

The dangers of microcystins in aquatic systems and progress of research into their detection and elimination¹

LIPING LIU ^{2*}, KANG LI ², YALING YUE ², JUN YAN ², YI YANG ² AND JAMES S. DIANA³

Microcystins (MC) are secondary metabolites of toxic cyanobacteria. The algae and metabolites often combine to cause strong discoloration of the water, accumulation at the surface in discrete scums and sometimes emit a strong odor (Figure 1, Figure 2A, Cai *et al.* 1997, Liang *et al.* 2001, Zurawell *et al.* 2005). MC belong to a family of extremely toxic compounds and are a health hazard to aquatic animals and even humans (Ding *et al.* 1998,1999, Falconer 1991, Hernandez *et al.*, 2000, Lawton *et al.* 1994). Researchers have identified blooms of cyanobacteria from eutrophic freshwater bodies in many parts of the world, and their occurrence can create a major water quality problem. For example, massive fish kills occasionally have been related to severe cyanobacterial blooms. Chronic damages, such as development of liver tumors may arise from long-term exposure to low concentrations of MC (Chen *et al.* 2006, Ding *et al.* 1998,1999, Ibelings and Chorus 2007, Lankoff *et al.* 2004, Li *et al.* 2007, Shen *et al.* 2003, Smith and Haney 2006, Zimba *et al.* 2006).

Characteristics of MC

Microcystins are cyanobacterium (blue-green algal) metabolites found world-wide in fresh, brackish and marine water environments. Cyanobacteria blooms occur especially in eutrophic freshwater bodies (Shen *et al.* 2003). It has been determined that MC belong to a family of extremely toxic compounds produced by species of freshwater cyanobacteria belonging to the genera *Microcystis*, *Anabaena*, *Nostoc* and *Oscillatoria* (Shen *et al.* 2003, Lankoff *et al.* 2004).

Microcystins are considered to be the most common and dangerous group of cyanotoxins. They possess a cyclic structure of the general composition where Adda, a special functional group in MC and an unusual amino acid, is responsible for the biological activity of the MC. So far, approximately 70 structurally different MC have been found, among which MC-LR is the most common and most toxic. The other compositions are MC-YR, MC-RR, MC-LF, and MC-LW. The abbreviations are: L-leucine, R-arginine, Y-tyrosine, F-benzodrine and W-tryptophan (Chen *et al.* 2006, Lawton *et al.* 1994, Sire'n *et al.* 1999).

Dangers of MC

Microcystins have led to mortalities in wild and domestic animals worldwide. They have been found to inhibit several

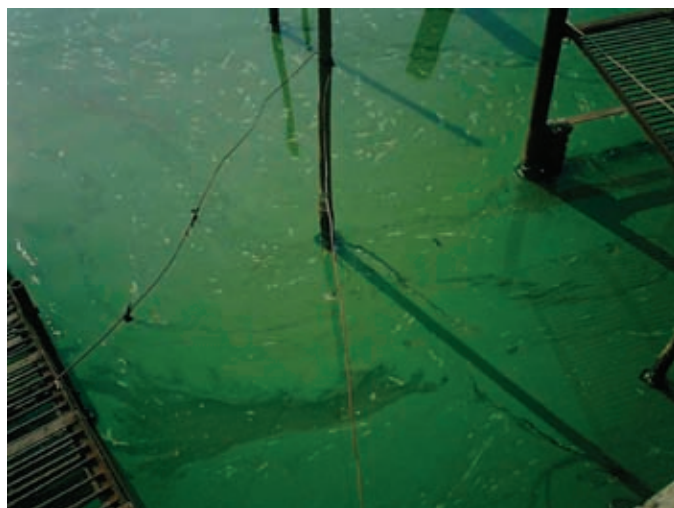


Fig. 1. *Microcystis aeruginosa* in a water system.

serine/threonine protein phosphatases including PP1 and PP2A, causing oxidative stress in many fishes (Mackintosh *et al.* 1990, Runnegar *et al.* 1993, Yoshizawa *et al.* 1990). Moreover, freshwater fishes as well as other aquatic organisms can not only be damaged by MC but the compounds can also bioaccumulate. Researchers have found MC can accumulate in certain species of freshwater mussels as well as in fish (Figure 3, Ibelings and Chorus 2007, Li *et al.* 2007).

MC have been associated with episodes of certain human diseases. When people consume contaminated water and or consume contaminated aquatic species, the toxins may cause such problems as nausea and liver damage (Chen *et al.* 2006; Ding *et al.* 1998, 1999; Lankoff *et al.* 2002; Hernandez *et al.* 2000; Roitt 1993). The World Health Organization has listed MC as drinking water contaminants which must be present at less than 1 mg/L for safe consumption. It has been reported that the incidence of primary liver cancer in some areas of China was related to the presence of MC in drinking water. Other studies have also suggested that vegetables can be another route for exposure to MC from using contaminated water for irrigation (Falconer 1994, Ibelings and Chorus 2007, Roitt 1993, Sire'n *et al.* 1997).



Fig. 2A. The contaminated water of Taihu.



Fig. 2B. The clear water of Taihu.



Fig. 3. *Carassias auratus* fed on *Micosystis aeruginosa*.

Research Progress on Detection and Elimination Methods

Over the past few years, several novel technologies have been explored to improve detection and identification of MC in biological and environmental samples (Table 1). HPLC (High Performance Liquid Chromatography) was used to detect MC.

At present, ELISA (Enzyme Linked Immunosorbent Assay) and LC/MS/MS (Liquid Chromatography/Mass Spectrum/Mass Spectrum) are two typical methods widely used for detecting MC. It has been found that ELISA is simpler and faster. In addition ELISA produces better recovery rates than HPLC for water samples. However, for trace concentrations of MC in water samples, LC/MS/MS can calculate the lower limit of detection (LLOD), as low as 1 ng/mL, which exhibits the best sensitivity. For fish flesh, both ELISA and LC/MS/MS have their own disadvantages, but LC/MS/MS is the most common analytical method for cyanobacterial toxin for processors and scientific research.

The efficiency of any technique for analyzing MC is influenced by the method used to extract the toxins from samples. Another important factor is the requirement for sample concentration and clean-up. More studies are focusing on improving detection efficiency. Recently, highly specific recognition molecules, such as antibodies and molecularly imprinted polymers have been used to preconcentrate trace levels of microcystins from water and fish flesh. This concentration of toxins not only aids in detection, but also has the potential to purify complex samples for subsequent analysis. New biosensor technologies are also becoming available, with sufficient sensitivity and specificity to enable rapid 'on-site' screening without the necessity for sample processing. However, the small number of available commercially purified MC variants relative to the high number of naturally occurring known MC variants limits the ability to quantify all MC present in a sample.

To minimize the risk of MC, it is important to control organic pollution of water systems. Fortunately, the government of China has realized the inherent danger of MC and has promulgated several laws to reduce water pollution. As a result, the blooms of cyanobacteria in Taihu Lake have been controlled effectively (Figure 2B). Furthermore we need to minimize the risk of human exposure to MC in water and food. To do this, we need a sensitive and reliable method to detect this class of toxin in food and water and eliminate it. Currently, some elimination methods have achieved positive results. Researchers have combined activated carbon and an ultrafiltration process to remove up to 95 percent of microcystin toxins in drinking water.

Fish, especially filter-feeding fish, and shellfish can accumulate and eliminate MC by themselves and the elimination rate is water temperature and time dependent (Jun Chen *et al.* 2007, Ronald *et al.* 2006; Zakaria *et al.* 2006). Different organs have different depuration rates (Smith and Haney 2006, Liqiang *et al.* 2004). It is useful but less effective to keep the contaminated fish and shellfish in MC-free water to depurate them (Ron-

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ald *et al* 2006, Mohamed and Hussein 2006, Soares 2004, Liqiang *et al.* 2004).

Conclusion

Microcystins are an important group of toxic compounds that are mainly produced by some cyanobacteria species. They have both acute and chronic toxic effects on aquatic animals in ponds, lakes and reservoirs. In addition, they threaten food safety and human health. At present, although various kinds of sensitive and reliable methods for detection of MC have been developed, the standardized methodology and MC standards necessary for comparing data across studies is still lacking (Tillmanns *et al.* 2007).

Notes

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²Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources of the Shanghai Ocean University and Ministry of Education, Shanghai Ocean University, Shanghai 201306, China

*Corresponding author. E-mail: lp-liu@shou.edu.cn

³School of Natural Resources and Environment, University of Michigan, Ann Arbor, Michigan 48109-1041 USA

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Table 1. Comparison of different detection methods for microcystins (McElhiney and Lawton 2005)

Method	Sensitivity (MCYST-LR)	Specificity for MCYSTS	Comments
Mouse Bioassay	LD50: 25- 150µg/kg	Non-specific	Requires animal license Being phased out in most countries
Protein phosphatase	Radiometric:0.1µg/L Colorimetric: 0.25-2.5µg/L Fluorometric:0.1µg/L	Non-specific	Radiometric assay requires specialized facilities Unable to distinguish between PPase inhibitors Purified enzyme can be expensive
Polyclonal antibodies	Anti-MCYST-LR:2.5µg/L Anti-Adda: 0.6µg/L	Specific	Dependent on laboratory animals Difficult to maintain a reproducible source
Monoclonal antibodies	Anti-MCYST-LR:0.1µg/L Anti-MCYST-LR:0.06µg/L	Very specific	Cross-reactivity depends on conjugation method Hybridoma techniques are labor intensive
Recombinant antibody fragments	4µg/L	Specific	Requires facilities for bacterial expression Sensitivity/cross reactivity can be modified
Molecularly imprinted polymers	Approx: 0.2µg/L	Very specific	Different polymers required for each variant Very stable; suitable for biosensor format
HPLC	No result	Non-specific	Sensitive detection and accurate identification, but expensive and time consuming
Thin Layer Chromatography (TLC)	Anti-MCYST-LR:10 ng	Non-specific	Simple and cost-effective
Artificial receptors	Anti-MCYST-LR: 0.1 µg /L	Very specific	Development sensors are extremely stable, capable of withstanding high temperatures, solvents and extremes of pH; but the polymer showed very poor cross-reactivity with microcystin-RR, microcystin-YR, and nodularin

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