



Spatial and temporal variations of microcystins in hepatopancreas of a freshwater snail from Lake Taihu

Dawen Zhang, Ping Xie^{*}, Yaqin Liu, Jun Chen, Zhourui Wen

Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Donghu South Road 7, Wuhan 430072, People's Republic of China

ARTICLE INFO

Article history:

Received 15 December 2007

Received in revised form

2 May 2008

Accepted 10 May 2008

Available online 16 July 2008

Keywords:

Microcystins

Snail

Bellamya aeruginosa

Lake Taihu

ABSTRACT

In this paper, spatial and temporal variations of three common microcystins (MC-RR, MC-YR, and MC-LR) in the hepatopancreas of a freshwater snail (*Bellamya aeruginosa*) were studied monthly in two bays of Lake Taihu. Microcystins (MCs) concentration in hepatopancreas was quantified by liquid chromatography–mass spectrometry (LC–MS). The MCs concentrations in hepatopancreas were higher at Site 1 than those at other sites, which was in agreement with the changes of intracellular MCs concentrations in the water column. There was a significant correlation between MCs concentrations in the hepatopancreas and that in the seston, suggesting that spatial variances of MCs concentrations in hepatopancreas among the five sites were due to spatial changes of toxic *Microcystis* cells in the water column. PCCA indicates that in addition to *Microcystis*, other factors (e.g., water temperature) also substantially affected the accumulation of MCs in hepatopancreas of the snail.

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1. Introduction

Toxin-producing cyanobacteria are found in several areas of the world and have shown increased incidences of blooms as a result of changes in the level of nutrients in the water (Kuiper-Goodman et al., 1999). Among cyanotoxins, microcystins (MCs) are considered to be the most common and dangerous group (Chorus and Bartram, 1999).

Microcystins are cyclic heptapeptides consisting of five D-amino acids that exhibit minor structural variations and two variable L-amino acids (Botes et al., 1984). More than 80 analogues of MCs have been identified so far (Dietrich and Hoeger, 2005). MCs are known to be potent hepatotoxins (Dawson, 1998) and tumor promoter (Nishiwaki-Matsushima et al., 1992). Exposure to MCs represents a health risk to aquatic organisms, wild life, domestic animals, and humans upon drinking or ingesting algae in the water (Malbrouck and Kestemont, 2006). Although microcystins are rarely ingested by human in amount high enough to a lethal acute dose, chronic toxic effects from exposure through food need to be considered, especially if there is long-term frequent exposure (Magalhães et al., 2001). For example, the high incidence of primary liver cancer in Haimen City (Jiangsu Province) and Fusui County (Guangxi Province) in China is considered to be related to microcystin in drinking water (Yu, 1989, 1995).

^{*} Corresponding author. Fax: +86 27 68780622.

E-mail address: xieping@ihb.ac.cn (P. Xie).

Microcystins are accumulated in a wide range of aquatic organisms such as fish (Williams et al., 1997a; Soares et al., 2004), zooplankton (Ferrão-Filho et al., 2002), bivalves (Williams et al., 1997b; Yokoyama and Park, 2002; Chen and Xie, 2005), and gastropods (Zurawell et al., 1999; Ozawa et al., 2003; Chen et al., 2005; Zhang et al., 2007). Recently, some studies have found that MCs can be transferred along the food web, although with the absence of biomagnification (Xie et al., 2005; Smith and Haney, 2006), indicating a potential threat to human health from consumption of MCs-contaminated aquatic products. Furthermore, snails are widely distributed in China with habitats of lakes, rivers, ditches, and ponds (Liu et al., 1993) and these organisms are an important food source not only for waterfowl, birds, and fish but also for mammals, including humans. This means that snails could be considered a better vector in the transferring of microcystins to higher trophic levels along the food chain.

So far, there have been a few studies on the MCs accumulation in gastropod in field (Zhang et al., 2007; Chen et al., 2005; Ozawa et al., 2003; Zurawell et al., 1999). To our knowledge, no information is available on spatial variations of MCs in invertebrate animals in Lake Taihu.

Therefore, it is necessary to investigate MCs concentration in snails within or among lakes with dense toxic blooms. This paper reports a field study on the spatial and temporal variations of MCs in the hepatopancreas of a freshwater snail (*Bellamya aeruginosa*) from Lake Taihu, a large shallow eutrophic lake with dense toxic *Microcystis* blooms, with discussion on the possible mechanisms underlying these variations.

2. Materials and Methods

2.1. Study areas

Lake Taihu (30°5′–32°8′N and 119°8′–121°55′E) is located in the east part of China. It is the third largest freshwater lake in China, and has a surface area of 2338 km², a mean water depth of 1.9 m and a maximum depth of about 2.6 m. This area is of historical importance in trade, politics, agriculture, and culture. About 35 million people inhabit the 36500 km² watershed of Taihu Lake (Qin et al., 2004). During the past decades, the lake has undergone a steady increase in eutrophication, and with a regular occurrence of cyanobacterial surface blooms in the warm seasons each year (Pu et al., 1998a,b). Meiliang Bay, a part of Lake Taihu, with water surface area of 125 km², accommodates municipal and industry wastewater from Wuxi City, and acts as principal water source for the city. Meiliang Bay is the most eutrophic part of the lake, characteristic of extremely dense accumulation of toxic *Microcystis* blooms by wind in the summer (Qin et al., 2004). Gonghu Bay (31°31′–23°N, 120°16′–35′E) is located at the northeast portion of Taihu Lake with abundant submerged plants. Five sampling sites were set up based on the Lake Taihu's eutrophication status and the investigation was carried out from November 2004 to October 2005 (Fig. 1).

2.2. Water quality

Water samples were collected monthly from the five sites in Lake Taihu with Tygon tubing fitted with a one-way valve from November 2004 to October 2005. Each integrated water sample was a mixture of two sub-samples from 0.5 m below the surface and from 0.5 m above the bottom, respectively. Transparency was measured by a Secchi disk. Total phosphorus (TP) and total dissolved phosphorus (TDP) were measured by colorimetry after digestion with H₂SO₄ (Ebina et al., 1983). Total nitrogen (TN) and total dissolved nitrogen (TDN) were determined by the Kjeldahl method. Ammonium (NH₄-N) was determined by the Nessler method and nitrite (NO₂-N) by the α -naphthylamine method (American Public Health Association et al., 1995). Subsamples for phytoplankton were preserved with 1% acidified Lugol's iodine solution concentrated to 30 ml after sedimentation for 48 h, and *Microcystis* cells were separated by sonication for 1 min with an ultrasonic probe (250 W, 20 kHz; Sonics JY88-II; Ningbo Scientz Biotechnology Co. Ltd, China) and counted microscopically in 100 microscopic fields. Biomass (expressed as mg fresh wt/l) was estimated from approximate geometric volumes of each taxon, assuming that 1 mm³ equals 10⁻⁶ μ g fresh weight. The relative abundance of *Microcystis aeruginosa* (% *M. aeruginosa* biomass in total phytoplankton biomass) at each site was calculated monthly during the study period.

2.3. Snail samples

About 200 snails (*B. aeruginosa*) were collected monthly at each site from November 2004 to October 2005. Average body weights of the snail was 4.68 ± 1.19 g with a shell height of 2.80 ± 0.28 cm ($n = 2700$). The collected snails

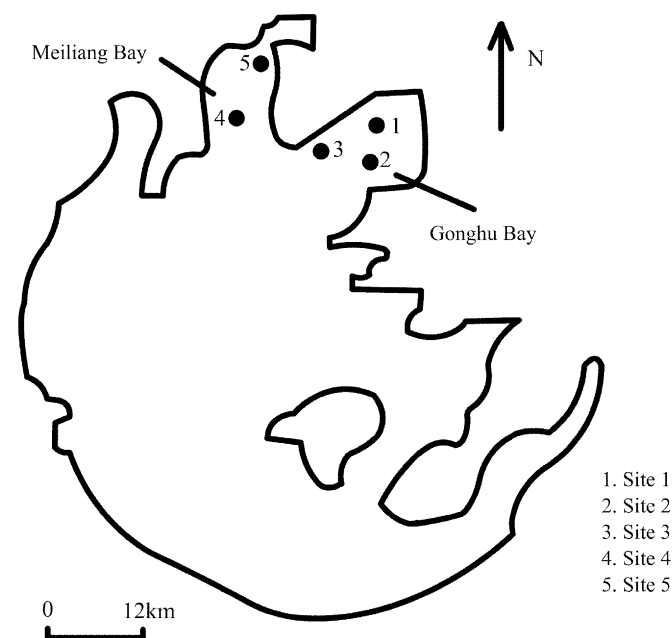


Fig. 1. Location of sampling sites in Lake Taihu.

were immediately frozen at -20 °C, and then the hepatopancreas were dissected from snails in the laboratory. The collected hepatopancreas samplings were frozen at -80 °C prior to microcystins analysis. To insure adequate sample, we pooled separate hepatopancreas materials of 50 snails into one composite sample.

2.4. Microcystins analysis

Microcystins in the seston of the water column was measured as intracellular and extracellular toxins according to the methods of Park and Lwami (1998). The intracellular toxins were extracted from cyanobacterial cells filtered from 1 l lake water on the glass-fiber filter (GF/C, Whatman, Brentford, UK). The filtrate (1 l) was used to detect the extracellular toxins.

All the frozen tissues and offspring of the snail were lyophilized prior to microcystins analysis. We extracted MCs from the snail tissues following the method of Zhang et al. (2007): lyophilized samples (~0.3 g dry wt for each tissue) were homogenized and extracted three times with 25 ml of butanol:methanol:water (1:4:15) for 24 h while stirring. The extract was centrifuged at 36290 × g and the supernatant was diluted with water.

Qualitative and quantitative analysis of MCs in the hepatopancreas of snails and waters were performed using a Finnigan LC-MS system comprising a Thermo Surveyor auto sampler, a Surveyor mass spectrum (MS) pump, a Surveyor photo diode array (PDA) system, and a Finnigan LCQ-Advantage MAX ion trap mass spectrometer (Thermo Electron, Waltham, MA, USA) equipped with an atmospheric pressure ionization fitted with an electrospray ionization source (ESI) (Thermo Electron). The instrument control, data processing, and analysis were conducted by using Xcalibur software (Thermo Electron). Separation was carried out under the reversed phase on Hypersil GOLD (Thermo Electron) 5 μ m column (2.1 mm i.d. × 150 mm). The isocratic mobile phase consisted of solvent A [water+0.05% (v/v) formic acid]/solvent B [acetonitrile+0.05% formic acid]. We used the following linear gradient program: 0 min 30% B, 2 min 30% B, 7 min 50% B, 11 min 100% B, 14 min 100% B, 15 min 30% B, 25 min 30% B. Sample injection volumes was typically 10 μ l. Mass spectrum tuning and optimization were achieved by infusing microcystin-RR and monitoring the [M+2H]²⁺ ion at m/z 520. The MS analytical conditions were as follows: ESI spray voltage 4.54 kV; sheath gas flow rate 20 unit; auxiliary gas flow rate 0 unit; capillary voltage 3.36 V; capillary temperature 250 °C; and multiplier voltage—853.19 V; and tube lens offset, 55v. Data acquisition was in the positive ionization centroid mode. The MS detection was operated in four segments: first, full scan mode with a m/z scan range between 400 and 1400, 4.2 min duration; second, two scan events: full scan mode as same as segment 1 and MS² mode with a m/z scan range between 140 and 1100, parent ion: 520; isolation width: 1; normalized collision energy: 37%; 4.8 min duration; third, three scan events: full scan mode as same as segment 1 and MS² mode with m/z scan range between 270 and 1100 and 285 and 1100, respectively; parent ion: 995.5 and 1045.5, respectively; isolation width: equal for both, normalized collision energy equal for both, 35%; 4.8 min duration; fourth, full scan mode as same as segment 1.

Standards of MCs (MC-LR, MC-YR, and MC-RR) used for LC-ESI-MS analysis were purchased from the Pure Chemical Industries, Osaka, Japan. The limit of detection for the MCs in hepatopancreas and seston were 0.003 μ g/g DW and 0.01 μ g/ml, respectively.

2.5. Statistical analysis

STATISTIC for Windows statistical software (Version 6.0) was used for all analyses. To characterize relationships between MCs content and environmental variables, we used principal component and classifying analysis (PCCA). One-way analysis of variance (ANOVA) was used to compare the differences of MCs concentrations in hepatopancreas of *B. aeruginosa* among the five sampling sites.

3. Results

Microcystins in the hepatopancreas of *B. aeruginosa* were determined by LC-ESI-MS. Based on total ion chromatogram of ESI LC-MS² measurement of microcystins in the hepatopancreas of *B. aeruginosa*, mass chromatograms monitored at m/z 520, and the presence of [M+H]⁺ ion at m/z 452 and 887, it is confirmed that peak obtained at 5.52 min was derived from MC-RR. Similarly, peaks obtained at 11.25 and 11.57 min were derived from MC-YR and MC-LR, respectively, as the peaks were detected by monitoring with m/z 1045.5 and m/z 995.5, respectively, and the mass chromatogram showed [M+H]⁺ ion at m/z 1045.5 and 599 for MC-YR and m/z 995.5 and 599 for MC-LR, respectively (Appendix Fig. 1).

Among the five sites, all nutritional parameters ($\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, TN, TDN, $\text{PO}_4\text{-P}$, TP, and TDP) of the lake water at Site 1 showed the lowest, while the highest was at Sites 4 and 5 (Table 1).

Among the five sites, density of *Microcystis* cells differed markedly in the patterns of spatial distribution and seasonal variations (Fig. 2A). The highest average *Microcystis* biomass was at Site 4 (2.10 mg/l), while the lowest was at Site 2 (0.3 mg/l). The first appearance of *Microcystis* cells in the water column was in June, and *Microcystis* reached the highest density in July at all sites except Site 2 (in September), remaining higher abundance until September.

The relative abundance of *M. aeruginosa* at each station changed markedly during the study period (Appendix Table 2). The relative abundance of *M. aeruginosa* ranged from 0% to 94.8% (average = 42.0%), 0% to 88.0% (average = 36.1%), 0% to 98.6% (average = 54.5%), 0% to 99.0% (average = 58.7%), 0% to 95.4% (average = 47.3%) at Sites 1–5, respectively. During the bloom period (from July to October 2005), *M. aeruginosa* showed an absolute dominance at all sites each month (Appendix Table 1).

Table 1
Annual means of physico-chemical parameters (mg/l) at five sites in Lake Taihu

	$\text{NH}_4\text{-N}$	$\text{NO}_2\text{-N}$	$\text{NO}_3\text{-N}$	TN	TDN	$\text{PO}_4\text{-P}$	TP	TDP
Site 1	0.430	0.012	0.983	1.908	1.400	0.001	0.067	0.023
Site 2	0.684	0.030	1.351	2.872	1.959	0.001	0.098	0.029
Site 3	0.522	0.024	1.448	2.730	2.262	0.001	0.117	0.034
Site 4	1.580	0.010	1.815	3.860	3.995	0.003	0.199	0.054
Site 5	1.508	0.014	1.804	3.919	2.951	0.002	0.163	0.041

Seasonal and spatial variations of the intracellular MCs (MC-RR+MC-YR+MC-LR) concentration in the water column were shown in Fig. 2(B) and Appendix Table 2. Intracellular MCs concentration reached the highest at Sites 1, 2, and 5 in July, but at Sites 3 and 4 in September. Annual average intracellular MCs concentration was highest at Site 1 (0.87 $\mu\text{g/g}$ DW), but lowest at Site 5 (0.24 $\mu\text{g/g}$ DW), whereas the average *Microcystis* biomass at Site 5 was relatively high (0.97 mg/l). A significant correlation was found between the *Microcystis* biomass and intracellular MCs concentration in the water column ($r = 0.599$, $p < 0.01$; $n = 60$), suggesting that the seasonal variations of intracellular microcystins in the water column were dependent on the changes of *Microcystis* biomass.

The monthly changes and spatial variances of MCs concentration in the hepatopancreas of *B. aeruginosa* were shown in Fig. 3. *B. aeruginosa* accumulated high amount of MCs in response to the high level of MCs concentration in seston in summer and autumn. However, MCs concentration in the hepatopancreas of the snail decreased to a low level in other seasons as the surface cyanobacterial blooms disappeared. MCs concentration in the hepatopancreas showed the highest peaks in July at Site 1, but in October at the other four sites. Average microcystins content in the hepatopancreas was 1.9–4.5 times higher at Site 1 than at the other four sites (Fig. 4). There was substantial variance in MCs content of the hepatopancreas among the five sites, although the differences were statistically not significant (ANOVA, $p = 0.184$).

There was significant correlation between MCs concentration in hepatopancreas and that in seston of the water column ($r = 0.606$, $P < 0.01$; $n = 55$), but no significant relationship was found between MCs concentration in hepatopancreas and extracellular toxins ($P > 0.05$; $n = 55$). MCs content in the hepatopancreas was

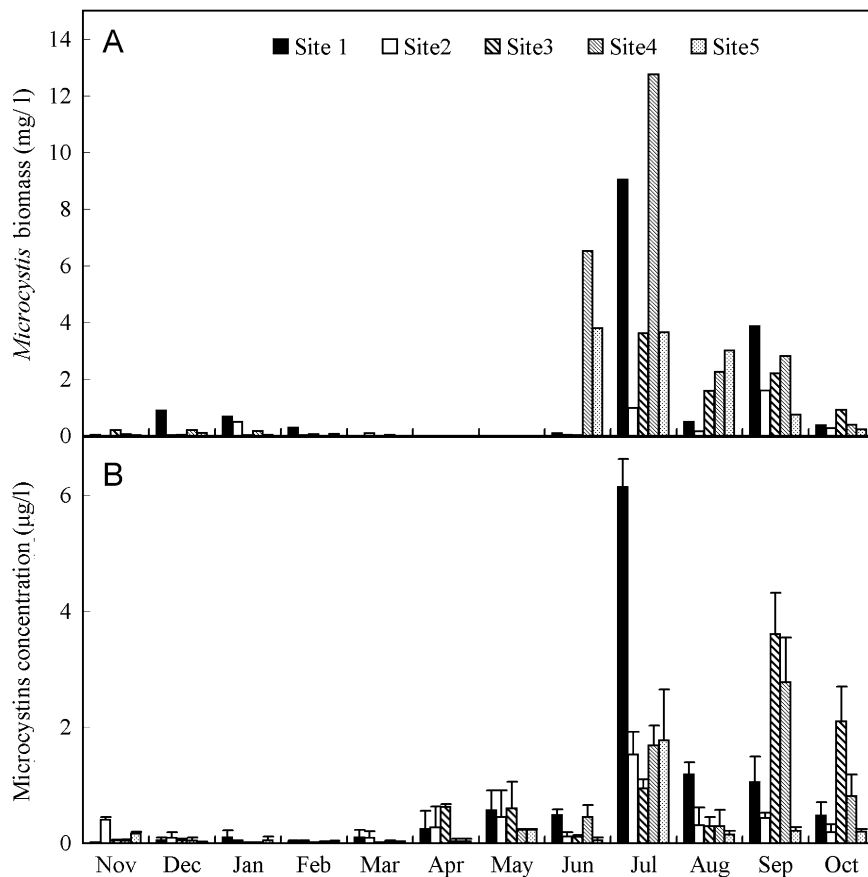


Fig. 2. Seasonal changes of *Microcystis* biomass (mg/l) (A) and seasonal changes of intracellular MCs content ($\mu\text{g/g}$) (B) at five sites of Lake Taihu.

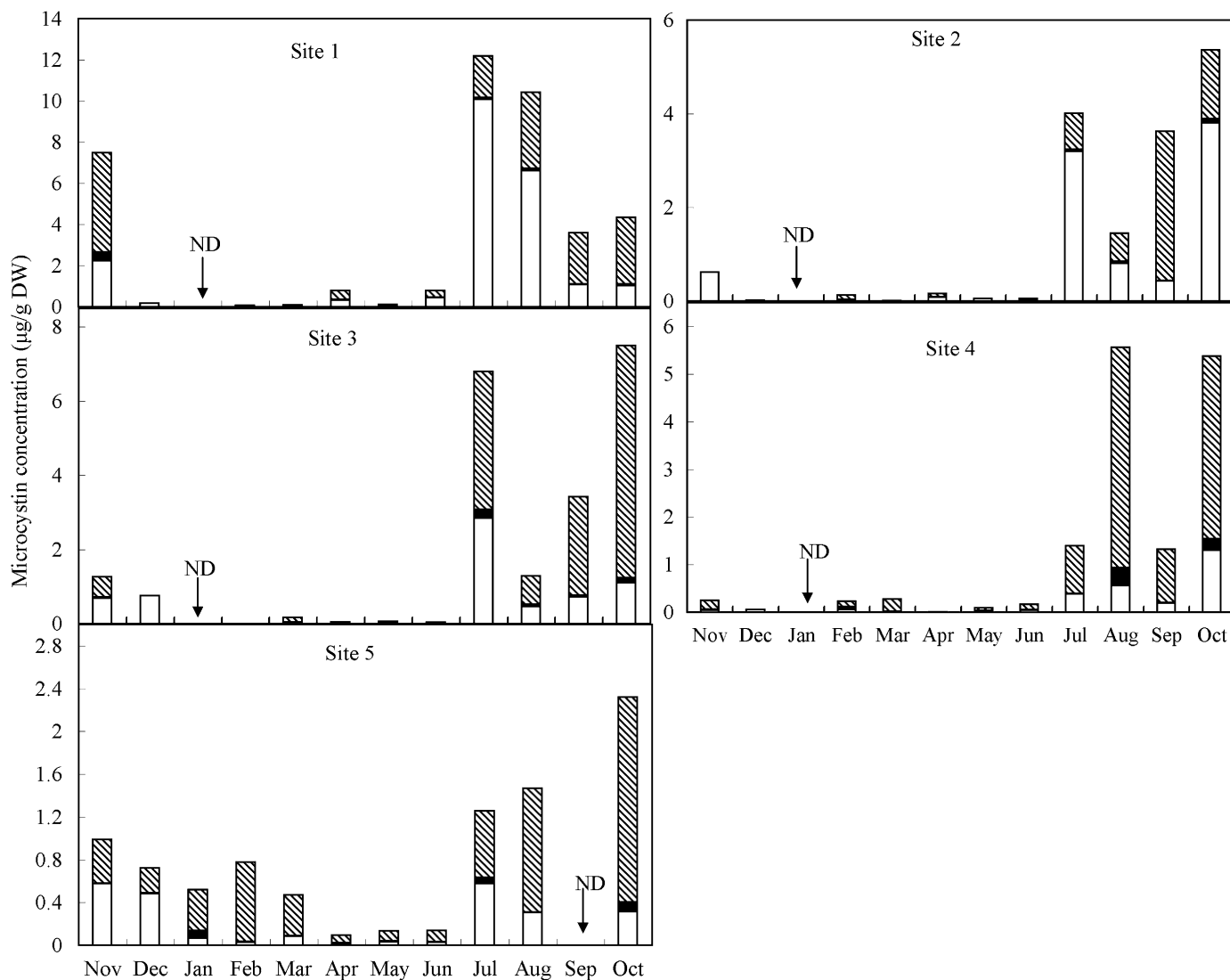


Fig. 3. The seasonal changes of microcystins (MC-RR, MC-YR, and MC-LR) in the hepatopancreas of *Bellamya aeruginosa* at five sites from Lake Taihu: ▨—LR; ■—YR; □—RR.

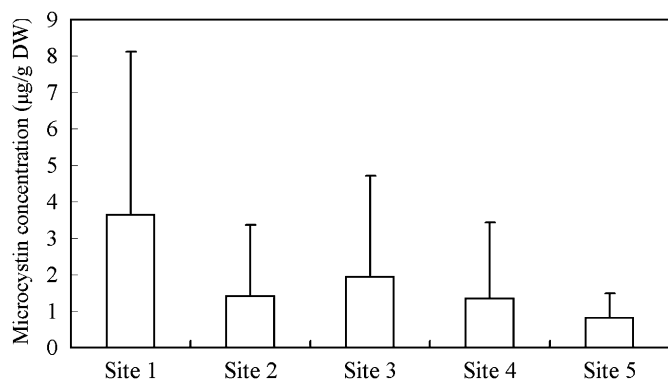


Fig. 4. Spatial variations of the annual mean microcystins (MC-RR+MC-YR+MC-LR) content in the hepatopancreas of *Bellamya aeruginosa* among the five sites from Lake Taihu.

significantly correlated with *Microcystis* biomass in the water column ($r = 0.327, P < 0.05; n = 60$).

During the period of dense cyanobacterial blooms (from July to October 2005), *Microcystis* was the absolute dominant species

(Appendix Table 1), and the other microcystin producing cyanobacterial genus such as *Anabaena*, *Oscillatoria*, *Anabaenopsi* were presented as minor components in the system (data not shown); and the MC-LR/MC-RR ratio of intracellular MCs in the water column ranged from 0.20 to 3.56 with an average of 1.44, while the MC-LR/MC-RR ratio in hepatopancreas varied from 0.20 to 8.26 with an average of 3.07.

With the principal components and classification analysis (PCCA), 55.2% of the environmental and biological variation in the data was explained by Component Axis 1 (39.2%) and Component Axis 2 (16.0%) (Fig. 5). Component Axis 1 had high positive weighting for chemical variables (such as N, P), but negative weighting for water temperature, *Microcystis* biomass, intracellular and extracellular toxin content, Secchi, and microcystins content in snail hepatopancreas. Component Axis 2 had high positive weighting for chemical variables (such as N, P), water temperature, *Microcystis* biomass, intracellular and extracellular toxin contents, and microcystins content in hepatopancreas, but negative weighting for Secchi. *Microcystis* biomass, intracellular and extracellular toxin contents, and microcystins content in hepatopancreas had high negative correlations with factor 1, but positive correlations with factor 2.

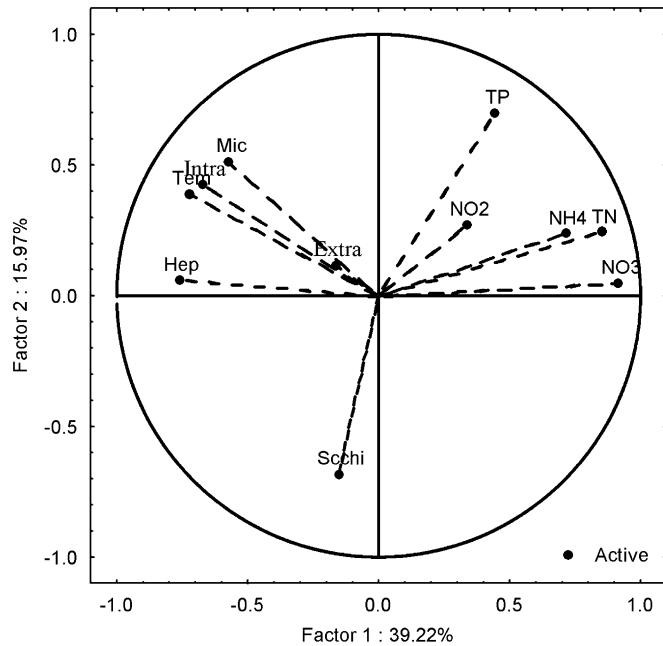


Fig. 5. Scatterplot of first two components from a PCCA performed with seven environmental variables and MCs variables in snail hepatopancreas, seston, and lake water. Extra: extracellular MCs content; Intra: intracellular MCs content; Mic: *Microcystis* biomass; Tem: temperature; Hep: MCs content in snail hepatopancreas; TN: total nitrogen; TP: total phosphors; Scchi: transparency depth.

4. Discussion

In the present study, peaks of *Microcystis* density or biomass did not strictly coincide with those of intracellular MCs concentration. Similar result was found in Ozawa et al. (2005). There were two possible explanations for this. First, the variation in the toxicity of blooms may be partly related with the relative proportion of toxic and nontoxic strains of *M. aeruginosa* (Carmichael and Gorham, 1977). Second, high concentration of MCs occurred during the exponential growth phase of the bloom (Park and Lwami, 1998). However, there was a significant correlation between *Microcystis* biomass and intracellular MCs concentration in the water column, suggesting that seasonal and spatial variations of intracellular microcystins in the water column were the result of the changes of *Microcystis* biomass. Kotak et al. (1993) showed that microcystin-LR concentration in phytoplankton can be highly dynamic, reflecting, at least partially, changes in the abundance or biomass of *M. aeruginosa* within a lake, temporally within and between years, and even over 24 h. Zurawell et al. (1999) reported that concentration of MC-LR in the phytoplankton varied within each lake probably in response to fluctuations in the relative and absolute abundance of *Microcystis* spp.

In the present study, there were substantial variances in MCs concentration of hepatopancreas among the five sampling sites, although the differences were statistically not significant, and significant correlation was found between MCs concentration in the hepatopancreas and intracellular toxins concentration and *Microcystis* biomass in the water column, suggesting that spatial variations of MCs concentration in hepatopancreas among the five sites were due to spatial changes of toxic *Microcystis* cells in the water column. Previous studies reported that snails are indiscriminate grazers searching for food (mainly detritus) (e.g., organic pieces, algae, silts) which adhere to the surface of macrophytes or the sediment or other substrata, and adapt their diets to the relative abundance of available sources (Elder and

Collins, 1991), and other studies showed that MCs content in invertebrates depended on the toxic level in their food sources (Chen et al., 2005; Chen and Xie, 2005; Zhang et al., 2007). Hence, we deduce that the relationship between intracellular toxin content and hepatopancreas toxin content is probably linked to the settling of cyanobacteria from the water column.

In the present study, MCs level in the hepatopancreas of the snail *B. aeruginosa* was high in summer and autumn (e.g., up to 12.19 $\mu\text{g/g}$ DW in July), but decreased to a low level in other seasons, suggesting that *B. aeruginosa* were quite tolerable to MCs and could depurate MCs efficiently. Similar results have also been obtained from other snails, mussels, and fish. In a laboratory experiment, *Sinotia histrica* accumulated the highest amount of MC-LR (436 $\mu\text{g/g}$ DW) on the 10th day of a 15 days' uptake, and MC-LR concentration in hepatopancreas declined to a low level (85.8 $\mu\text{g/g}$ DW) during the following 15-days' depuration period (Ozawa et al., 2003). Four freshwater mussels (*Anodonta woodiana*, *Hippocratea cumingii*, *Cristaria plicata*, *Lamprotula lei*) accumulated high amount of MCs in the hepatopancreas (e.g., up to 38.5 $\mu\text{g/g}$ DW for *C. plicata*) in summer, but declined to a low MC level in other seasons. Xie et al. (2004) found that liver of silver carp accumulated MCs quickly in a 40-days' uptake experiment, but MCs concentration in silver carp decreased to a low level in the following 40-days' depuration experiment.

In the present study, the ratio of MC-LR/MC-RR in seston of the water column (namely food source of snails) was lower than that in the hepatopancreas of the snails, suggesting (1) that the intestinal tract of the snails had low ability to inhibit the transportation of MC-LR, so MC-LR could accumulate in the snails quickly and easily, and (2) that MC-LR might be more resistant to degradation by the snails or/and snails might depurate MC-RR efficiently due to its more polar nature. In Lake Chaohu, China, the ratio of MC-LR/MC-RR increased from digestive tract (0.44) of *B. aeruginosa* to their hepatopancreas (0.66) (Chen et al., 2005). On the contrary, Xie et al. (2004) found that there was a decline in the ratio of MC-LR/MC-RR from 0.57 in *Microcystis* cells to 0.07–0.17 in the intestines and to zero in the blood of silver carp in the laboratory condition. In Lake Chaohu, MCs concentration (MC-RR+MC-LR) was as high as 137 $\mu\text{g g}^{-1}$ DW (more than 30 μg MC-LR/g DW) in the gut content of *Hypophthalmichthys molitrix*, but in their livers, only less than 2 μg MC-RR/g DW and no MC-LR were found (Xie et al., 2005). However, Xie et al. (2007) demonstrated that *S. histrica* (collected from Lake Suwa, Japan) is capable of depurating MC-LR efficiently. These results suggest that accumulation patterns and metabolism mechanisms of MCs seem likely to vary among different species of snails or other aquatic animals.

Generally speaking, the main uptake routs of microcystins for aquatic organisms are via the diet (toxic cyanobacterial cells), not the dissolved toxins in the water column. In the present study, the statistical analysis showed that MCs concentration in hepatopancreas was significantly correlated with the intracellular MCs concentration and *Microcystis* biomass in the water column, but not with the extracellular MCs content. Similarly, studies with the three species of gastropod (*Lymnaea stagnalis*, *Helisoma trivolvis*, and *Physa gyrina*) (Zurawell et al., 1999), three freshwater Unionid bivalves (*A. Woodiana*, *C. plicata*, and *Unio douglasiae*) (Yokoyama and Park, 2002) indicated that the concentration of microcystins in the tissue of the invertebrates was correlated with the toxin in phytoplankton, but not with extracellular aqueous microcystins. Meanwhile, some studies with the freshwater clam, *Anodonta grandis simpsoniana* (Prepas et al., 1997), the blue mussel, *Mytilus edulis* (Novaczek et al., 1991), and the gastropods (Lance et al., 2006) pointed out that the primary routes of uptake of algal toxins are through diet, and to a lesser extent, via uptake of dissolved toxins.

In the present study, PCCA indicates that among biological variables, *Microcystis* biomass showed closer positive relationships with intracellular and extracellular toxins content than with MCs content in snail hepatopancreas, suggesting that in addition to *Microcystis*, other factors (e.g., water temperature) also substantially affected the accumulation of MCs in snail hepatopancreas. In the present study, temperature had close correlations both with intracellular toxin content, and with MCs content in snail hepatopancreas, suggesting the importance of water temperature on the MCs accumulation in snail hepatopancreas. Yokoyama and Park (2003) reported that *Unio douglasiae* accumulated much higher concentration ($250 \pm 40 \mu\text{g/g DW}$) at 25°C than that at 15°C ($130 \pm 11 \mu\text{g/g DW}$).

Gastropods are common and abundant in fresh waters (Habdija et al., 1995), and are an important food source for fish, waterfowl, crayfish, and amphibians (Stein et al., 1984), and even for human. Therefore, the risk of MCs transfer along the food chain is possible. Hence, for the sake of human health, spatial and temporal variations of MCs in edible snails should be monitored in lake ecosystem management. The risk of the possible transfer of MCs along the food web should be evaluated in our future study.

5. Conclusions

Spatial variations of MCs concentration in hepatopancreas were found in Lake Taihu, and these variations were due to spatial changes of toxic *Microcystis* cells in the water column. PCCA indicates that in addition to *Microcystis*, other factors (e.g., water temperature) also substantially affected the accumulation of MCs in the snail hepatopancreas. It is likely that accumulation patterns and metabolism mechanisms of MCs seem to vary among different species of snails and other aquatic animals.

Acknowledgments

We would like to express our sincere thanks to Prof. Dr. Irena Twardowska and two anonymous reviewers for their useful comments and suggestions on this manuscript. Sincere thanks are also given to Zhou Qiong, and Qiu Tong for their help in the fieldwork. This research was supported by a fund of National Natural Science Foundation of China (Grant no. 30530170).

Disclaimer: Experimental animals used in the present study were conducted in accordance with national and institutional guidelines for the protection of animal welfare.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2008.05.014.

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