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Relationship Between Cyanobacterial Biomass and Total Microcystin-LR Levels in Drinking and Recreational Water

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Abstract Cyanobacterial biomass, chlorophyll-*a*, and microcystin-LR levels were monitored in drinking and recreational water in Seoul, South Korea and three satellite cities from Oct 2006 to Aug 2007. Total microcystin-LR was the sum of particulate and dissolved microcystin. Except during cold periods, toxic cyanobacteria, including *Anabaena flos-aquae,* were found at all sites. The total microcystin-LR levels were below guideline danger levels (<1.0 μg/L) except one time (1.27 μg/L in October), whereas chl-*a* (111.7 μg/L) and cell levels (2.6×10^5 cells/mL) were at 'vigilance' and 'alert' levels for drinking water and at 'guidance' level for recreational water, respectively. Discrepancies in these parameters may thus lead to frequent unnecessary alerts, thereby increasing water management costs.

Keywords Cyanobacteria · Microcystin-LR · Drinking and recreational waters · Alert system

Microcystin, a small hepatotoxic peptide produced by cyanobacteria, poses a threat to wildlife, livestock, and humans (Carmichael et al. 1988). The provisional guideline value of 1 μg/L microcystin for drinking water has been debated by researchers since it was first proposed by the World Health Organization (WHO 1998; Figueiredo et al. 2004; Hoeger et al. 2004). While some countries adopted the value of 1 μg/L (Chorus 2005; Vardaka et al. 2005), others developed their own guideline values for microcystin in drinking water. Notably, this level was set at 1.3 μg/L in Australia (NHMRC 2004) and 1.5 μg/L in Canada (HC 2002). For recreational water or water for bathing, the WHO considers 20–100 μg/L microcystin to be the acceptable range for chronic exposure for humans (WHO 2003); however, Germany considers >100 μg/L microcystin unacceptable for recreation or bathing water, and the cutoff is >10 μg/L in Australia (Wood et al. 2006).

Based on the WHO and Australian guidelines, the Korean Ministry of the Environment adopted an alert system for the 20 drinking water reservoirs in Korea, but have not yet established acceptable levels for cyanobacteria, chlorophyll-*a* (chl-*a*), and microcystin. Seoul is the capital city of South Korea, with a population of approximately 10 million people and 40 natural or artificial ponds. The seven satellite cities include 70 natural or artificial ponds and 150 reservoirs (SDI 2001); most of these ponds and lakes are not regularly or systematically monitored for cyanobacteria, chl-*a*, and microcystin levels.

This study reports the results of bi-monthly monitoring of cyanobacterial biomass, chl-*a,* and microcystin levels in drinking and recreational waters in Seoul and its satellite cities and investigates the relationship of these three parameters with the current alert system.

Materials and Methods

Ten bodies of water used for drinking water and recreational purposes in Seoul city and three satellite cities (Ilsan, Seongnam, and Gwangju city) were monitored bi-monthly

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Table 1 Characteristics of 10 bodies of water used for drinking water and recreational purposes in Seoul and its satellite cities in South Korea

Sampling sites	Surface (1,000 m^2)	Volume (1,000 m^3)	Mean depth (m)	Use	Visitors (1,000 ind./year)	Sources	Year installed
[S1] Gyeongan str*	598	ca. 300,000	2–5	D	10,000	rw	1980
[S2] Paltang lake*	23.8	ca. 240,000	5–20	D, R	10,000	rw	1973
[S3] Gildong ep	2.5	2.5	1–2.5	R	100	gw	1998
[S4] Bundang pp	4.2	127.3	1–1.5	R	1,000	rw	1994
[S5] Cheonho brid.*	2.9	ca. 10,000	3–5	D, R	15,000	rw	1976
[S6] Seokchon lake	147.4	737	4–5	R	15,000	rw, gw	1971
[S7] Gyeonghoeru pp	11.6	0.7	0.5–2	R	1,000	gw, tw	1412
[S8] Yongsan pp	6.7	6.0	0.5–2	R	2,500	tw	1959
[S9] Yeouido ep	0.6	0.5	0.5–1	R	10,000	gw, tw	1997
[S10] Ilsan pp	300.0	453	0.5–2	R	3,500	gw, tw	1995

* Directly connected with the Han River; *str*, stream; *ep*, ecological pond; *pp*, park pond; *R*, recreational water; *D*, drinking water; *gw*, ground water; *tw*, tap water; *rw*, river water

from October 2006 to August 2007 (Table 1). Three sites (S1, S2, and S5) are connected directly to the Han River, one of the largest rivers in Korea and the source of drinking water for approximately 20 million people in two cities. Because of the alert system for cyanobacterial blooms, three sampling sites are monitored monthly by a branch of the Korean Ministry of the Environment. The other eight sites, which are not monitored, are mostly artificial ponds that are supplied by ground and tap water from residences. Of these eight sites, five sites (S3, S6–S9) are in Seoul and two sites (S4 and S10) are in the satellite cities. The air temperatures during the study period showed seasonal variation, ranging from −10 to 33°C (http://www.kma.go.kr/index.jsp).

Water temperature, dissolved oxygen, pH, and electric conductivity were measured at each site using a portable meter (YSI 6920, Yellow Springs Inc., OH, USA). Water samples were taken at a depth of 0–50 cm bi-monthly from October 2006 to August 2007 using a 5 L Van Dorn sampler (Go-Flo, General Oceanics, Inc.) and sent to our laboratory for analysis. Chl-*a* levels were determined using a spectrophotometer (DU 800, Beckman Coulter, Inc., USA) after filtration with GF/C filters (pore size 1.2 μm, Whatman, UK) and 90%-acetone extraction for 24 h (APHA, 1995). To count phytoplankton cells, 100 mL aliquots of each sample were fixed with 1% (v/v) Lugol's iodine, and cells were counted under an inverted microscope using sedimentation chambers. The biovolume of the phytoplankton species was calculated by measuring the dimensions of 20 individuals or cells based on the geometric solids that most closely approximated the observed shapes (Hillebrand et al. 1999).

The concentration of cellular microcystin (p-MC) and dissolved microcystin-LR (d-MC) was quantified in each sample by enzyme-linked immunosorbent assay (ELISA; Nagata et al. 1997; Kim et al. 2008). The total concentration (t-MC) was calculated as the sum of the p-MC and d-MC values. Because anti-microcystin monoclonal antibody (MAb) M8H5 used in the ELISA assay reacts equally with all major microcystin derivatives (i.e., microcystin-LR, microcystin-RR, and microcystin-YR), we used 250 μg of the most widely studied microcystin, microcystin-LR ($C_{49}H_{74}N_{10}O_{12}$, MW = 995.17, Wako), as the ELISA standard. Briefly, to quantify the concentrations of p-MC, 2 L of water was taken from each sample, filtered through a 25-mm glass filter (Whatman GF/C), and extracted with 10 mL of 90% methanol for 24 h at room temperature. A portion of each methanol-extracted sample (10–50 μL) was neutralized or diluted with distilled water (90–50 μL). The samples or standards were mixed with an appropriate amount of M8H5 MAb and placed in a 96-well microtiter plate (Coaster) that was pre-coated with a microcystin-bovine serum albumin conjugate. The plates were washed, and the bound MAb was detected with horseradish peroxidase-labeled goat anti-mouse IgG (TAGO 4550) and its substrate (0.1 mg/mL of 3,3′,5,5′-tetramethylbenzidine, 0.05% H_2O_2 in 0.1 M acetate buffer, pH 5.0). Finally, absorbance was measured at 450 nm. The microcystin concentration was determined from a standard competitive curve with a detection limit of 50 pg/mL. For d-MC, a small portion (50–100 μL) of the 2 L of filtered water was used directly for the measurement (without methanol extraction or dilution with water). The protocol for measuring d-MC was otherwise the same as for measuring c-MC.

To understand the relationship of cyanobacterial biomass, chl-*a*, and microcystin-LR levels with the WHO guidelines for drinking and recreational water, a Spearman rank correlation analysis was performed using $p < 0.05$ as the significance level (SPSS Inc., ver. 12.0.1, released in 2004).

Results and Discussion

The cyanobacteria count (biovolume) ranged between 0 and 2.6×10^5 cells/mL (ca. 9.316 cm^3/m^3) and was especially high in the three recreational ponds, S6, S7, and S8. The biomass was greater at these sites during warm seasons, with the exception of the December 2006 sampling at S7 (Fig. 1). The cyanobacterium *M. aeruginosa* was predominant at S6 in October 2006. Over the course of the study, the chl-*a* concentration fluctuated between 0.3 μg/L at S10 in June 2007 and 111.7 μg/L at S1 in April 2007. The identified cyanobacteria included toxic algae such as *Aphanocapsa elachista*, *Merismopedia elegans*, *Microcystis* (*M. aeruginosa*, *M. wesenbergii*, and *Microcystis* spp.), *Oscillatoria agardhii* and *Phormidium tenue,* and *Planktolynbya contorta* (Table 2). Of these, *M. wesenbergii* and *P. contorta* overwhelmingly dominated at S7 (Gyeonghoeru pond) between December 2006 and August 2007.

The highest chl-*a* concentration (111.7 μg/L) was due to two diatoms, *Stephanodiscus hantzschii* and *Aulacoseira granulata*, while the second highest value (68.7 μg/L) was associated with high levels of green algae (*Coelastrum sphaericum*) and cyanobacteria (*Merismopedia elegans*) rather than diatoms. Other relatively high chl-*a* concentrations (>40 μg/L) were associated with different phytoplankton species, including *Microcystis aeruginosa* and *Aulacoseira granulata* in October 2006 (S6 and S9), *Scenedesmus quadricauda* in April 2007 (S4), and *Oscillatoria* sp. in August 2007 (S7).

We found that t-MC concentrations correlated better with *Microcystis* levels (including *M. aeruginosa*, *M. wesenbergii*, and *Microcystis* spp.) and with the total number of cyanobacteria cells (including non-toxic cyanobacteria), rather than with chl-*a* concentrations. According to the WHO guideline values, the cyanobacteria cell numbers and the chl-*a* levels measured in the present study fell within the alert level 2 range for drinking water and the guideline level 2 range for recreational waters. The total microcystin levels reached the guideline level for drinking water but remained below guideline level 1 for recreational water. These results indicate that although the maximum cyanobacterial cells and chl-*a* level may reach alarm-triggering levels that are considered dangerous to humans, the corresponding microcystin levels may be below the concentration associated with adverse health effects. Thus, the results for these three parameters are not always consistent with each other.

The p-MC and d-MC concentrations ranged from 0 to 1.27 μg/L and from 0 to 0.22 μg/L, respectively (Fig. 2). Except for samples with t-MC > 1 μg/L (taken from S6 and S7 in October 2006), there were no other high t-MC concentrations identified during the study period. Over the course of the study, the t-MC concentration showed clear

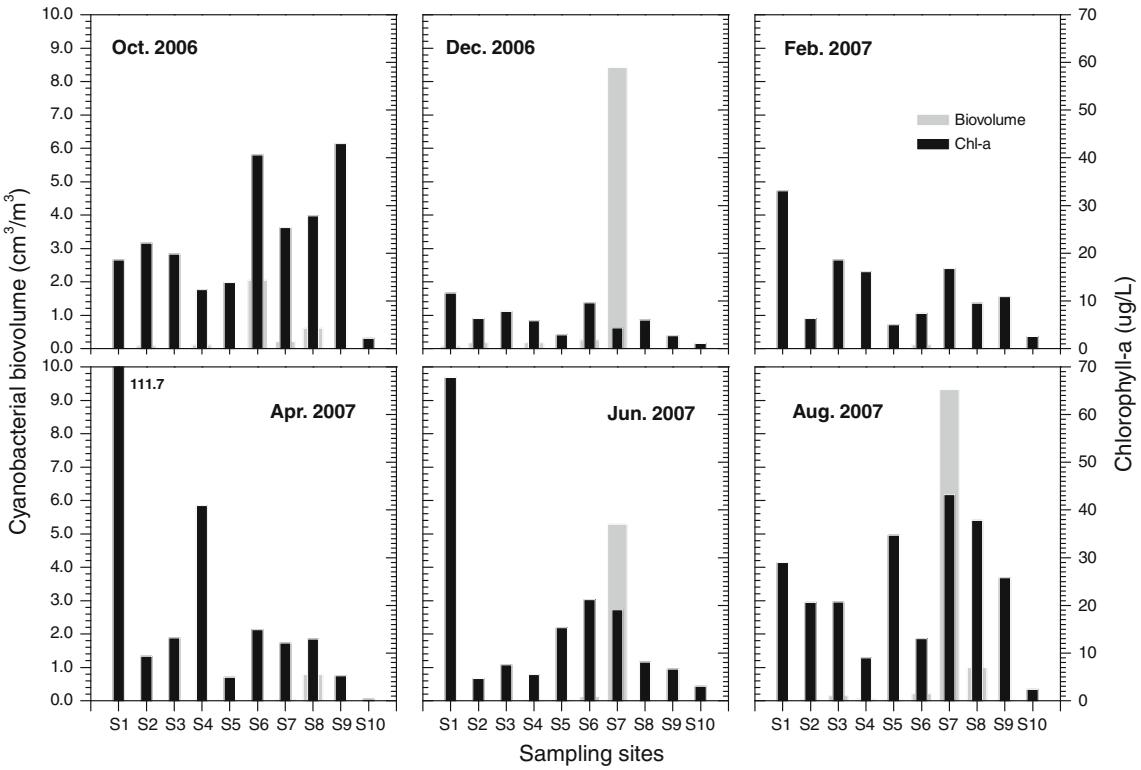

Fig. 1 Monthly changes in cyanobacterial cell numbers (biovolume; *grey bars*) and chlorophyll-*a* concentrations (*black bars*) in drinking and recreation waters in Seoul and its satellite cities in South Korea between October 2006 and August 2007

Table 2 Microcystin levels and dominant cyanobacteria in drinking and recreational water in Seoul and its satellite cities in South Korea from October 2006 to August 2007

Sites	Water	Max. t-MC (μg/L)	Cyanobacteria species
[S1]	Stream	0.190	*Anabaena flos-aquae, Microcystis wesenbergii, Phormidium tenue, Planktothrix agardhii*
[S2]	Lake	0.094	*Anabaena flos-aquae, Phormidium tenue, Planktothrix agardhii*
[S3]	Pond	0.218	*Phormidium tenue*
[S4]	Pond	0.170	*Chroococcus turgidus, Aphanocapsa elachista, Merismopedia glaucum, Oscillatoria* sp.
[S5]	Stream	0.203	*Phormidium tenue, Planktothrix agardhii, Merismopedia glaucum, Oscillatoria* sp.
[S6]	Pond	0.553	*Merismopedia glaucum, Aphanocapsa elachista, Microcystis aeruginosa*
[S7]	Pond	1.390	*Anabaena flos-aquae, Planktolyngbya contorta, Microcystis aeruginosa, Merismopedia wesenbergii, Oscillatoria* sp.
[S8]	Pond	0.294	*Anabaena flos-aquae, Microcystis aeruginosa, Merismopedia wesenbergii, Aphanocapsa elachista, Oscillatoria* sp.
[S9]	Pond	0.170	*Phormidium tenue, Merismopedia glaucum, Aphanocapsa elachista*
[S10]	Pond	0.074	*Planktothrix agardhii, Oscillatoria* sp.

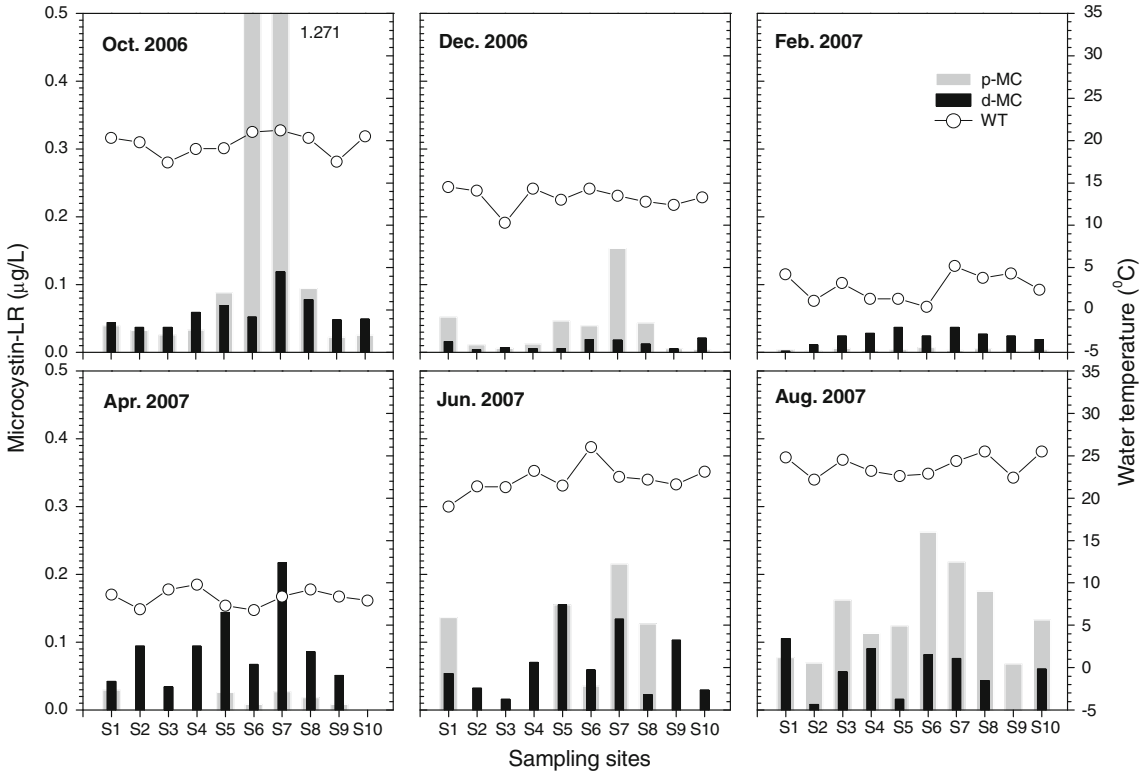

Fig. 2 Monthly changes in particulate microcystin (p-MC) and dissolved microcystin (d-MC) and in the water temperature (WT) of drinking and recreation water in the capital and its satellite cities of South Korea between October 2006 and August 2007

seasonal variations. The patterns of the p-MC and d-MC concentrations showed distinct differences over time: Higher concentrations of p-MC were associated with temperatures over 10°C between October and December 2006, whereas higher d-MC concentrations were recorded when temperatures fell below 10°C between February and April 2007. The t-MC dynamic showed a significant correlation with cyanobacterial cell numbers ($R = 0.49$, $p = 0.0103$) and *Microcystis* cell numbers ($R = 0.59$, $p = 0.0342$), not chl-*a* concentration ($R = 0.0504$, $p > 0.5$) (Fig. 3).

One might expect a close correlation between algal biomass (or chl-*a*) and t-MC concentrations, but this was not the case in this study. There are a few possible explanations. First, cyanobacteria that produce relatively little microcystin (such as non-toxic *Microcystis* species) (Yasuno et al. 1998) would increase the total biomass level

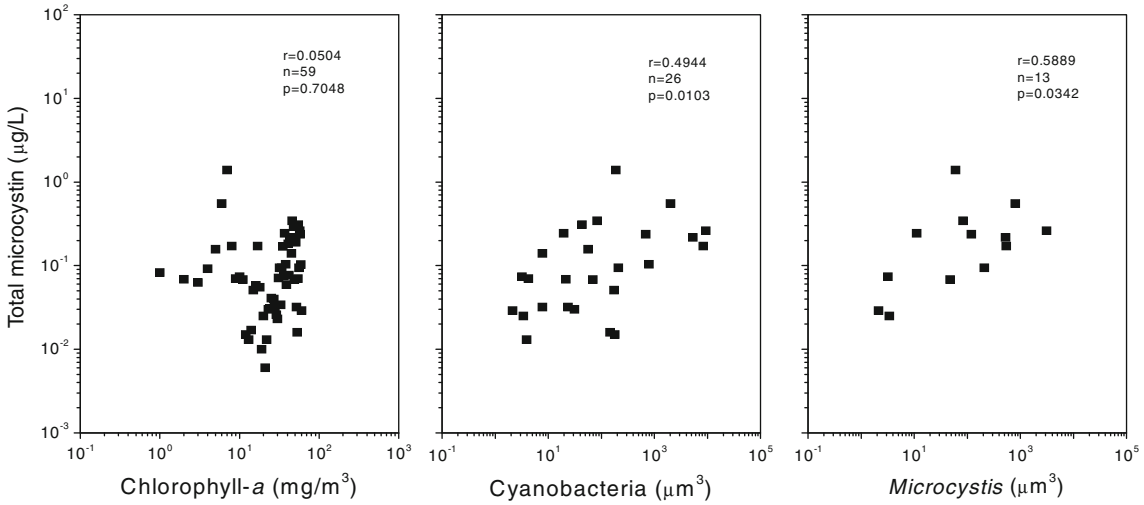

Fig. 3 Relationship between the total microcystin concentration and total chlorophyll-*a* concentration, total cyanobacterial biovolume, and total biovolume of *Microcystis* (*M. aeruginosa, M. wesenbergii* and *Microcystis* spp.) in drinking and recreation water in Seoul and its satellite cities in South Korea between October 2006 and August 2007

but would not increase the d-MC by much; that would result in a low d-MC concentration but a high *Microcystis* biomass. Second, d-MC can be released from toxic cyanobacteria, such as *Anabaena, Oscillatoria*, and *Aphanizomenon* (Carmichael et al. 1988), resulting in high d-MC levels but lower *Microcystis* biomass; this suggests that *Microcystis* is senescent when it is colder. Third, there may be yet-identified microcystin-producing cyanobacteria species that contribute to the d-MC levels but are not considered to be cyanobacterial cells.

Discrepancies in the levels of cyanobacterial biomass, chl-*a*, and microcystin-LR may result in inappropriate conservation efforts, including over-zealous protection of blue-green waters and frequent but unnecessary responses to *Microcystis* blooms. Notably, however, the February 2007 measurements at S7 revealed a non-*Microcystis* bloom in which *Planktolyngbya contorta* comprised approximately 45–74% of the total phytoplankton cells, yet the waters had the highest p-MC levels of the entire study (1.27 μg/L). The dense yellow-green *Planktolyngbya*

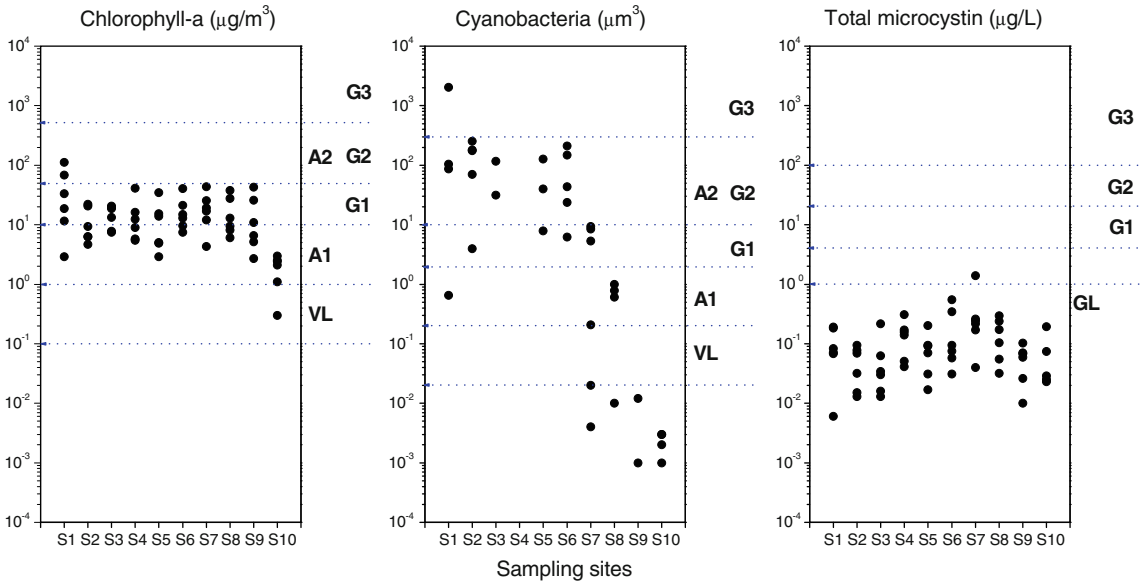

Fig. 4 Comparison of the chlorophyll-*a*, cyanobacterial biovolume, and total microcystin levels in drinking and recreation water in Seoul and its satellite cities in South Korea between October 2006 and August 2007. The transverse *dotted lines* show the WHO guideline values: vigilance and alert levels (VL, A1, and A2) for drinking water, and guidance levels (GL, G1, G2, and G3) for water used for recreational purposes

contorta bloom was accompanied by cyanobacteria other than *M. aeruginosa*, including *Oscillatoria* sp. *M. wesenbergii*, and *Aphanizomenon* sp. Recently, there have been *Planktolynbya* blooms (previously called *Lyngbya*) also reported as cyanotoxin producer in marine and fresh water (Teneva et al. 2003; Zavoruyev and Zotina 2003). In addition, t-MC production at S6 was associated with the filamentous cyanobacterium, *Anabaena flos-aquae,* while *Oscillatoria* sp. were found at S5, S8, and S10 (Table 2). Moreover, *Aphanocapsa elachista* contributed to the t-MC microcystin at S8; this species is known to produce low levels of microcystin (Domingos et al. 1999). Collectively, these results indicate that two kinds of cyanobacteria, *Planktolyngbya contorta* and *Aphanocapsa elachista*, can be either minor or major microcystin producers.

The chl-*a* concentrations at the sampling sites ranged between the WHO guideline alert level 2 (A2) and the virulent level (VL) for drinking water, and between WHO guideline level 2 (G2) and guideline level 1 (G1) for recreational water (Fig. 4). S1 reached A2 and G2 for drinking and recreational water, respectively, and S10 reached VL and G1, respectively; except for these two sites, the water at all sites fell between these alert levels. In terms of the cyanobacterial biovolume level for drinking and recreational water, many sites (S1–S6) fell within A1–A2 and G1–G3, and the other four sites (S7–S10) were within VL–A1 and G1, respectively. In contrast, the levels of t-MC reached only the guideline level (GL) for drinking water and remained below the G1 for recreational water.

In conclusion, the t-MC level in drinking and recreational water in Seoul and its satellite cities in South Korea was below the WHO guideline 'danger' level, but the chl-*a* and cyanobacterial cell counts were within the vigilance and alert level for drinking water and the guidance level for recreational water, respectively. These inconsistencies may result in unnecessary and costly alerts in countries that have not yet adopted their own guidelines for drinking and recreational waters.

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