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Short communication

Risk assessment of microcystins in silver carp (*Hypophthalmichthys molitrix*) from eight eutrophic lakes in China

Dawen Zhang [a,b], Xuwei Deng [a], Ping Xie [a,*], Jun Chen [a], Longgen Guo [a]

[a] *Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China*

[b] *Institute for Quality & Safety and Standards of Agricultural Products Research, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, PR China*

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ABSTRACT

Bioaccumulation and risk assessment of microcystins (MCs) in muscle of silver carp (*Hypophthalmichthys molitrix*) from eight eutrophic lakes along the Yangtze River of China were examined by using liquid chromatography electrospray ionisation mass spectrometry. MCs contents in seston collected from these eutrophic lakes ranged from 0.02 to 21.7 μg/L. MCs concentrations in silver carp muscle samples varied from 0.014 to 0.036 μg/g DW with an average of 0.028 μg/g DW. The total length of silver carp showed a significant negative correlation with MCs concentrations in their muscle ($r = -0.85$, $p < 0.05$), suggesting that MCs accumulation in silver carp muscle seems to be size dependent. EDI values of MCs in fish muscle from these eight eutrophic lakes varied from 0.0027 to 0.0071 μg/kg day, which was much lower than the TDI value of 0.04 μg/kg day previously established by WHO, indicating that it is safe to consume silver carp muscle from eutrophic lakes in China.

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1. Introduction

Eutrophication accompanied with the frequent occurrence of cyanobacteria blooms in water bodies has been recognised as a worldwide problem (Codd, 1995). Many of the bloom-forming cyanobacteria are capable of producing a wide variety of toxins, including neurotoxins, hepatotoxins, cytotoxins and lipopolysaccharide endotoxins (Wiegand & Pflugmacher, 2005). Generally, the most common and toxic cyanotoxins in water bodies are microcystins (MCs; Chorus & Bartram, 1999). To date, more than 90 analogues of MCs have been identified (Ufelmann, Krüger, Luckas, & Schrenk, 2012). MCs are known to cause poisoning or death of fish, birds, domestic and wild animals (Carmichael, 2001), as well as illnesses and mortality in human (Azevedo et al., 2002).

Lake eutrophication has become a serious environmental problem in China (Jin, 2003). According to the survey of Jin (2003), more than 66% of lakes and reservoirs in China are eutrophic and toxic cyanobacterial blooms occur regularly in these freshwater ecosystems in the warm season every year. However, freshwater aquatic product is about 50% of the total amount of aquatic product and is one of the most important food sources in China. Unfortunately, extensive studies have confirmed that MCs can accumulate in the edible part of various aquatic animals and, thus, enter into the food chain (Gkelis, Lanaras, & Sivonen, 2006; Papadimitriou, Kagalou, & Leonardos, 2012; Peng et al., 2010; Xie et al., 2005; Zhang, Xie, Liu, Chen, & Liang, 2007; Zhang, Xie, Liu, & Qiu, 2009). While previous studies on MCs accumulation in fish in China was just limited to some large lakes (e.g., Lake Taihu, Chaohu and Dianchi) (Chen, Xie, Zhang, Ke, & Yang, 2006; Peng et al., 2010; Xie et al., 2005; Zhang et al., 2009) and there is little or no information on MC contamination in aquatic product from other Chinese eutrophic lakes.

Silver carp (*Hypophthalmichthys molitrix*) is one of the most important phytoplanktivorous fish in China and has been introduced worldwide for aquaculture, comprising as much as 12% of the total freshwater fish production of the world (FAO, 1991). This fish consumes phytoplankton directly as food and can be used for biological management of cyanobacteria blooms (Xie & Liu, 2001). In addition, MCs accumulation in muscle of silver carp have been confirmed under laboratory and field conditions (Chen et al., 2006; Xie et al., 2004, 2005; Zhang et al., 2009). Thus it is essential to study the accumulation levels of MCs in silver carp from different eutrophic lakes and evaluate the potential risk to human health.

The aims of the present study were to examine the accumulation levels of MCs in the muscle of silver carp collected from eight eutrophic lakes along the Yangtze River of China and evaluate the potential risks of MCs in silver carp to human health.

* Corresponding author. Address: Institute of Hydrobiology, Donghu South Road 7, Wuhan 430072, PR China. Tel./fax: +86 27 68780622.
E-mail address: xieping@ihb.ac.cn (P. Xie).

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2. Materials and methods

2.1. Study area

The eight shallow lakes are located in four provinces (Hubei, Hunan, Jiangsu and Anhui) in the middle and lower reaches of the Yangtze River. All these lakes are eutrophic or hypereutrophic and dense cyanobacteria blooms occur in the warm season every year in these lakes except Lake Donghu, where a large amount of phytoplanktivorous silver and bighead carp was stocked (Jin, 2003; Wang & Dou, 1998; Xie & Liu, 2001). The GPS coordinates of the studied lakes are in Table 1.

2.2. Sample preparation

Water samples were collected with Tygon tubing fitted with a one-way valve. To estimate intracellular toxins concentration in the water column, 1 L of the water sample was filtered through a Whatman glass–fibre filter ([GF/C], Whatman, Brentford, UK). Seston on the glass–fibre filter was used to detect intracellular toxins.

Silver carp (*H. molitrix*; body weight: 988.2 ± 372.9 g; body length: 35.76 ± 4.93 cm; total length: 44.25 ± 5.53 cm) were collected from these eight eutrophic lakes in July and August, 2008. Three silver carp were captured from each lake. The collected fish were measured, weighed and sacrificed immediately; fish muscle was dissected in the field and all samples were stored in iceboxes and transported to the laboratory, finally frozen at −20 °C immediately in the laboratory.

All samples were lyophilised in a Christ® Alpha 2–4 freeze dryer (Martin Christ, Osterode, Germany). These lyophilised samples were extracted and purified following the methods of Xie and Park (2007): the lyophilised samples (~0.1 g dry weight (DW) for each sample) were extracted three times with 5 mL of 0.01 M EDTA–Na_2 −5% acetic acid by sonicating for 3 min (30% amplitude, 60 W, 20 kHz; Sonics VC130PB, Newtown, CT) at 0 °C and then centrifuged at 14,000 rpm (BR4; Jouan, Winchester, VA) at 4 °C. The supernatant was first applied to an Oasis HLB cartridge (500 mg; Waters, Milford, MA), which had been preconditioned by washing with 10 mL 100% MeOH and 10 mL distiled water. The column containing sample was washed with 20 mL water followed by 20 mL 20% MeOH and then eluted with 20 mL 100% MeOH. The eluant collected was evaporated to dryness and the residue was dissolved in 100% MeOH. This solution was applied to a Sep-Pak silica gel cartridge (2 g; Waters) which had been preconditioned with 100% MeOH. The column was washed with 20 mL 100% MeOH and analytes then eluted with 20 mL of 70% MeOH. This fraction was evaporated to dryness and redissolved in 100 μL of the LC mobile phase and used for the final detection and identification of MCs by liquid chromatography–mass spectrometry (LC–MS).

2.3. Analysis of MCs

MCs in the seston of water column were analysed quantitatively according to Park and Lwami (1998). Qualitative and quantitive analysis of MCs (MC-RR, -YR and -LR) strictly followed the methods of Zhang et al. (2009). The limit of detection (LOD) of microcystins (MC-RR, -YR and -LR) was 0.005 μg/g DW.

2.4. Recovery experiment

Recovery experiments were carried out in quadruplicate, spiking 100 mg of freeze–dried fish muscle samples with MC-RR, -YR and -LR solution at 0.5 μg/ml. The extraction and analysis were performed as described above and the recovery and the relative standard deviation of the analytical method were calculated.

2.5. Risk assessment

Risk assessment in the present study was calculated using the tolerable daily intake (TDI) previously established by WHO (Chorus & Bartram, 1999). The Estimated Daily Intake (EDI; μg/kg body weight/day) was calculated using the following equation:

$$\mathrm{EDI} = \frac{C_{\mathrm{MC}} \times D_{\mathrm{intake}}}{\mathrm{bw}}$$

where C_{MC} = average MC-LR equivalents ($MC\text{-}LR_{equivalent}$) concentrations in fish muscle (μg/g ww), D_{intake} = daily fish consumption (300 g/day) and bw = average body weight (60 kg). As is described by Gupta, Pant, Vijayarghavan, and Rao (2003), the i.p. medium lethal dose (LD_{50}) in mice for MC-RR and -YR is about 5- and 2.5-fold that for MC-LR, corresponding to 0.2 and 0.4 MC-LR equivalents, respectively. Hence $MC\text{-}LR_{equivalent}$ = MC-LR + (MC-RR ∗ 0.2) + (MC-YR ∗ 0.4). In the present study, the average ratio of dry weight to wet weight was 0.20, so the coefficient of 0.20 was used to convert dry weight to wet weight of silver carp muscle.

2.6. Statistical analysis

Pearson correlation analysis was conducted to determine the relationships between MCs concentrations in silver carp muscle and in seston or the total length of silver carp, using SPSS for Windows (Ver 13.0; SPSS, Chicago, IL). The relationships were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Recovery experiment

The average recoveries from fish muscle were 66.5% (ranging from 59.3% to 73.4%), 82.8% (ranging from 78.4% to 87.6%) and 86.2% (ranging from 82.5% to 92.6%) for MC-RR, -YR and -LR, respectively. The relative standard deviations (RSDs) of MC-RR, -YR and -LR were 7%, 3% and 8%, respectively.

3.2. MCs in seston

MCs contents in seston from these eight lakes were demonstrated in Fig. 1. Due to the absence of seston samples in Lake Cha-

Table 1
GPS coordinates for eight eutrophic lakes along the Yangtze River area (cited from Wang & Dou, 1998).

Lake name	Longitude *E*	Latitude *N*	Area (km^2)	Mean depth (m)	Locations
Huanggai lake	113°30′–113°38′	29°39′–29°48′	86	4.2	Hunan Province
Bajiao lake	113°11′–113°15′	29°39′–29°31′	12.3	1.95	Hunan Province
Gehu lake	119°44′–119°53′	31°29′–31°42′	146.5	1.9	Jiangsu Province
Dianshan lake	120°53′–121°01′	31°04′–31°12′	62	2.1	Shanghai
Taihu lake	30°55′40″–31°32′58″	119°52′32″–120°36′10″	2427.8	1.9	Jiangsu Province
Donghu lake	114°21′–114°28′	30°31′–30°36′	33.7	2.8	Hubei Province
Wushan lake	115°31′–115°37′	29°53′–29°57′	16.1	3.1	Hubei Province
Chaohu lake	117°16′–117°5′	31°25–31°43′	769.55	2.69	Anhui Province

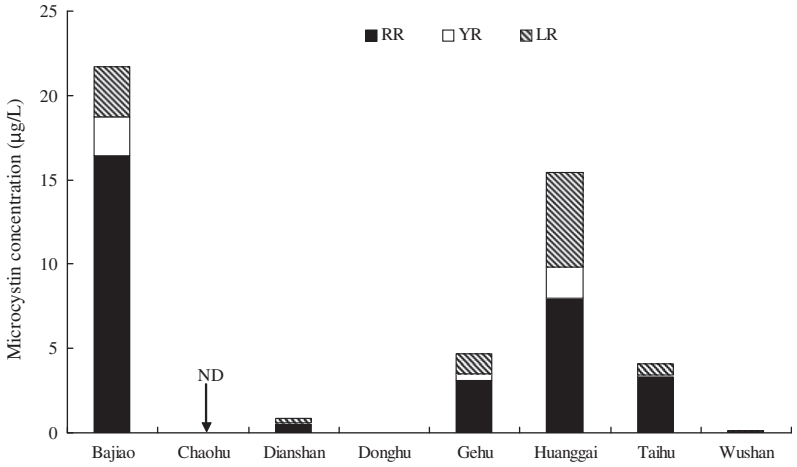

Fig. 1. MCs (MC-RR, -YR, -LR) concentrations in seston collected from eight eutrophic lakes along the Yangtze River, China. ND = not detected.

ohu, the MCs content in seston from Lake Chaohu was shown as ND (not detected). The MCs values in seston (intracellular MCs) varied considerably among lakes, ranging from 0.02 to 21.7 μg/L and the maximum was observed in Lake Bajiao (21.7 μg/L), followed by Lake Huanggai (15.4 μg/L), Lake Gehu (4.71 μg/L), Lake Taihu (4.11 μg/L), Lake Dianshan (0.84 μg/L), Lake Wushan (0.14 μg/L) and Lake Donghu (0.021 μg/L).

3.3. MCs in fish muscle

In the present study, MC-YR was just detected qualitatively in samples from Lake Dianshan and although MC-LR was detected qualitatively in all the samples from these eight lakes, all the values were below detectable level, only the MC-RR concentrations in all the silver carp muscle samples from these eight lakes were above the detectable level, suggesting that MC-RR is the most prominent MC in silver carp muscle. These results are in accordance with other studies (Lei et al., 2008; Nyakairu, Nagawa, & Mbabazi, 2010; Semyalo, Rohrlack, Naggawa, & Nyakairu, 2010; Xie et al., 2004, 2005; Zhang et al., 2009). The MC-RR in Nile tilapia muscle accounted for 70% of detected MCs in Lake Mburo and 52% in Lake Victoria, Uganda (Semyalo et al., 2010). Zhang et al. (2009) studied the accumulation of microcystins (MC-RR, -YR and -LR) in the muscle of six species of fish (*Neosalanx tangkahkeii taihuensis, Culter ilishaeformis*, *Carassius auratus*, *Cyprinus carpio, H. molitrix*) in Lake Taihu and found that only MC-RR was detected in muscle samples of all the fish except *C. carpio*. Lei et al. (2008) showed that only MC-RR was found in the muscle of *C. auratus* after intraperitoneal (i.p.) injection with MCs extract. In a sub-chronic toxicity experiment, no MC-LR was detectable in the muscle samples of silver carp (*H. molitrix*) in spite of the abundant presence of this toxin in the intestines and the maximum MC-RR in silver carp muscle was up to 1.77 μg/g DW (Xie et al., 2004).

Previous studies pointed out that the MCs content in fish varies widely not only within species but also within specimens of the same species (Vasconcelos, 1999) and the MCs variant and study area play important roles in MCs accumulation in fish muscle (Deblois, Aranda-Rodriguez, Giani, & Bird, 2008). In the present study, MCs concentrations in silver carp muscle samples varied from 0.014 to 0.036 μg/g DW with an average of 0.028 μg/g DW (Table 2). These results were in the same range of MCs levels in different species of fish muscle from various eutrophic water bodies (Deblois et al., 2008; Magalhaes et al., 2003; Romo, Fernandez, Ouahid, & Sola, 2012; Zhang et al., 2009), but lower (Gkelis et al., 2006; Papadimitriou et al., 2012; Peng et al., 2010; Xie et al., 2005), or higher (Amé et al., 2010; Nyakairu et al., 2010; Semyalo et al., 2010) than MCs contents previously found in fish muscle from other water bodies across the world.

In the present study, a significant negative correlation was observed between MCs concentrations in silver carp muscle and total length ($r = -0.85$, $p < 0.05$), suggesting that fish with lower total length accumulated higher amounts of MCs in comparison with fish of higher total length and MCs accumulation in silver carp muscle seems to be size dependent. The possible explanation should be that different total length of silver carp has different detoxification and/or excretion efficiency of MCs. A similar result was observed by Papadimitriou et al. (2012), who reported that significant negative correlations were found between MCs concentrations in all tissues and the total length of *Rutilus panosi* from Lake Pamvotis, Greece. However, Semyalo et al. (2010) reported that no significant correlation was found between MCs concentration in Nile tilapia muscle and the weight or length of fish in Lake Victoria and Lake Mburo, Uganda. These results indicate that the response of fish to MCs exposure is species-specific and that extensive studies on the response of fish to MCs under natural exposure are needed in the future.

In the present study, there was a significant negative correlation between MCs content in seston and in silver carp muscle ($r = -0.89$, $p < 0.01$). This result might be attributed to the influence of the total length of silver carp on MCs accumulation in fish muscle (Papadimitriou et al., 2012). In the present study, the total length of silver carp showed a significant negative correlation with MCs concentration in their muscle ($r = -0.85$, $p < 0.05$) and there was a positive correlation between total length of silver carp and MCs content in seston ($r = 0.78$), although statistically not significant ($p = 0.070$). Magalhaes et al. (2003) reported that there was a significant correlation between MCs concentration in seston samples and in the muscle of *Tilapia rendalli* collected from Sepetiba Bay of Brazil, while no significant correlation was found between MCs concentration in seston and in foot (muscle) of snail in Lake

Table 2
MC-RR concentrations (μg/g DW) in muscle of silver carp collected from eight lakes along the Yangtze River, China.

Bajiao	Chaohu	Dianshan	Donghu	Gehu	Huanggai	Taihu	Wushan	Mean
0.014 ± 0.009	0.028 ± 0.018	0.036 ± 0.006	0.027 ± 0.016	0.034 ± 0.015	0.024 ± 0.021	0.025 ± 0.014	0.034 ± 0.006	0.028 ± 0.007

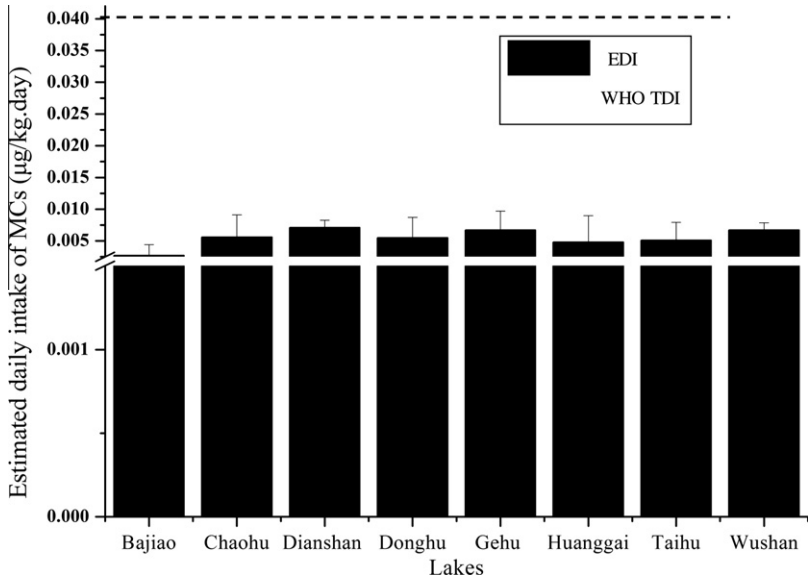

Fig. 2. Estimated daily intake of microcystins (EDI) by a 60-kg person consuming 300 g of muscle of silver carp collected from eight eutrophic lakes along the Yangtze River, China. TDI = tolerable daily intake (0.04 μg/kg day) proposed by the WHO (Chorus & Bartram, 1999). Data were expressed as the mean value and standard deviations for three replicate determinations.

Taihu of China (Zhang et al., 2007). Additionally, there was no significant correlation in MCs concentration between the intestine and muscle of silver carp (Chen et al., 2006) stocked in Lake Taihu, China.

3.4. Risk assessment

MCs are known to be potential risks to human health. To avoid potential health risk, the World Health Organization (WHO) established the tolerable daily intake (TDI) of 0.04 μg/kg body weight per day for MC-LR (Falconer et al., 1999). In the present study, the EDI values of MCs in fish muscle from these eight lakes varied from 0.0027 to 0.0071 μg/kg day, which was much lower than the TDI value of WHO (Fig. 2), indicating that it is safe to consume the muscle of silver carp from the eutrophic lakes in China.

4. Conclusions

Our results indicated that MC-RR is the most prominent MC in silver carp muscle and it is safe to consume silver carp muscle from eutrophic lakes in China, MCs accumulation in silver carp muscle seems to be size dependent.

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