population and cell death (apoptosis) can contribute to the decline of a bloom (Bidle and Falkowski, 2004; Veldhuis and Brussaard, 2006); (4) dissolved oxygen depletion and stress at high cell densities due to a reduction in photosynthesis and also increased biological oxygen

demand due to microbial processes in the sediments and water column as a result of the bloom; (5) other biotic effects such as grazing by protozoans or zooplankton, lytic bacteria or viruses that can infect cyanobacteria.

3.3. Variation of odorous compounds

Analysis of major odorous compounds was performed by GC/MS analysis under scan mode. The most abundant cyanobacteria-related compounds were geosmin and β -cyclocitral, together with others such as β -ionone, camphor, hexanal, octanol, nonanal, 5-hepten-2-one, nonanol, DL-menthol, etc., present at a much lower level. The odor of β -cyclocitral is described as tobacco or smoky with an OTC (odor threshold concentrations) of 19.3 μ g L⁻¹, while the odor of geosmin is described as earthy or musty with a lower OTC (4 ng L⁻¹) (Watson, 2004). Based upon the significant occurrence of geosmin and the association with *A. spiroides*, it was likely that geosmin was mainly responsible for the heavy musty odor of source water reported throughout the period of the investigation.

Total geosmin concentration in the water intake is shown in Fig. 5. The variation in geosmin was closely related to the cell density of *A. spiroides* (Fig. 4), further suggesting that geosmin produced by *A. spiroides* was responsible for the odor in the reservoir. Total geosmin level increased rapidly with a 1–2-day lag behind the increase in *A. spiroides* cell density, and dropped equally quickly with the decrease of cell density. The maximum geosmin concentration of 7100 ng L⁻¹ was observed on 3 July, 1 day after *A. spiroides* reached its maximum cell density. This is one of the highest geosmin levels ever reported. Previously, 4000 ng L⁻¹ in Australia (Jones and Korth, 1995) and 3170 ng L⁻¹, in South Africa (Wnorowski and Scott, 1992) have been reported in natural waters.

Fig. 5. Geosmin variation in the water intake surface layer.

Fig. 6. Correlation of geosmin concentration with *Anabaena spiroides* density, $n = 40$. $Y = 0.999 \times X \approx 1 \times X$; slope standard error = 0.049; $R^2 = 0.912$.

Fig. 7. Variations of dissolved cyanotoxins in the water intake surface layer.

The total geosmin concentration is plotted against *A. spiroides* density in Fig. 6. Geosmin concentration was very strongly correlated with cell numbers of *Anabaena* ($R^2 = 0.912$), further demonstrating that geosmin was produced mainly by *A. spiroides*. In addition recent molecular analysis work using the method of Steven et al. (2008) has confirmed that the gene responsible for geosmin synthesis was found in the preserved field samples collected from Yanghe Reservoir (unpublished). Based upon this correlation the average geosmin cell quota for *A. spiroides* was about 0.1 pg cell^{-1} , which was 10 times higher than $0.01 \text{ pg cell}^{-1}$ that was reported in an Australian bloom associated with *Anabaena* sp. (Jones and Korth, 1995). In a study of the dynamics of odor compounds in the pilot experimental area of Lake Dianchi, southern China (Li L. et al., 2007; Li W. et al., 2007), correlations between particulate geosmin and the biomass of *Anabaena* were found. But the maximum geosmin concentration was only 10 ng L^{-1} .

It should be noted that the concentration of geosmin dropped dramatically from the peak value of 7100 ng L^{-1} on 3 July to as low as 1080 ng L^{-1} on 4 July which was associated with the disappearance of *A. spiroides*. A range of factors can be important for the loss of geosmin in natural waters including biodegradation and volatilization (Lin et al., 2007). Some gram-negative (Hoeftel et al., 2006) and gram-positive (Saadoun and el-Migdadi, 1998) bacteria, *Pseudomonas* sp., *Alphaproteobacterium* sp., *Sphingomonas* sp. (Ho et al., 2007) were reported to be involved in the biodegradation of geosmin. A population of *Pseudomonas* sp. and *Sphingomonas* sp. has been identified in Yanghe Reservoir water by molecular analysis techniques (unpublished data). The reason for the sudden disappearance of geosmin in the case of Yanghe Reservoir, however, requires further investigation.

Some samples were analyzed for both dissolved and total geosmin which includes the intracellular content for selected dates throughout the investigation (Fig. 5). In the conventional water treatment process the intracellular geosmin is readily removed together with the algal cells in the coagulation and flocculation step (Chow et al., 1999). As shown in Fig. 5, in the Yanghe Reservoir case, 85–97% of the total geosmin was intracellular, which means that less than 15% of the geosmin was present in dissolved form.

With such a high intracellular geosmin concentration, special care should be taken to prevent geosmin from being released from cells within the water treatment process. The waterworks at Qin-huangdao City stopped prechlorination during the period, which was very important to prevent *A. spiroides* cells from being broken up or lysed by chlorine. On 3 July, the total geosmin was 7100 ng L^{-1} , and the dissolved geosmin was only 473 ng L^{-1} . The water treatment system was able to successfully control geosmin in drinking water to below 10 ng L^{-1} by addition of powdered activated carbon (PAC) into the source-water intake and the coagulation tank in succession with a total PAC dose of 80 mg L^{-1} .

3.4. Cyanotoxins

Variations of cyanotoxins in water intake over the period 25 June–24 August are shown in Figs. 7 and 8. All of the four cyanotoxins were detected, and most of cyanotoxins were located within the cells, indicating that appropriate treatment was required to prevent cells from being disrupted (Watanabe et al., 1992; Kenefick et al., 1993; Daly et al., 2007; Sakai et al., 2007). The maximum concentrations found for the four individual cyanotoxins were: dissolved cyanotoxins: MC-RR 1.56 µg L^{-1} , MC-YR 0.066 µg L^{-1} , MC-LR 0.544 µg L^{-1} , anatoxin-a 0.106 µg L^{-1} ; intracellular cyanotoxins: MC-RR 70.1 µg L^{-1} , MC-YR 3.76 µg L^{-1} , MC-LR 24.6 µg L^{-1} , anatoxin-a 0.184 µg L^{-1} . These values occurred on 2 July, the day which coincided with the maximum total cyanobacterial density ($81,000 \text{ cells mL}^{-1}$), whereas the maximum *Microcystis* spp. density ($28,000 \text{ cells mL}^{-1}$) occurred on 30 June. It was clear that MC-RR was the dominant cyanotoxin, followed by MC-LR, while MC-YR and anatoxin-a were at a significantly lower level. More than 90% of cyanotoxins were intracellular or cell-bound during the cyanobacterial bloom period, except for MC-RR. For MC-RR, the intracellular ratio was in a wide range between 15% and 95% and was related to the abundance and concentration of *Microcystis* spp. and the stage of the bloom over the period of the study. For example the intracellular MC-RR ratio was 95% on 25 June (*Microcystis* spp.: $18,000 \text{ cells mL}^{-1}$) but had fallen to 15% on 13 July (*Microcystis* spp.: $2000 \text{ cells mL}^{-1}$). The reason proposed for this

Fig. 8. Variations of intracellular cyanotoxins in the water intake surface layer.

Table 2Parameters from correlation analysis of cyanotoxin concentrations and cyanobacterial cell numbers (pg toxin cell⁻¹).

	MC _s - <i>Microcystis</i>	MC-RR- <i>Microcystis</i>	MC-YR- <i>Microcystis</i>	MC-LR- <i>Microcystis</i>	Anatoxin-a- <i>Anabaena</i>
Total toxins					
Slope	1.24	0.973	0.053	0.215	0.0058
Stand error	0.159	0.117	0.0065	0.0408	0.0009
Adj. R ²	0.714	0.738	0.727	0.527	0.572
Intracellular toxins					
Slope	1.08	0.790	0.054	0.234	0.0046
Stand error	0.089	0.065	0.004	0.024	0.0009
Adj. R ²	0.852	0.851	0.882	0.784	0.507

is that with the collapse of the *Microcystis* population after 30 June the MC-RR was released and remained in the water as free dissolved toxin into July. Cyanotoxins were correlated with cell densities of cyanobacteria for the 25 samples collected throughout the investigation as shown in Table 2. The Adj. R² for the correlations between total toxins and cell numbers of corresponding cyanobacteria were 0.527–0.738. The Adj. R² between intracellular cyanotoxins and the corresponding cell numbers were higher (0.507–0.882). It should be noted that the correlation between the intracellular MCs and *Microcystis* was relatively strong (0.784–0.882), indicating that *Microcystis* was likely to have been responsible for the production of MCs. In addition, the *Microcystis* population contained non-microcystin producing *M. wesenbergii* (Xu Y. et al., 2008) which constituted between 10% and 30% range of total *Microcystis* population and the MC production of *Microcystis* was influenced by environmental factors and water quality. Also further work has shown that the *mcy E* gene responsible for microcystin synthesis was detected (Vaitomaa et al., 2003) in *Microcystis* but not in *A. spiroides* (unpublished data) in preserved field samples from Yanghe Reservoir. This suggested that the *A. spiroides* population would be likely to be unable to produce microcystin in this case. Based upon the slopes of the correlation curves the average MCs production potential per cell could be estimated to be approximately 1 pg cell⁻¹ and about 80% of that was MC-RR.

To put the Yanghe Reservoir data into context, the dissolved MC concentrations were only about 1/3–1/2 of that reported in an *Anabaena* bloom in Brazil (Sotero-Santos et al., 2008). Two separate studies of the seasonal variation of microcystin toxins associated with *Microcystis* blooms in Taihu Lake in southern China showed maximum toxin levels of 10.4 µg L⁻¹ (Shen et al., 2003) and 15.6 µg L⁻¹ (Xu Q. et al., 2008; Xu Y. et al. (2008)). These concentrations were determined using ELISA and were calibrated to MC-LR, and compared with the toxin levels measured in Yanghe Reservoir, where the range of variants were determined and quantified.

Although MC-RR, the dominant microcystin in Yanghe Reservoir is not as toxic as MC-LR (Dittmann and Wiegand, 2006), the very high MC-RR concentration on July 2 (70.1 µg L⁻¹) can be regarded as hazardous for water supply. Fortunately, the cyanotoxins can also readily be adsorbed by PAC as is geosmin, and the cyanotoxin concentrations in tap water derived from Yanghe Reservoir remained at a very low level during the cyanobacterial bloom period (for example on 16 July: the MC-RR 1.154 ng L⁻¹, MC-YR 0.371 ng L⁻¹, MC-LR 0.563 ng L⁻¹, anatoxin-a not detected). This study has shown that both geosmin and cyanotoxins should be considered as a potential water quality hazards when a cyanobacterial bloom dominated by *Anabaena* and *Microcystis* occurs.

4. Conclusions

This study investigated the occurrence of both odor compounds and cyanotoxins monitored at high intensity during a

cyanobacterial bloom in Yanghe Reservoir in north China. The bloom changed in composition with initial dominance by microcystin producing *M. aeruginosa* followed by *A. spiroides* which produced a very high concentration of geosmin (up to 7100 ng L⁻¹) as well as the neurotoxin anatoxin-a. *A. spiroides* had a geosmin production potential of 0.1 pg cell⁻¹, and more than 85% geosmin was intracellular. Among the four detected cyanotoxins produced by cyanobacteria, MC-RR was the most abundant and the maximum dissolved concentration was 15.6 µg L⁻¹. Most of cyanotoxins were located within the cells although as the bloom collapsed significant amounts of MC-RR were found free and dissolved in the water. By contrast the cell density of *A. spiroides* declined quickly after it reached the peak value, without perceptible release of geosmin and cyanotoxins. This study showed the relatively complex requirement for monitoring cyanobacterial blooms in this lake in China, where both the range of odors (e.g. geosmin) and cyanotoxins (MC-LR, RR, YR and anatoxin-a) can be produced in concentrations which can change rapidly over a short time along with the bloom composition. It also demonstrated that both odor compounds and cyanotoxins should be considered when a bloom dominated by *Anabaena* and *Microcystis* occurs.

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