ACCUMULATION OF HEPATOTOXIC MICROCYSTINS IN FRESHWATER MUSSELS, AQUATIC INSECT LARVAE AND OLIGOCHAETES IN A LARGE, SHALLOW EUTROPHIC LAKE (LAKE CHAOHU) OF SUBTROPICAL CHINA

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ABSTRACT

This paper studied the seasonal changes of two common microcystins (MCs), MC-RR and -LR, in the commercially important mussel *Corbicula fluminea* in Lake Chaohu, where there occurred dense cyanobacteria. Occasional measurements were also made for MC in the mussel *Arconaia lanceolat*, the oligochaete *Limnodilus hoffineisteri* and the insect larva *Chironomus* sp. Mean MC of *C. fluminea* was much higher in hepatopancreas than in intestine and foot. Our study is the first to report accumulation of MCs in oligochaetes and aquatic insect larvae. The high contents of MCs in the insect larvae suggest a great possibility for the transfer of MCs to benthos-feeding omnivores like common carp. According to the provisional standard by the WHO, 28.6 % of the collected *C. fluminea* were harmful for human consumption, assuming a daily consumption of 300 g by a person. It is recommended that edible mussels should not be collected for human consumption during toxic cyanobacterial blooms in Lake Chaohu.

KEYWORDS:
Microcystin-RR and -LR; edible mussel; organ and inter-specific patterns; oligochaetes; aquatic insect larvae; Lake Chaohu.

INTRODUCTION

Cyanotoxins produced by toxic cyanobacteria, such as *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc*, in eutrophic freshwaters are causing serious ecological and health problems worldwide. Among these toxins, the cyclic peptide hepatotoxic microcystins (MCs) are probably the most important in terms of human health, because they are potent inhibitors of protein phosphatase 1 and 2A, and are considered to be potent tumor promoters [1]. So far, much more concerns have been on MC intake through drinking water [2-3], whereas the risk of chronic intoxication through ingestion of contaminated fisheries products (e.g., fish, mollusks) has not been well studied [4-5].

Limited information exists regarding MC accumulation by freshwater mollusks in the field [6–9], probably because freshwater mollusks usually are not consumed by humans in Europe and North America. Mollusks are considered to play an important role in the transfer of MCs to humans through food consumption in estuaries of eutrophic rivers [10-11], and also may affect terrestrial food webs [6]. Up to now, studies with bivalves frequently have been limited to MC accumulation in the hepatopancreas [8] or the whole body [6, 9], whereas little attention has been paid to MC distribution in various organs. And at present, little is known concerning MC accumulation in freshwater bivalves in China, where freshwaters are threatened by heavy MC contamination.

The present study was conducted in a large, shallow eutrophic Chinese lake, Lake Chaohu, and the purposes are to measure concentrations of two common microcystins, MC-RR and MC-LR, in freshwater mussels, aquatic insect larvae and oligochaetes, and to describe the seasonal changes of MCs in the commercially important mussel *Corbicula fluminea* with comments on their risk for human consumption, and with discussion on the distribution patterns of MCs in various organs of the mussel.

MATERIALS AND METHODS

Lake Chaohu, one of the three important renovated lakes (Chaohu, Taihu and Dianci) in China, is a large (760 km^2), shallow (mean depth 3.06 m) eutrophic lake located in Anhui Province in the subtropical China. In recent decades, cyanobacterial blooms, mainly composed of *Microcystis* sp. and *Anabaena* sp., have occurred densely in the warm seasons of each year.

Corbicula fluminea (Mollusca: Corbiculidae), is a freshwater species inhabiting southern and eastern Asia (China, Russia, Thailand, Philippines), and over its native range, the *C. fluminea* is marketed fresh or dry for human consumption, and as feed for domestic fowl [12]. In Lake Chaohu, *C. fluminea* is the most abundant mollusk with a biomass of 78.4 g wet weight (ww) m^{-2}, and a total production (whole lake) of 61690 tons ww) in 2002 (Daogui Deng, unpublished data). It is commercially important because it traditionally has a high food value for local residents, and is exported as food to Japan, and is also used to feed fowl or crab in culture.

During June to December 2003, *C. fluminea* were collected monthly near Zhongmiao, which is a littoral area between western and eastern parts. In this area, surface cyanobacterial blooms frequently accumulate densely by wind. *C. fluminea* are small mussels with a shell width of 2.1 ± 0.1 cm and a shell height of 2.01 ± 0.1 cm. The collected *C. fluminea* were immediately frozen at –20 °C, and then dissected into three parts (intestines, hepatopancreas and foot) in the laboratory. The collected organs were frozen at –80 °C prior to MC analysis. Whole body of the clam (excluding shell) was also sampled at the same time. Since there were insufficient toxins in the intestines, hepatopancreas and foot to allow for individual analysis, each value represents an average amount of MCs in the organs of 60 individuals.

Occasional samplings were also made in the same sampling site for other benthos. Samples of *Limnodilus hoffineisteri* (Oligochaetes) and *Chironomus* sp. (Chironomidae) were taken in June and June/July, respectively, with a 0.0625 m^2 modified Petersen grab, and the collected material was sieved through a 450-μm net. The animals were separated in laboratory by forceps. *Arconaia lanceolata* (Mollusca: Unionidae) were collected in December, and were dissected into gill, foot and hepatopancreas in the laboratory.

Extraction and analysis of MCs in mussels (various organs), oligochaetes and insect larvae basically followed the method of [5]. Recovery experiments were performed in triplicate spiking 500 mg of homogenized freeze-dried *C. fluminea* samples (hepatopancreas and foot) with a mixed MC solution of the two commercial standards (MC-RR and MC-LR; Wako Pure Chemical Industries - Japan) at 2.5 μg/g. The extraction was performed as described previously, and the recovery and the relative standard deviation (RSD) of the analytical method were calculated. The average recoveries (n=6) from different parts of mussel samples were 78 % (range 67–82 %; RSD range 10-17 %) for MC-RR and 81 % (range 72–88 %; RSD range 9-15 %) for MC-LR.

According to a provisional tolerable daily intake (TDI) (0.04 μg kg^{-1} body weight (bw) or 2 μg for an adult with 60 kg bw) for MC-LR by WHO [2-3], we estimated the edible security of *C. fluminea*. Ratios of dry weight to wet weight for various organs were calculated on a subset of twenty individuals, and the ratios (mean ± standard deviation) of the hepatopancreas, intestine, foot, and the whole body were 0.16 ± 0.05, 0.21 ± 0.05, 0.23 ± 0.02, and 0.17 ± 0.03, respectively. A coefficient of 5 was used to convert the dry weight of *C. fluminea* tissue to wet weight. And since the intraperitoneal median lethal dose in mice for MC-RR is approximately 5.0-fold higher than that for MC-LR [13], coefficient of 0.2 was used to convert MC-RR into the MC-LR equivalent (MC-LReq).

Pearson correlation analysis was conducted to determine the relationship of MC content between cyanobacterial blooms and whole body of the *C. fluminea* using SPSS® for Windows (Ver 11.5; SPSS, Chicago, IL, USA). No data transformation was performed before analysis.

RESULTS

The chromatograms of the MC-LR and -RR standards, as well as the extracts of hepatopancreas of the *C. fluminea* are compared in Figure 1. This shows that the toxins were taken up by the mussel, and were extractable in the organs. Figure 2 shows the ESI LC/MS analysis of microcystins in the hepatopancreas of the *C. fluminea*. Based on total ion chromatogram, mass chromatograms monitored at m/z 1,038, and the presence of the $[M+H]^+$ ion at m/z 1,038 confirmed the presence of MC-RR. Similarly, MC-LR was deduced to be derived from the mussel, as the peak was detected by monitoring with m/z 995, and the mass chromatogram showed $[M+H]^+$ ion at m/z 995.

Table 1 shows the seasonal changes of MC-RR and MC-LR contents in intestines, hepatopancreas, foot and whole body of the *C. fluminea* during June and December, 2003. The critical amount that is necessary to ingest to reach a tolerable daily intake (TDI) for MC was estimated, and the figures are also given in Table 1. In general, mean MC levels were remarkably higher in the hepatopancreas (1.76 μg/g dry weight = dw) than in the intes-

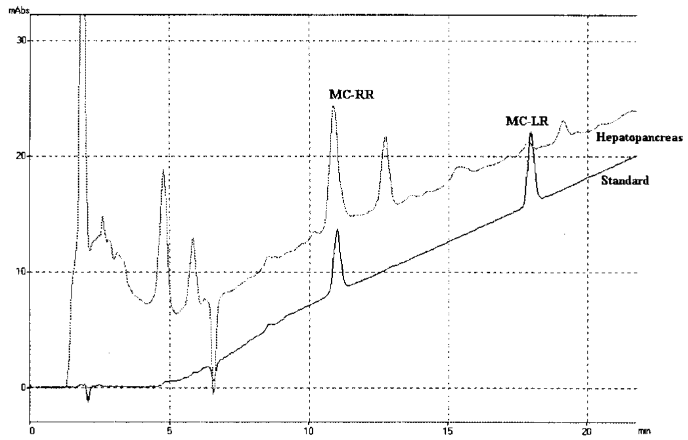
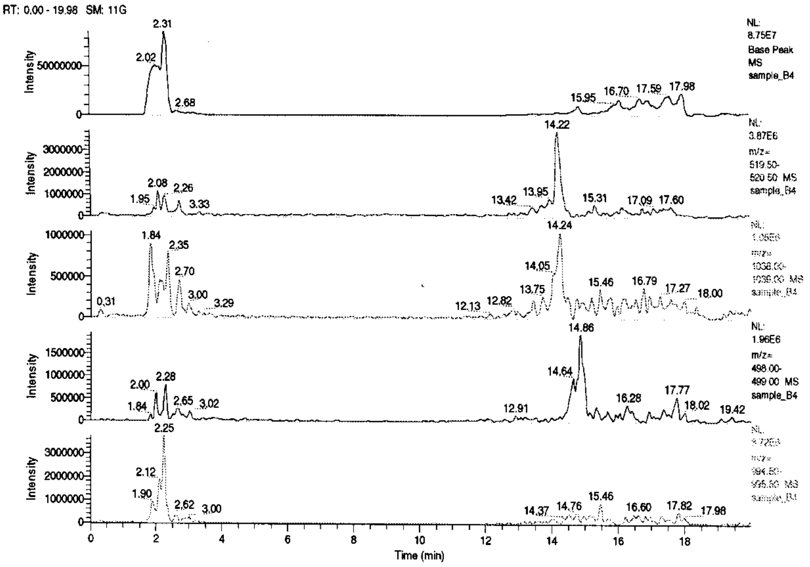
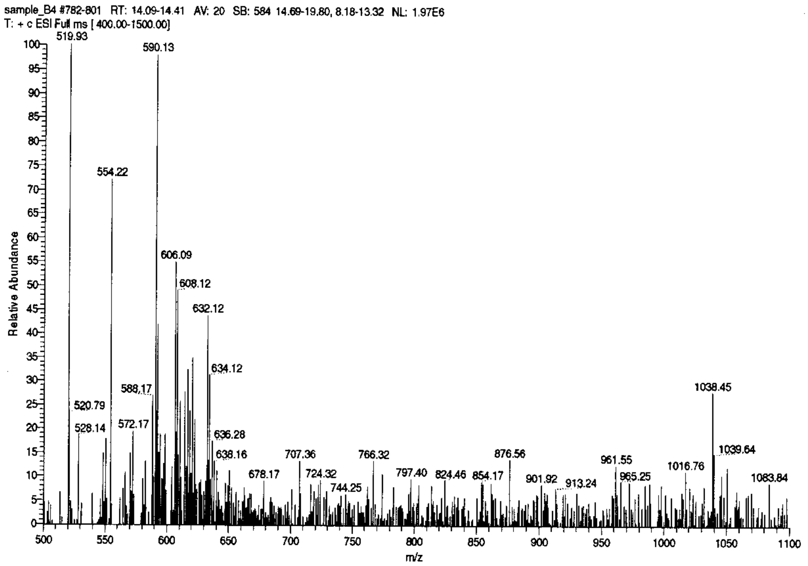


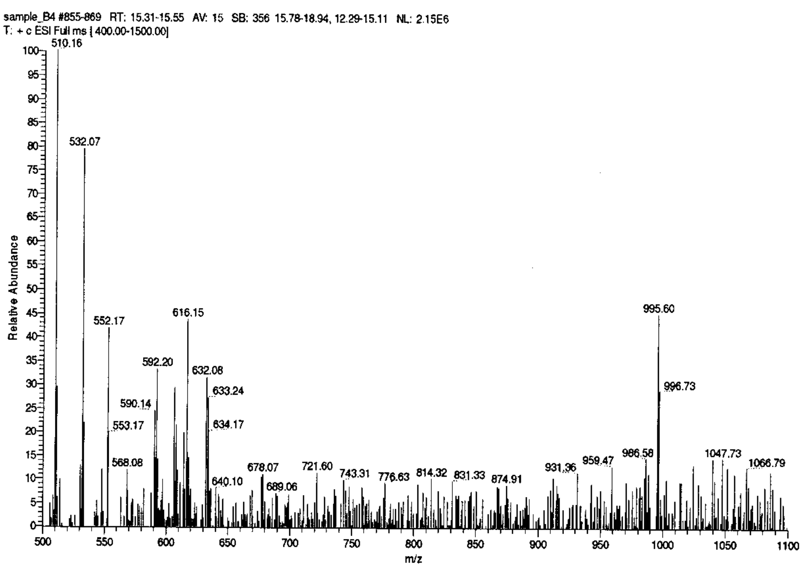
FIGURE 1 - High performance liquid chromatograms monitored at 238 nm and measured for the standards MC-LR and -RR, and the extract of hepatopancreas of *Corbicula fluminea* collected from Lake Chaohu in October 2003.



mass chromatograms monitored at m/z 520, 1038, 498 and 995



ESI mass spectrum at 14.22 min (microcystin-RR)



ESI mass spectrum at 15.46 min (microcystin-LR)

FIGURE 2 - ESI LC/MS analysis of microcystins in the hepatopancreas of *Corbicula fluminea* (October 2003).

TABLE 1 - Microcystin contents (μg/g dw) in tissues of *Corbicula fluminea* in Lake Chaohu during June-December 2003, and the critical amount (CA) that is necessary to ingest, in order to reach a tolerable daily intake (TDI) for MC.

Months	Intestine			Hepatopancreas			Foot			Whole body			CA
	RR	LR	Total	RR	LR	Total	RR	LR	Total	RR	LR	Total	
June	---	---	---	0.74	0.00	0.74	---	---	---	---	---	[1]0.09	
July	---	---	---	---	---	---	0.02	0.00	0.02	0.00	0.00	0.00	
Aug.	---	---	---	4.33	0.85	5.18	0.13	0.05	0.18	0.32	0.21	0.53	36
Sep.	---	---	---	0.12	0.00	0.12	0.04	0.03	0.07	0.11	0.00	0.11	455
[2]Oct.	0.95	0.21	1.16	3.95	0.42	4.37	0.00	0.00	0.00	0.35	0.04	0.39	91
Nov.	0.38	0.00	0.38	0.14	0.00	0.14	0.15	0.00	0.15	0.03	0.00	0.03	1667
Dec.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Mean			0.51			1.76			0.07			0.16	

[1] Estimated from MC contents in the hepatopancreas and the remaining tissue (0.74 and 0.04 μg/g dw, respectively) and their dw proportions (7.4 and 92.6%, respectively) in June
[2] No microcystins were detected in the gill
--- No data

TABLE 2 - Microcystins contents (μg/g dw) in the whole bodies of oligochates and insect larvae, and various tissues of the mussel *Arconaia lanceolata* in Lake Chaohu.

Limnodilus hoffineisteri (Oligochaeta)									
	RR	LR	Total						
June	0.23	0.00	0.23						
Chironomus sp. (Insect larvae of the family Chironomidae)									
	RR	LR	Total						
June	5.92	5.62	11.54						
July	0.71	0.95	1.66						
Arconaia lanceolata (a bivalve of the family Unionidae)									
	Gills			Foot			Hepatopancreas		
	RR	LR	Total	RR	LR	Total	RR	LR	Total
December	1.22	0.47	1.69	0.23	0.00	0.23	14.79	3.22	18.01

tines (0.51 μg/g dw) and feet (0.07 μg/g dw). In the hepatopancreas, the maximum content was in August (5.18 μg/g dw), and in the whole body, there were two maximums: in August (0.53 μg/g dw) and October (0.39 μg/g dw), respectively. If the average daily consumption of the mussel by a person is taken to be 300 g, MC contents in 28.6 % of the collected *C. fluminea* (in August and October) were above the risk levels for human consumption.

Great seasonal variations of MC-LR and MC-RR content were observed in whole body of the *C. fluminea* (Table 1). Whole body of the clam showed maximum MC content in August, while MC levels during cyanobacterial blooms reached a maximum synchronously. Statistical analysis shows that there was a positive correlation between MC in cyanobacterial blooms and MC in the whole body of *C. fluminea* ($r=0.5699$), although statistically not significant ($P=0.182$), suggesting that, to some extent, accumulation of MC in *C. fluminea* depended on MC level in the food resources.

Table 2 shows a comparison of MC contents in the whole bodies of oligochaetes and aquatic insect larvae, and various tissues of the mussel *Arconaia lanceolata* from occasional samplings. MC contents in the June sample of *Chironomus* sp. were rather high (11.54 μg/g dw), with MC-RR and -LR being almost equally important. MC content in the oligochaete was the lowest. The hepatopancreas of *Arconaia lanceolata* accumulated as much as 18.01 μg MCs/g dw, 10.2 times the mean MC content of the *C. fluminea*. In *A. lanceolata*, MC levels were much higher in the hepatopancreas than in the gills and feet.

DISCUSSION

In the present study, *A. lanceolata* accumulated much more MCs in the hepatopancreas (18.01 μg/g dw) than in the gill (1.69 μg/g dw) or foot (0.23 μg/g dw), and *C. fluminea* also accumulated much more MCs in the hepatopancreas (1.76 μg/g dw) than in the foot (0.07 μg/g dw). In Lake Taihu, the maximum MC contents in the hepatopancreas, intestine, visceral mass, gill, foot and rest of four edible freshwater mussels (*Anodonta woodiana, Hyriopsis cumingii, Cristaria plicata and Lamprotula leai*) were 38.48, 20.65, 1.70, 0.64, 0.58, and 0.61 μg/g dw [14]. In Lake Suwa, the mussel *Unio douglasiae* (Unionidae) accumulated higher MCs in the hepatopancreas (2.72 μg/g dw) than in the gill and muscle (2.00 μg/g dw), but the difference was not as great as in our study lake [7]. These facts indicate that in mussels, hepatopancreas is the target organ by MCs in the field. On the other hand, difference in MC levels between gill and foot or muscle was variable. In agreement with our results, in a laboratory experiment, the saltwater mussel *Mytilus galloprovincialis* accumulated higher MC-LR contents in gills (0.29 μg/g dw) than in foot (0 μg/g dw) [15]. In Driedmeat Lake where *Anodonta grandis simpsoniana*, after being placed for 24 days, accumulated an average MC-LReq concentration of 0.369±0.047 μg/g dw, but with higher toxin level in the muscle tissue (foot and adductor muscles) than in the gill [6].

In the present study, mean MC levels of *C. fluminea* were much lower in the intestines (0.51 μg/g dw) than in the hepatopancreas (1.76 μg/g dw). In Lake Taihu, mean MC levels were much lower in intestines than in the hepatopancreas for *Cristaria plicata* and *Lamprotula leai*, but *Anodonta woodiana* and *Hyriopsis cumingii* had higher MCs in intestines than in the hepatopancreas [14]. In a laboratory experiment, saltwater mussels, *Mytilus galloprovincialis*, were fed with toxic *Microcystis aeruginosa* cells, and MC-LR in digestive tract (digestive glands + stomach) of the mussel reached 27.6 μg/g dw; toxin burden reached 96.5 % of the whole body, although the weight of digestive tract was only 16 % of the whole body weight [15].

Compared with other mollusks, species of the genus *Cobicula* seem to accumulate less MCs under the same environmental conditions. For instance, in a Japanese lake (Lake Biwa), MCs in the hepatopancreas of a snail (*Sinotaia histrica*) reached 3.2 μg/g dw, but no MCs were detected in that of *Corbicula sandai* [16]. In Lake Chaohu, MC contents in the hepatopancreas of the snail *Bellamya aeruginosa* (collected at the same time and the same site) varied between 1.06 and 7.42 μg g^{-1} dw, with an average of 4.14 μg g^{-1} dw [4]. In the present results, the bivalve *A. lanceolata* accumulated as much as 18.01 μg MCs g^{-1} dw in the hepatopancreas, whereas MC contents in the hepatopancreas of *C. fluminea* only varied between 0-5.18 μg g^{-1} dw, with an average of 1.76 μg g^{-1} dw.

To our knowledge, our study is the first to report the accumulation of MCs in oligochaetes and aquatic insect larvae. The high contents of MCs in the insect larvae suggest a great possibility for transfer of MCs to benthos-feeding omnivores like common carp, which is confirmed by the presence of high MCs contents in various organs of common carp [17]. The significant difference in MC content among mussels, oligochaetes and aquatic insect larvae might be related to their divergence in food habits or in physiology, which needs to be clarified in our future study.

WHO has established a provisional tolerable daily intake (TDI) of 0.04 μg kg^{-1} bw per day for MC-LR [2-3]. Since MCs are not broken down by cooking [17], and the *C. fluminea* are traditionally eaten as a whole in some Chinese foods, and if the average portion of *C. fluminea* eaten by a person is taken to be 300 g wet weight, 16.4 and 6.6 μg MC-LReq would be ingested daily for the mussels collected in August and October, respectively (Table 1), which are about 8.2 and 3.3 times the recommended TDI for MC-LR. In other words, 28.6 % of the collected *C. fluminea* were harmful for human consumption in Lake Chaohu. In Lake Taihu, during October 2003 and September 2004, the daily intakes from four bivalves (*Anodonta woodiana, Hyriopsis cumingii, Lamprotula leai* and *Cristaria plicata*) reached, respectively, 0.32, 0.94, 0.48 and 0.66 μg MC-LReq kg^{-1} bw (8, 23.5, 12 and 16.5 times the TDI value suggested by WHO), when the bivalves are eaten as a whole [14]. Apparently, bivalves from Lake Taihu are also very harmful for human consumption. It should be also noted that the local fishermen in Lake Chaohu are at high risk because they usually consume high amounts of mussels or other fisheries products and also use the MC-contaminated lake water at the same time. It is recommended that in Lake Chaohu, edible mussels should not be collected for human consumption during toxic cyanobacterial blooms and that more attention should be paid to the potential harmful effects of MCs on human health by multiple exposure routes through aquatic food, drinking water and swimming in lakes with toxic cyanobacterial blooms.

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