Bull. Environ. Contam. Toxicol. (2004) 73:698–706

DOI: 10.1007/s00128-004-0482-1

Bulletin of Environmental Contamination and Toxicology

Variation of Intracellular and Extracellular Microcystins in a Shallow, Hypereutrophic Subtropical Chinese Lake with Dense Cyanobacterial Blooms

L. Zheng, P. Xie, Y. L. Li, H. Yang, S. B. Wang, N. C. Guo

Donghu Experimental Station of Lake Ecosystems, State Key Laboratory for Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, People's Republic of China

Received: 9 April 2004/Accepted: 16 July 2004

Microcystins (MC) production by cyanobacteria results from cyanobacterial blooms caused by abundant nutrients and favorable conditions for cyanobacterial growth. There have been only a few reports on the relationships between MC concentrations and the N and P concentrations in natural waterbodies. An indirect effect of total phosphorous (TP) on MC concentration through influencing cyanobacteria biomass or the size of MC-producing cyanobacteria was indicated or proposed (Kotak, 2000; Chorus 2001). However, The information is very limited and field evidences are also inadequate. Such studies are still lacking in Chinese lakes in spite of the abundant presence of cyanobacteria blooms in many eutrophic lakes in China. Lake Lianhuahu (30° 33'N, 114° 16'E) is a small shallow lake with a gate connected to the Yangtze River in the subtropical Wuhan City, China. The surface area of the lake is about 0.02 km^2 with an average depth of 1.2m. It has been seriously eutrophicated due to sewage input. Cyanobacteria bloom occurred regularly in the warm seasons each year. The main purposes of this paper are (1) to describe the variations of intracellular and extracellular MC in cyanobacteria in the shallow hypereutrophic lake during cyanobacteria blooms, and (2) to discuss the possible mechanisms underlying these variations.

MATERIALS AND METHODS

Three sites were chosen for sampling. Two sites were at the center of the lake, and one site was near the shore where dense algal slurry often accumulated by wind. Water samples were collected from the surface and near the bottom from 20 September to 26 October 2002 at an interval of three days. Dissolved oxygen (DO), water temperature and pH were determined in situ with an Orion 810 dissolved oxygen meter and an Orion 210 pH meter. Phosphate in filtered (through GF/C glass fiber filter) lake water was analyzed by colorimetry after reaction with ammonium molybdate and stannous chloride. TP in the lake water was measured by colorimetry after digestion of the total samples with $K_2S_2O_8$+NaOH to

---

Correspondence to: P. Xie

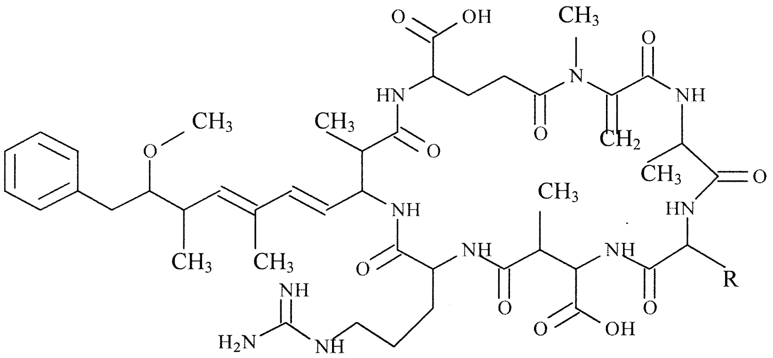

Figure 1. Chemical structures of microcystin-LR and microcystin-RR. MC-LR: R = $CH_2CH(CH_3)_2$, MC-RR: R = $CH_2CH_2NH(=NH)NH_2$.

orthophosphate (Ebina, 1983). After digestion simultaneously with TP, total nitrogen (TN) was measured as nitrate and absorbance was measured at 220 nm. Similarly, total dissolved nitrogen (TDN) and total dissolved phosphorous (TDP) were determined after digestion of filtered lake water. Ammonium (NH_4-N) was determined by the Nessler method, and nitrite by the a-naphthylamine method. Chlorophyll a (Chl.a) concentration was determined by the spectrophotometric method (Zhang, 1991). Subsamples for phytoplankton were preserved with 1% acidified Lugol's iodine solution and concentrated to 30ml after sedimentation for 48 hours. After mixing, 0.1ml concentrated samples were counted directly under 400×magnification. *Microcystis aeruginosa* cell counts were made separately on samples previously subjected to a 10-30 s treatment in a high-speed blender (Ultra-Turrax). Taxonomic identification was made according to Hu et al. (1979) and biomass was estimated from approximate geometric volumes of each taxon, assuming that 1 mm^3 equals 10^{-6} μg fresh weights (Shei, 1993). The geometric dimensions were measured on 10-30 individuals for each dominant species.

MC in the lake water was fractionated to intra- and extracellular MC, and was measured according to Park (1998) and Martin (1999). The intracellular toxins were extracted from cyanobacterial cells filtered from 500 ml lake water on the glass-fiber filter (GF/C, Whatman, UK). The filtrate (500 ml) was used to measure the extracellular toxins. Both extra- and intracellular MC were determined by a reverse-phase high-performance liquid chromatography (HPLC) equipped with an ODS column (Cosmosil 5C18-AR, 4.6×150 mm, Nacalai, Japan) and a SPD-10A UV-vis spectrophotometer set at 238 nm. The sample was separated with a mobile phase consisting of 65% aqueous methanol with 0.05% trifluoroacetyl (TFA) at a flow rate of 1 ml/min. MC concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR and MC-RR, Wako Pure Chemical Industries-Japan) (Fig.1).

RESULTS AND DISCUSSION

A pronounced bloom was observed during Sep. 20 and Oct. 10 in the lake. After a heavy rain on Oct. 14, cyanobacteria showed considerable declines (Fig. 2, 3). In general, water temperature, DO and pH decreased during the study period. Both TP and TN remained at high levels, corresponding to a hypereutrophic status. NO_2-N concentrations were generally low, not exceeding 0.01 mg/L in most cases. PO_4-P was main component of TDP, and was usually lower than 0.02 mg/L. NH_4-N was always below 0.6 mg/L during the study period with the exception of an extremely high value on Oct. 14.

Among cyanobacteria, four species dominated during the study period. *A. flos-aquae* showed a general increase before Oct. 2, and then declined gradually, reaching a low level of less than 0.5 mg/L after Oct.14. During the study period, *M. aeruginosa* remained relatively stable in biomass, whereas *Oscillatoria sp.* showed a steady decline in biomass. Biomass of *Merismopedia sp.* was below 0.7 mg/L throughout the study period (Fig. 3a). Temporally, *A. flos-aquae, M. aeruginosa* and *Oscillatoria sp.* were predominant before Oct. 14, and then *M. aeruginosa* dominated. The ratio of cyanobacteria to phytoplankton remained high during Sep. 20 and Oct. 10, followed by a sudden drop on Oct. 14 after a heavy rain. The ratio decreased further afterwards. Chlorophyll a concentration remained high, with a maximum of 263 mg/L on Sep. 28 (Fig. 3b).

The toxins of the samples collected from Lake Lianhuahu were identified as MC-LR and RR, and MC-LR was absolutely important in quantity (Fig. 3c, 3d), whereas, MC-RR was only occasionally detected in cyanobacteria cells. A similar case was also reported by Poon et al. (2001). It remains unknown why MC-RR was undetectable in spite of the presence of relatively high amount of intracellular MC-RR in some cases. Mean intracellular MC content was usually below 5 μg/L, but an extremely high value (17 μg/L) was observed on Oct. 14. The maximum intracellular MC was 33.2 μg/L at the shore site on Oct. 10. Spatially, intracellular MC content was higher at the shore site than at the other sites. Surface blooms of cyanobacteria mainly *Microcystis aeruginosa* were collected on Oct. 26 and the MC content was 78 μg/g dry weight (MC-LR). Extracellular MC was high on Sep. 20 and Oct. 6-10, but was low or under the limit of detection (< 0.02 μg/L) on the other sampling dates. It may be because intracellular toxins are released mostly in ageing blooms during cell lysis (Fromme, 2000). In the present study, high extra-MC only lasted for several days. Studies conducted in natural waters in the United Kingdom indicate that five days are required for the destruction of 50% of the toxin (Fawell, 1998). Biodegradation and photolysis are means by which released MC naturally decreases in concentration (Kenefick, 1993; Tsuji, 1994).

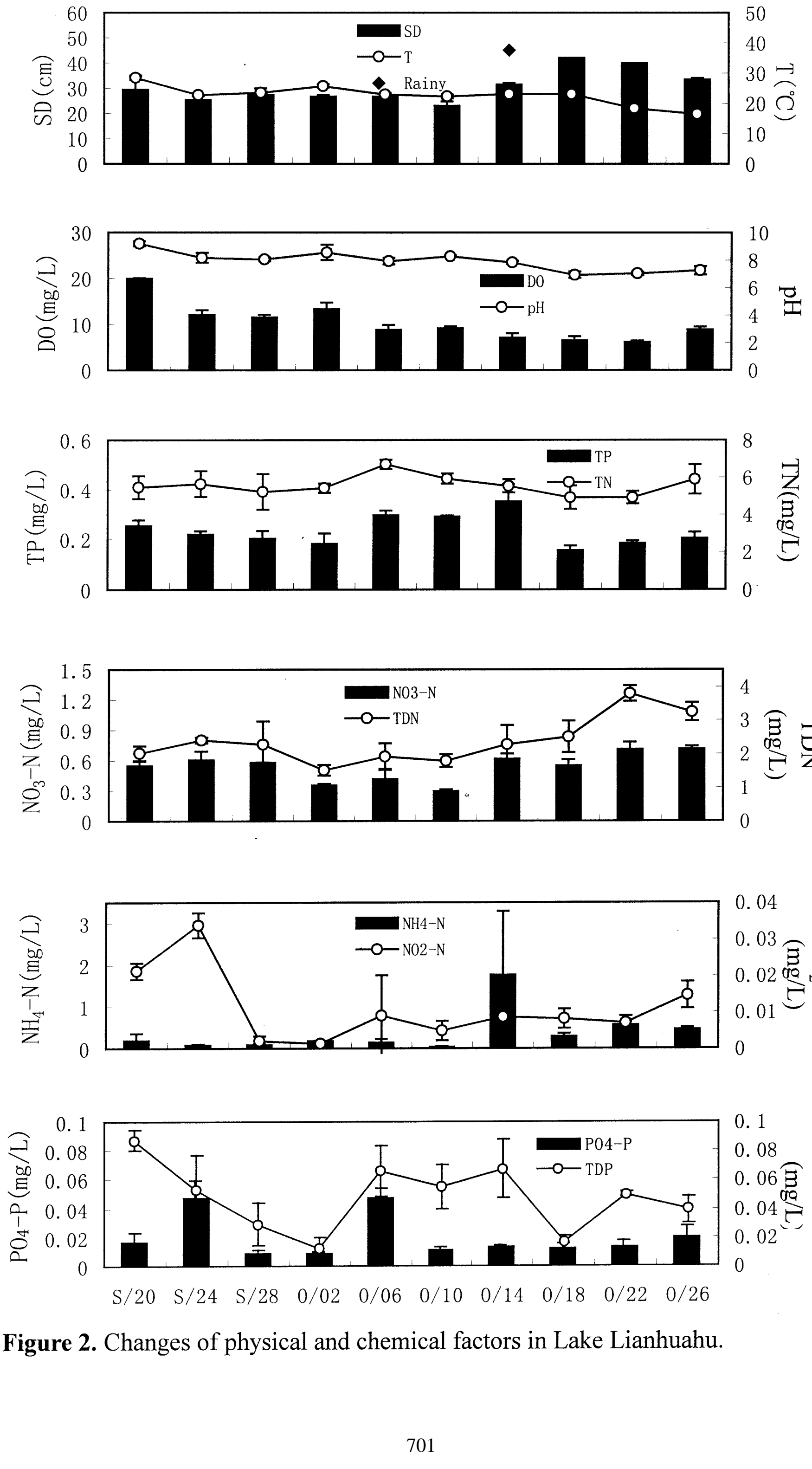

Figure 2. Changes of physical and chemical factors in Lake Lianhuahu.

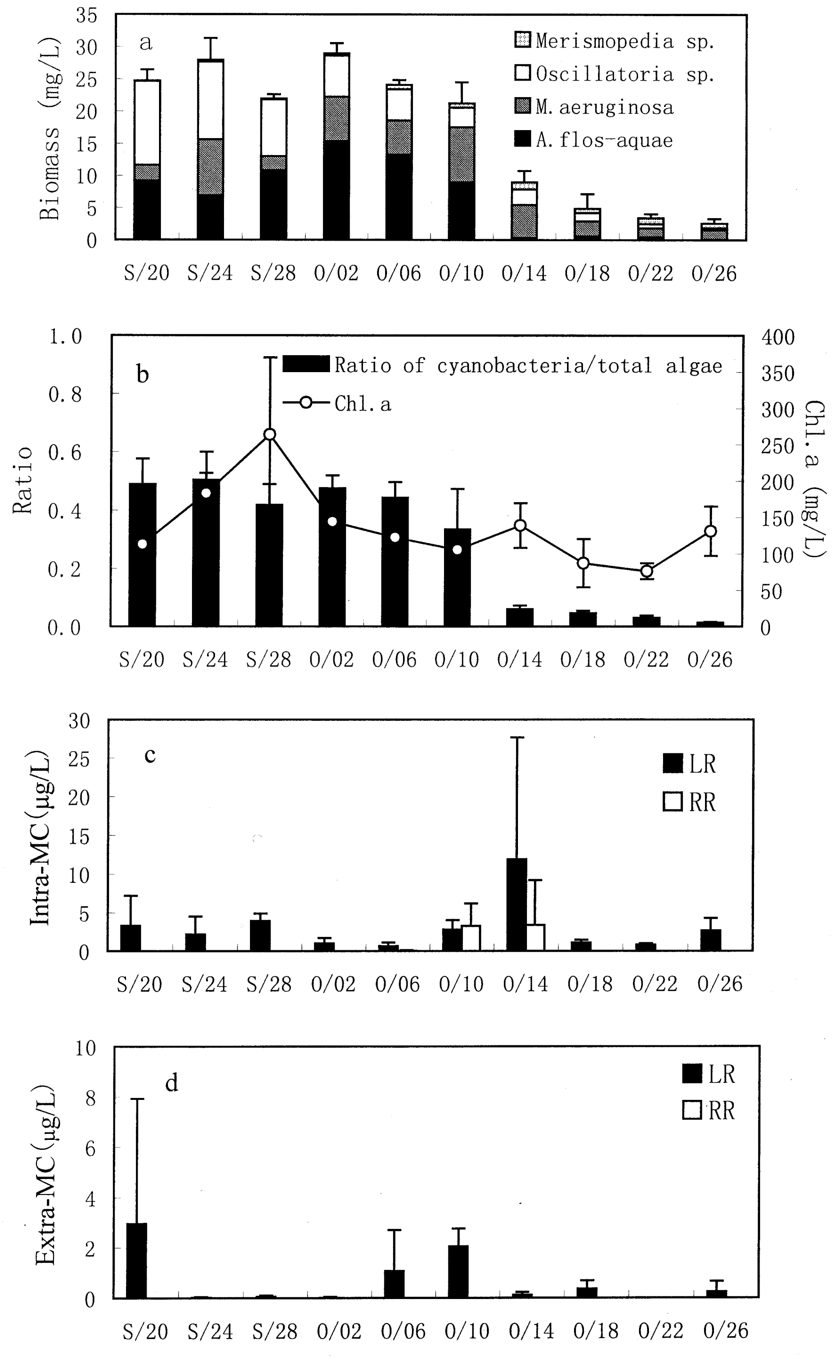

Figure 3. Changes in biomass of cyanobacteria, biomass ratio of cyanobacteria/total algae, Chl.a and microcystin concentrations in Lake Lianhuahu during September and October, 2002.

Table 1. The correlations between MC and physical, chemical and biological factors during the cyanobacteria blooms in Lake Lianhuahu (Boldface words indicate significant linear relationship at the 95% confidence level, N=10.).

	Extra-MC	Chl.a	CB	AB	MB	T	SD	pH	DO
Intra-MC	-0.01	0.11	-0.18	-0.32	0.16	0.07	-0.13	0.09	-0.17
Extra-MC	----	-0.32	0.32	0.28	0.08	0.50	-0.31	0.61	0.58
	TN	TDN	NO_3-N	NH_4-N	NO_2-N	TP	TDP	PO_4-P	N/P
Intra-MC	0.02	-0.14	0.08	**0.83**	-0.09	**0.74**	0.36	-0.26	**-0.76**
Extra-MC	0.30	-0.40	-0.41	-0.29	0.16	0.38	0.61	-0.02	-0.43

CB: cyanobacteria biomass, AB: *A. flos-aquae* biomass, MB: *M. aeruginosa* biomass.

There were significant linear relationships ($P<0.05$) between nutrient conditions (ammonium, TP and N/P ratio) and intra-MC (Tab. 1, Fig. 4). Maria et al. (2003) report that there was a negative correlation between ammonium-nitrogen and MC concentration in a reservoir, Argentina. In our results, an unusually high ammonium concentration was present on Oct.14. If this data was excluded, negative correlation was present between ammonium and MC concentration. Kotak et al. (2000) indicate an effect of TP on MC concentration through an indirect effect of TP on the biomass of *M. aeruginosa*. Chorus I (2001) proposes that MC concentration in water depends largely on the population size of MC-producing cyanobacteria, and thus TP exerts more strongly on their population size than on MC concentrations. However, no significant correlation ($P<0.05$) was present between cyanobacteria biomass and intra-MC. The relationships between biomass of cyanobacteria (total cyanobacteria, *A. flos-aquae*, and *M. aeruginosa*) and intra-MC are also showed in Figure 4. It suggests that the effect of TP on MC concentration may not through the influence on the biomass of cyanobacteria in our case. Similarly, Jacoby et al. (2000) found no clear relationship between algal biomass and MC concentrations in Lake Steilacoon.

Table 2. A comparison of the maximum concentrations of MC, TN, TP and N/P ratio during cyanobacteria blooms in several eutrophic Chinese lakes.

Lakes	Taihu	Chaohu	Dianchi	Dianshanhu	Lianhuahu
MC (μg/L)	11^a	4^b	1.9^c	55.4^a	33.2^b
TN (mg/L)	8.3	2.25	2.98	1.89	5.53
TP (mg/L)	0.55	0.15	0.26	0.15	0.35
N/P ratio	15	15	11	13	16
References	Shen (2003)	d	d	Wang (1995)	Present study

a: ELISA method, b: HPLC method, c: mg/g d.w. bloom cell. d: unpublished data.

It should be noted, however, that October 14 was a turning point of phytoplankton community in Lake Lianhuahu, i.e., cyanobacteria blooms collapsed, companied

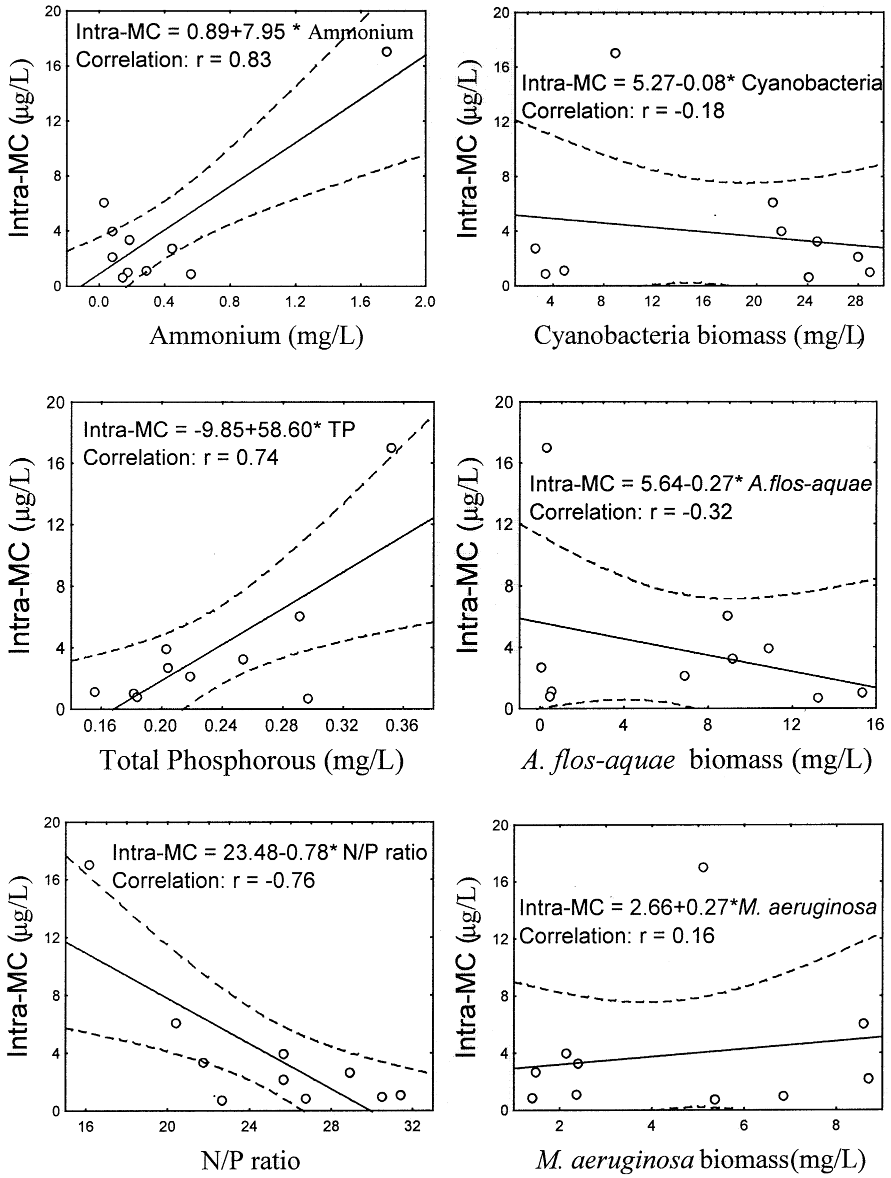

Figure 4. Relationship between intra-MC and ammonia, total phosphorus, N/P ratio, total cyanobacteria, *A. flos-aquae*, and *M. aeruginosa* biomass at the 95% confidence level (N=10).

with a high concentration of intracellular MC. This may be explained by a change in the ratio of toxic to nontoxic strains, as indicated by Ohtake (1989). Genetic works have shown that MC is constituent to some strains, but not to all (Moore, 1996). The maximal MC concentrations, TN, TP and N/P ratio in different eutrophic Chinese lakes are compared in Table 2. These lakes usually have a high TP concentrations and a lower N/P ratio. Possibly, some nutrients such as TP may be associated with the competition between toxic and nontoxic strains.

Acknowledgments. This work was supported by a key project of the Chinese Academy of Sciences (Grant No. KZCXI-SW-12) and by a fund National Natural Science Foundation of China (Grant No. 30123004).

REFERENCES

Chorus I (2001) Cyanotoxins — occurrence, causes, consequences. Springer, Berlin

Ebina J, Tsutsui T, Shirai T (1983) Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. Wat Res 17: 1721-1726

Fawell JK (1998) Personal communication. Water Research Centre, Medmenham, UK

Fromme H, Kohler A et al. (2000) Occurrence of cyanobacterial toxins microcystins and anatoxin-a in Berlin water bodies with implication to human health and regulations. Environ Toxicol 15: 120-130

Hu, H, Li R, Wei Y et al. (1979) Freshwater algae in China. Science Press, Shanghai (in Chinese)

Jacoby JM, Collier DC et al. (2000) Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. Canadian J Fish Aquat Sci 57: 231-240

Kenefick SL, Hrudey SE, Peterson HG et al. (1993) Toxin release from *Microcystis aeruginosa* after chemical treatment. Water Sci Technol 27: 433-440

Kotak, BG, Lam AKY et al. (2000) Role of Chemical and physical variables in regulating microcystin-LR concentrations in phytoplankton of eutrophic lakes. Canadian J Fish Aquat Sci 57: 1584-1593

Maria VA, et al. (2003) Occurrence of toxic cyanobacteria blooms in San Roque Reservoir (Cordoba, Argentina): a field and chemometric study. Environ Toxicol 18: 192-201

Martin W, Sigrid H et al. (1999) Hepatotoxic cyanobacteria in the shallow lake Muggelsee. Hydrobiologia 408/409: 263-268

Moore RE (1996) Cyclic peptides and depsipeptides from cyanobacteria: a review. J Ind Microbiol 16: 134-143

Ohtake A, Shirai M et al. (1989) Toxicity of *Microcystis* species isolated from natural blooms and purification of the toxin. Appl Environ Microbiol 55:3202–3207

Park HD, Lwami C (1998) Temperal variabilities of the concentrations of intra- and extracellular microcystin and toxic microcystis species in a hypertrophic lake, Lake Suwa, Japan (1991-1994). Environ Toxicol Water Qual 13: 61-72

Poon KF, Lam MH et al. (2001) Determination of microcystins in cyanobacterial blooms by solid-phase microextraction-high-performance liquid chromatography. Environ Toxicol Chem 20: 1648-55

Shei P, Lin WL, Wang SM, Liu JK (1993) Plankton and seston structure in a shallow, eutrophic subtropic Chinese lake. Arch Hydrobiol 129: 199-220

Shen PP, Shi Q et al. (2003) Analysis of microcystins in cyanobacteria blooms and surface water samples from Meiliang Bay, Taihu Lake, China. Environ Int, 29: 641-647

Tsuji K, Naito S, Kondo F et al. (1994) Stability of microcystins from cyanobacteria: effect of light on decomposition and isomerization. Environ Sci Technol 28: 173-177

Wang HB, Song WM, Zhu HG (1995) The dynamic of phytoplankton and microcystins in Lake Dianshanhu, Shanghai. J Environ Health 12: 196-199 (in Chinese)

Zhang ZS, Huang XF (1991) A manual on methods for plankton research in Fresh waters. –Science Press, Beijing (in Chinese)