Bull. Environ. Contam. Toxicol. (2006) 77:367–374

DOI: 10.1007/s00128-006-1075-y

Environmental Contamination and Toxicology

Seasonal Variation of Microcystin Concentration in Lake Chaohu, a Shallow Subtropical Lake in the People's Republic of China

H. Yang, P. Xie, J. Xu, L. Zheng, D. Deng, Q. Zhou, S. Wu

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: 15 March 2006/Accepted: 20 July 2006

Toxic cyanobacterial blooms occur frequently in eutrophic brackish and fresh water body all over the world (Paerl et al. 2001). Cyanotoxins have been considered to cause poisoning and death of wild and domestic animals and also human health (Carmichael et al. 2001). Neurotoxins and hepatotoxins are two main groups of toxins (Carmichael 1992). Microcystins (MCs) produced by *Microcystis, Anabaena, Oscillatoria, and Nostoc* are the most studied hepatotoxins (Dawson 1998). To date, more than 60 variants of microcystins have been found (Sivonen and Jones 1999). Microcystin have been reported to inhibit protein phosphatase (Mackintosh et al. 1990), and are regarded as tumor promoting agents (Nishiwaki et al. 1992). The long-term exposure to MCs is thought to be a cause for high levels of primary liver cancer in China (Yu 1995).

Many studies have examined the influence of various environmental factors (e.g, light, temperature, nutrients) on microcystin production in cyanobacteria. The effects of temperature (Vander der Westhuizen and Eloff 1986; Rapala and Sivonen 1998) and light (Utkilen and Gjolme 1992; Song et al. 1998) on microcystin production were the most commonly studied parameters. It has been shown that nitrogen had a pronounced effect on the production of microcystin (Rapala et al. 1997; Downing et al. 2005) and MC concentrations were also associated with phosphorous (Kotak et al. 1995; Oh et al. 2001). However, the regulation of microcystin production in cyanobacteria is not well understood in Chinese big shallow lakes. Lake Chaohu, the fifth largest lake in China, located in Anhui Province of southeastern China. It is an important fishery resource and supplies the drinking water. It has a surface area of 760km^2, and a mean depth of 3.10m. It consists two basins: western basin (240km^2, mean depth 3.0m maximum 5.8m depth), and eastern basin (420 km^2, mean depth 3.0m maximum depth 6.0m). The water flows from west to east and connects with the Yangtze River (Fig. 1). Since the 1980s, cyanobacteria (mainly *Microcystis* spp. and *Anabaena* spp.) have occurred massively in the warm seasons of each year.

The purposes of our study were to investigate distributions and seasonal variation of MC concentrations in Lake Chaohu, and to discuss the possible mechanisms underlying these variations.

Correspondence to: P. Xie

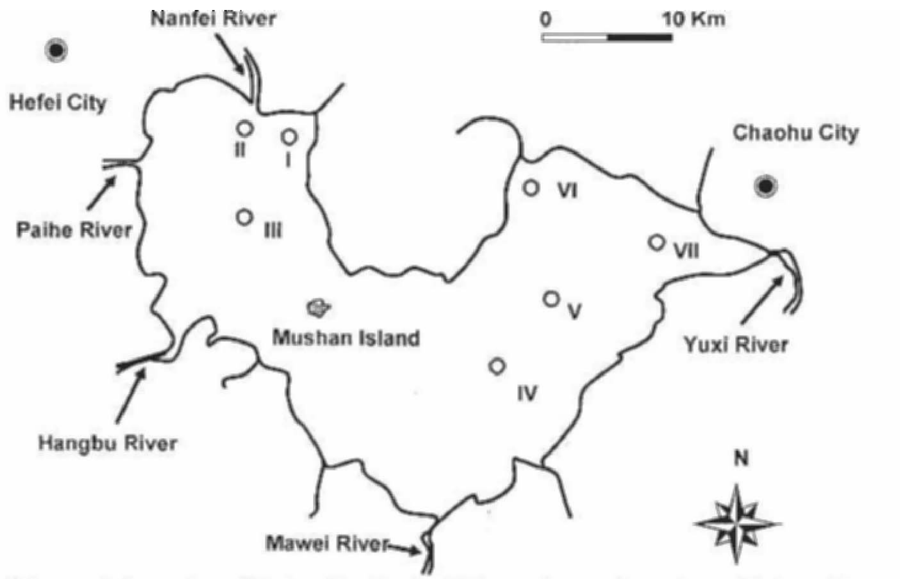

Figure 1. Location of Lake Chaohu in China and sampling sites of this study.

MATERIALS AND METHODS

Seven stations were chosen for sampling. Water samples were collected from the surface and near the bottom from October 2002 to July 2003 monthly. Water temperature, pH, dissolved oxygen and transparency were measured by a thermometer, metrohm, OXYMETER ysl MODEL 57 and Secchi disk, respectively. Ammonium (NH_4-N) was determined by the Nessler method, nitrate (NO_3-N) by ultraviolet sepectrophotometric screening method, and nitrite (NO_2-N) by the α-naphthylamine method. Total nitrogen (TN) was measured by alkaline potassium persulfate digestion-UV spectrophotometric method. Ortho-P was measured using Murphy and Riley (1962) procedure. Total phosphorus and total dissolved phosphorus were digested with potassium persulfate and measured by molybdenum blue colorimetric method (Murphy and Riley, 1962).

1L water sample was filtered through a glass microfiber filter (Whatman GF/C) and adjusted to pH 7. The filter were homogenized and extracted with 90% aqueous methanol and shaken 3 hours using a shaker screen for three times. After centrifugation the supernatant was applied to ODS cartridge (0.5g Dalian Institute of Chemical Physics, CAS). The cartridge was eluted with 90% aqueous methanol, and the eluate was evaporated to dryness and the residue was dissolved in methanol and then injected to HPLC system for determination (Zheng et al., 2004).

RESULTS AND DISCUSSION

Fifteen parameters were measured from each of the 84 collected samples. The trophic state in western basin was higher than that in eastern basin (Table 1). Eighty percent of the samples collected contained MC. Microcystin-LR (MC-LR) and Microcystin-RR (MC-RR) were detected in lake samples, and MC-LR was the dominant species. The mean MC concentration was higher in western basin than in eastern basin. The highest MC concentration (17.29 μg L^{-1}) was from a water sample collected from eastern basin on Jun 2003 (Fig. 2).

In the present study, MC concentration varied from undetectable concentration to 17.29 μg L^{-1}. MC concentration in western lake was higher than in eastern lake.

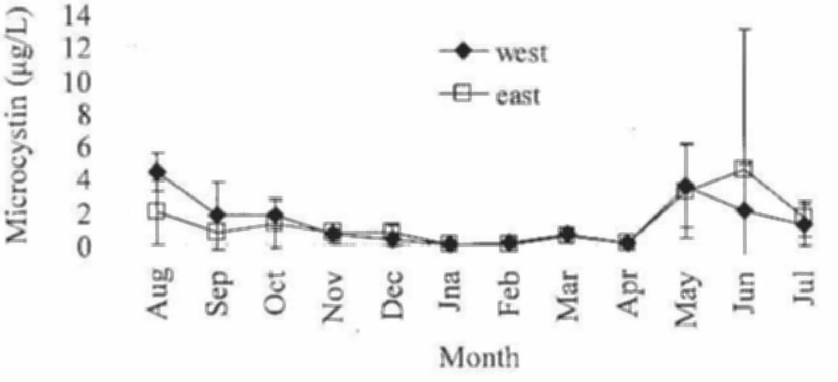

Figure 2. Seasonal variation of MC concentrations in Lake Chaohu.

Table 1. Limnological variables of Lake Chaohu.

Variables	Western Lake (average)	Min.-Max	Eastern Lake (average)	Min.-Max
Secchi depth (cm)	36.5	18-76	68.4	28-126
Water depth (m)	2.77	1.7-5.8	3.31	2-6
pH	7.84	6.54-10.39	7.87	3.71-8.98
Dissolved oxygen (mg/L)	10.96	7.9-15.4	11.84	6.9-23
Conductivity	29.66	14.41-46.1	25.03	15.09-40.06
Water Temperature (°C)	16.08	3.3-27.7	17.61	4.2-30.9
TN (mg/L)	5.16	1.77-11.2	2.27	0.83-4.92
NO_3-N (mg/L)	2.0	0.49-4.06	1.11	0.13-3.26
NO_2-N (mg/L)	0.05	0-0.18	0.01	0-0.11
NH_4-N (mg/L)	0.98	0.13-3.15	0.26	0.11-0.88
TDN (mg/L)	4.27	0.78-10.29	1.86	0.43-4.91
TP (mg/L)	0.17	0.07-0.31	0.06	0.03-0.11
TDP (mg/L)	0.05	0.07-0.13	0.02	0-0.06
PO_4-P (mg/L)	0.03	0-0.10	0.01	0-0.03
Chl *a* (mg/L)	30.72	1.64-128.7	15.16	1.64-35.49

Table 2. Correlations between total MC, Microcystin-LR (MC-LR), and Microcystin-RR (MC-RR) Concentration ($\mu g\ L^{-1}$) and limnological variables and cyanobacterial species biomass in western and eastern Lake Chaohu (Note: *P<0.05; **P<0.01; ***P<0.005; ns, not significant).

	western Lake Chaohu						eastern Lake Chaohu					
	total-MC		MC-RR		MC-LR		total-MC		MC-RR		MC-LR	
Variable	r	P	r	P	r	P	r	P	r	P	r	P
Secchi depth	0.37	*	0	ns	0.37	*	0.14	ns	0.01	ns	0.18	ns
Water depth	0.21	ns	0.5	***	0.1	ns	0.15	ns	0.46	***	0	ns
pH	0.04	ns	-0.29	ns	0.26	ns	0.23	ns	0.19	ns	0.22	ns
Dissolved oxygen	-0.61	***	-0.09	ns	-0.52	***	-0.18	ns	-0.09	nd	-0.14	ns
Conductivity	0.24	ns	0.42	**	-0.02	ns	0.3	*	0.46	***	0.18	ns
Water Temperature	0.6	***	0.43	**	0.47	***	0.34	*	0.4	*	0.25	ns
TN	-0.46	***	-0.32	ns	-0.43	***	0.03	ns	-0.06	ns	-0.07	ns
NO_3-N	-0.48	***	-0.13	ns	-0.59	***	0.08	ns	-0.09	ns	0	ns
NO_2-N	-0.15	ns	0.15	ns	-0.1	ns	-0.05	ns	0.18	ns	-0.1	ns
NH_4-N	-0.25	ns	-0.04	ns	-0.38	*	-0.2	ns	-0.21	ns	-0.11	ns
TDN	-0.45	**	-0.31	ns	-0.45	**	0.04	ns	-0.16	ns	0.04	ns
TP	-0.3	ns	0	ns	-0.37	*	0.36	*	0.4	*	0.16	ns
TDP	-0.09	ns	-0.07	ns	-0.21	ns	0.15	ns	0.03	ns	0.13	ns
PO_4-P	-0.08	ns	-0.07	ns	-0.28	ns	-0.08	ns	-0.16	ns	-0.01	ns
Microcystis	0.07	ns	-0.06	ns	0.16	ns	0.12	ns	0.49	***	0.03	ns
Anabaena	-0.02	ns	0.29	ns	-0.09	ns	0.25	ns	0.5	***	0.01	ns
Cyanobacteria	0.02	ns	0.17	ns	0.01	ns	0.21	ns	0.55	***	0	ns
Chl *a*	-0.04	ns	0.08	ns	0.05	ns	0.27	ns	0.59	***	0.12	ns

This may be due to massive cyanobacteria accumulation near ZhongMiao during the warm seasons.

In the present study, MC concentration was correlated with dissolved oxygen, TN, NO_3-N, TDN, and water temperature in western basin and with conductivity, water temperature and TP in eastern basin (Table 2). Of all the physiochemical variables, the significant correlation was with water temperature (r=0.60), suggesting that MC in phytoplankton partly depended on water temperature. Shen et al. (2003) found that higher microcystin contents were coincident with high water temperature (>25℃) in Lake Taihu, China. Wicks and Thiel (1990) found that microcystin concentration in phytoplankton of Hartbeespoort Dam, South Africa, was positively correlated with water temperature (r=0.60). These results agree with us. The significant correlation could be explained by the fact that water temperature accelerated growth of cyanobacteria including the MC-producing strains.

There was no relationship between MC and *Microcystis* and *Anabaena* biomass. However, MC-RR concentration was significantly correlated with *Microcystis, Anabaena* and Chl *a* in eastern basin. MC-LR was strongly correlated with dissolved oxygen, TN, NO_3-N, TDN and water temperature in western basin. MC-RR was related with TP in eastern basin.

Wicks and Thiel (1990) found that microcystin concentration in phytoplankton was strongly correlated to primary production, Kotak et al. (1995) reported that MC-LR concentration in phytoplankton was strongly correlated with the biomass of *M. aeruginosa* (r=0.52). However, in the present study, no significant correlation was found between MC and *Microcystis* biomass. It is likely that toxic and nontoxic strains coexist in blue green algae in lakes and MCs mainly depend on some species of MC-producing cyanobacteria.

In the present study, there was a significantly negative correlation between MC concentration and TN, NO_3-N, NH_4-N in the western basin. Kotak et al. (1995) reported that MC-LR concentration of cyanobacteria in three Canadian lakes was negatively correlated with nitrate concentration. In contrast, Sivonen (1990) found that production of MC-RR in *O. agardhii* increased with increasing NO_3-N concentration. Toxin production did not seem to be affected by phosphorus concentration in two basins in Lake Chaohu, which was similar with those of Sivonen (1990). The differences in the response of toxin production to nitrogen or phosphorus manipulation were not surprising because the response might be expected to be species-dependent (Kotak et al. 2000).

Our results revealed that MC-RR tended to dominate in higher TP level and MC-LR in lower TP level both in eastern basin and western basin (Fig. 3), which suggested that MC-RR was the dominant variants when TP concentration was relatively high and MCs and TP did not show linear correlations.

Lake Chaohu was not only used as the drinking resource, but also as important

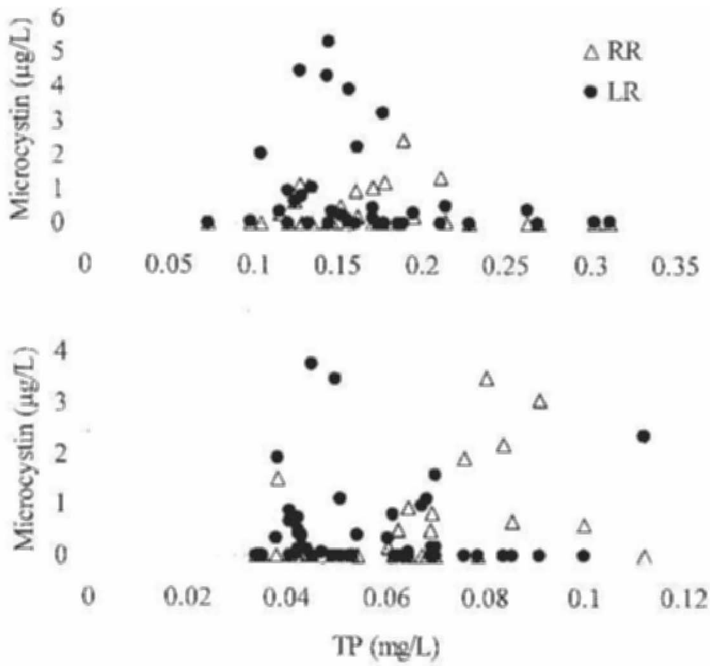

Figure 3. Relationship between TP and MC-LR and MC-RR concentrations in Lake Chaohu.

recreational place. The microcystin concentration in phytoplankton was relatively high during May to October, suggesting that there can be a potential health risk for both animals and human. More attention needs to be directed to the ecology of toxin-producing cyanobacteria. Further studies are needed to identify factors regulating MC-producing cyanobacteria and microcystin production.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (Grant No. 30225011) and a key project of the Chinese Academy of Sciences (Grant No. KZCX1-SW-12).

REFERENCES

Carmichael WW, Yu MJ, He ZR, He JW, Yu JL (1988) Occurrence of the toxic cyanobacterium (blue-green algae) *Microcystis aeruginosa* in central China. Arch Hydrobiol 114: 21-30

Carmichael WW, Azevedo SM, An JS, Molica RJ, Jochimsen EM, Rinechart KI, Shaw GR, Eaglesham GK (2001) Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ Health Perspect 39: 341-344

Dawson RM (1998) The toxicology of microcystins. Toxin 36: 953-962

Downing TG, Meyer C, Gehringer MM, van de Venter M (2005) Microcystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. Environ Toxicol 20: 257 – 262

Kotak BG, Lam AK-Y, Prepas EE, Kenefick SL, Hrudy SE (1995) Variability of the hepatotoxin microcystin-LR in hypereutrophic drinking water lakes. J Phycol 3: 248-263

Kotak BG, Lam AK-Y, Prepas EE, Hrudey SE (2000) Role of chemical and physical variables in regulating microcystin-LR concentration in phytoplankton of eutrophic lakes. J. Fish. Aquat. Sci. 57: 1584-1593

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta, 27: 31-36

Nishiwaki-Matsushima R, Ohtake T, Nishiwaki S, Sukanuma M, Kohyama K, Ishikawa T, Carmichael WW, Fuiji H (1992) Liver cancer promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. J Cancer Res Clin Oncol 118: 420-424

Oh HM, Lee SJ, Kim JH, Yoon BD (2001) Seasonal variation and indirect monitoring of microcystin concentrations in daechung reservoir, Korea. Appl Environ Microbiol 67: 1484-1489

Paerl HW, Fulton RS, Moisander PH, Dyble J (2001) Harmful freshwater algal blooms,with an emphasis on cyanobacteria. Sci Wordld J 1: 76-113

Rapala J, Sivonen K., Lyra C, Niemelä SI. (1997) Variation of microcystins, cyanobacterial hepatotoxins, in *Anabaena* spp. as a function of growth stimuli. Appl Environ Microbiol 63: 2206-2212

Rapala J, Sivonen K (1998) Assessment of environmental conditions that favor hepatotoxic and neurotoxic *Anabaena* spp. strains cultured under light limitation at different temperatures. Microbial Ecol 36: 181-192

Shen PP, Shi Q. Hua ZC, Kong FX, Wang ZG., Zhuang SX, Chen DC (2003) Analysis of microcystins in cyanobacteria blooms and surface water samples from Meiliang Bay, Taihu Lake, China. Environ Int 29: 641-647

Sivonen K (1990) Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth and hepatotoxin production by *Oscillatoria agardhii* strains. Appl Environ Microbiol 56: 2658-2666

Sivonen K, Jones GJ (1999) Cyanobacterial toxins. In: Chrous, I and Bartram, J (eds.) Toxic cyanobacteria in water, E & FN Spoon, London, pp 41-111

Song LR, Sano T, Li RH, Watanabe MM, Liu YD, Kaya K (1998) Microcystin production of *Microcystis Viridis* (cyanobacreia) under different culture conditions. Phycol. Res. 46: 19-23

Utkilen H, Gjolme N (1992) Toxin production by *Microcystis aeruginosa* as a function of light in continuous cultures and its ecological significance. Appl Environ Microbiol 58: 1321-1325

Van der Westhuizen AJ, Eloff JN (1986) Effect of temperature and light (fluence rate) on he composition of the toxin of the cyanobacterium *Microcystis aeruginosa* (UV-006). Archiv Hydrobiol 108: 145-154

Wicks RJ, Thiel PG (1990) Enviromental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir. Environ Sci Technol 24: 1413-1418

Yu SZ (1995) Primary prevention of hepatocallular carcinoma. J Gastroenterol Hepatol 10: 674-682

Zheng L., Xie, P. Li Y. L., Yang H., Wang S. B., Guo N. C. (2004) Variation of intracellular and extracellular microcystins in a shallow, hypereutrophic subtropical Chinese lake with dense cyanobacterial blooms. Bull Environ Contam Toxicol 73:698-706