

Note

Sorption of dissolved microcystin using lanthanum-modified bentonite clay

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INTRODUCTION

Microcystins (MCs) are a class of cyanobacterial toxins ubiquitous in fresh waters throughout the world (Codd et al. 2005). These toxins primarily accumulate in the liver and kidneys of exposed mammals and fish and can cause severe health risks to these organisms (Ueno et al. 1996, Briand et al. 2003). Acknowledging risks associated with MC exposures has spurred many agencies to set guidelines for drinking and recreational waters (WHO 2003, USEPA 2015, USEPA 2016). Guideline concentrations are based on total toxin (i.e., in the cell and in the water). Toxins housed intracellularly can accumulate in nearshore areas subsequently concentrating the toxin and increasing risks to humans and wildlife. Specific concerns regarding dissolved MCs has garnered enhanced regulatory scrutiny on managing toxic blooms, and restricted applied management initiatives due to potential release of toxins (Kenefick et al. 1993, Greenfield et al. 2014, USEPA 2018). Though dermal toxin sorption of dissolved toxin is an insignificant route of exposure for humans (USEPA 2016), and released MC often dilutes and biodegrades (Jones and Orr 1994, Lahti et al. 1997, Edwards et al. 2008), there is still concern associated with MC release. Some reasons often include difficulty to treat in drinking water systems and increased exposure routes to aquatic biota. The release of intracellular MCs following some chemical treatments has been documented, which often restricts approval of these approaches (Iwinski et al. 2016). However, depending on the growth stage of cyanobacteria, MCs may also be found extracellularly without treatment (Lahti et al. 1997, White et al. 2005, Lehman et al. 2013). In toxic cyanobacterial blooms, the release of accumulated toxin is often an unavoidable concern as the bloom naturally senesces (Chorus and Bartram 1999) or if exposed to natural stressors (ultraviolet light, salinity, physical damage; Ross et al. 2006). This natural toxin release can be of greater intensity than if it was managed at an earlier growth stage.

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Therefore, methods that mitigate dissolved toxin are important in managing risks associated with toxic cyanobacteria independent of, or in concert with, other management approaches.

Sediments have been found to adsorb MCs, though this adsorption is dependent on several characteristics, such as particle size, pH, and clay and organic matter content (Munusamy et al. 2012, Song et al. 2014). Many studies have documented the removal of MCs by association with the clay fraction of sediments (Miller et al. 2001, Mohamed et al. 2007, Chen et al. 2008, Maghsoudi et al. 2015). Chen et al. (2006) suggests interaction of nitrogen and oxygen atoms in MCs with metal ions on the clay surface as an avenue for adsorption. A common phosphorous (P) mitigation product, lanthanum-modified bentonite clay (LMB; Phoslock),¹ has been used throughout the world to bind phosphorus, alter nutrient ratios, and shift the algal assemblage toward less cyanobacteria (Spears et al. 2016, Bishop and Richardson 2018). LMB is a specifically designed and patented formulation to enhance water-column P reaction and removal with lanthanum while minimizing potential for offsite movement and risks related to dissolved lanthanum (Copetti et al. 2016). Prochazka et al. (2013) found bentonite clay to have the ability to remove MCs, and this comprises 95% of the LMB formulation. With increasing LMB additions, up to 100% adsorption and removal of water-soluble *Prymnesium parvum* Carter ichthyotoxins has been documented (Seger et al. 2015). However, little work has been done regarding cyanobacterial toxins and direct interaction with LMB. The overall goal of this work was to evaluate the ability of LMB to adsorb and remove dissolved MC-LR (the most abundant MC) from the aqueous phase. Specific objectives of this research were to measure 1) dissolved MC-LR removal extent with differing LMB amendments and 2) capacity of LMB to remove MC-LR from the water column.

MATERIALS AND METHODS

High-performance liquid chromatography-grade MC-LR toxin standards and enzyme-linked immunosorbent assay (ELISA) kit² were purchased from Abraxis. In order to test the effects of LMB on dissolved MC-LR, an MC-LR stock solution (10 $\mu\text{g ml}^{-1}$ in methanol) was dissolved into sterilized tubes containing deionized water and diluted to 50, 100, and 500 ppb in triplicate. A stock solution of LMB (1 g L⁻¹) was made into a slurry to mimic field application

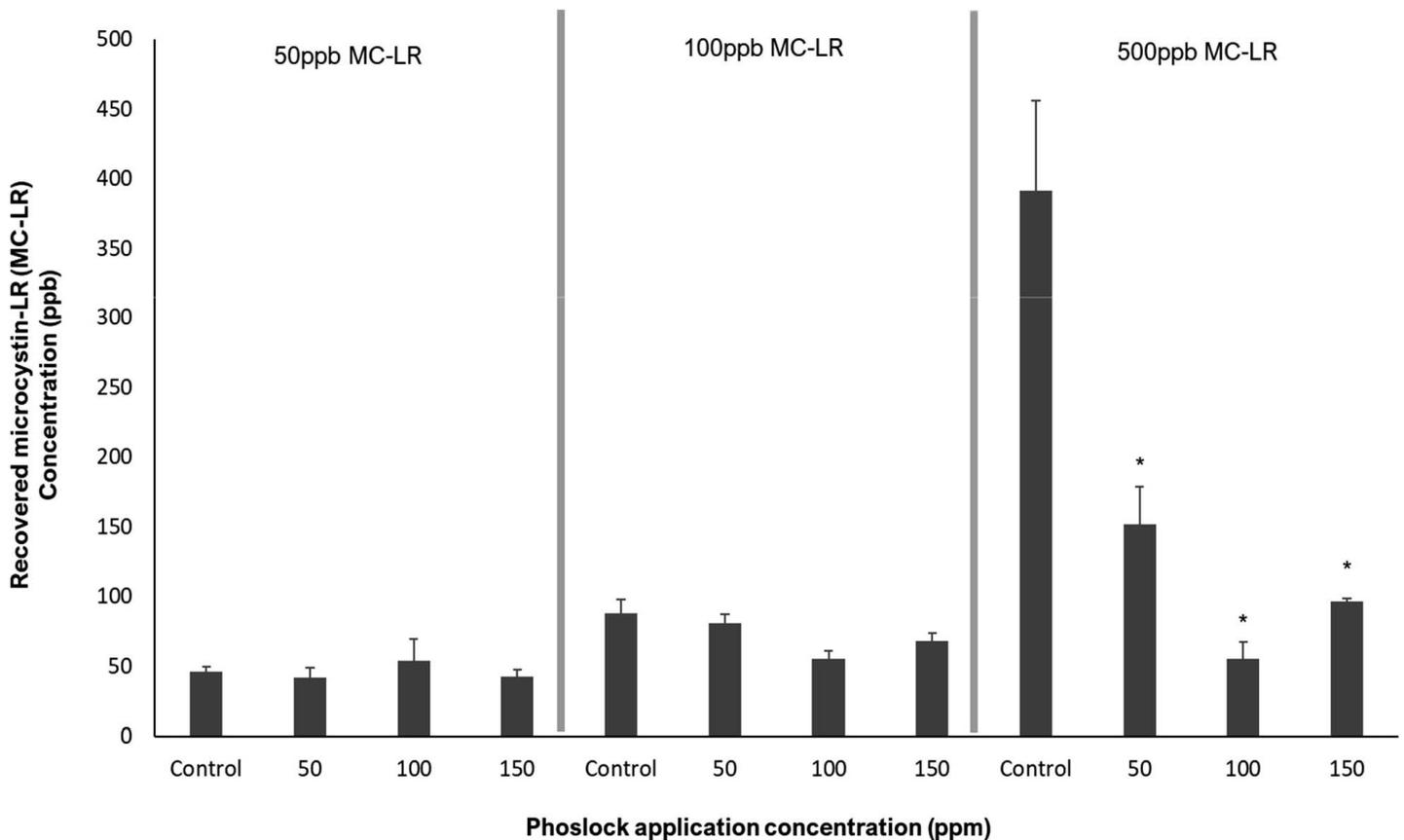


Figure 1. Graph indicating the effects of lanthanum-modified bentonite clay application rates (0, 50, 100, and 150 ppm) on recovered microcystin-LR concentrations (50, 100, and 500 ppb) 48 h post application. *Denotes significant values from relative control at $\alpha = 0.05$.

methods, using sterilized deionized water and kept homogenized on a magnetic stirrer, and from the stock applied into triplicate tubes containing MC-LR (targeting 50, 100, and 150 ppm of LMB); the control tubes contained MC-LR concentrations but no LMB. Concentrations of LMB selected represent common operational application amounts to target water-column and/or sediment P inactivation (Bishop and Richardson 2018). The tubes were sealed with Parafilm, then vortexed and inverted to homogenize the contents. The tubes were incubated at room temperature for a 48-h period in the dark. After incubation, a 50- μ l aliquot of each treatment was removed from the tubes carefully without disturbing the contents and diluted (10 \times , 20 \times , and 100 \times , respectively) with sterile deionized water to reach quantifiable MC-LR concentrations for ELISA before quantification. Microcystin concentration (ppb) was extrapolated using a MC-LR-based standard curve ($r^2 = 0.99$). To test for the possible natural sedimentation or settling of the toxin, the control replicate tubes with MC-LR concentration (100 ppb) were vortexed and tested for MC-LR concentrations. No background sedimentation or settling of MC-LR was observed during this experiment. In order to determine the effects of LMB on dissolved MC-LR, a one-way analysis of variance with a *post-hoc* Tukey analysis was applied to discern differences between the control without LMB and the treatments with increasing LMB concentrations ($P < 0.05$).

RESULTS AND DISCUSSION

After the incubation period, we observed a natural decrease in MC-LR in comparison to the inoculated concentrations (Figure 1); however the treatments with lowest MC-LR concentration (50 ppb) remained the lowest and those with the highest (500 ppb) remained highest. This decrease in MC-LR indicates that there was natural degradation to a certain degree. Several possible sources for toxin degradation could be the exposure to light during inoculation and analysis, the use of plastic pipette tips that possibly adsorb the compound, and/or the use of deionized water without buffering solutes.

The application of LMB reduced MC-LR concentrations from the water column, though the effect on reduction differed depending on initial MC-LR concentration. LMB did not significantly decrease MC-LR at the lowest (50 and 100 ppb) concentrations from the water column. However, there was a significant decrease at the highest MC-LR concentration of 500 ppb. Figure 1 demonstrates that as the application rate of LMB increases, the concentration of MC-LR in the water significantly decreased. The application of LMB (50, 100, and 150 ppm) decreased the MC-LR concentrations by 61.2, 86.0, 75.4% relative the controls, respectively. All LMB concentrations significantly reduced dissolved MC-LR at 500 ppb concentration ($P = 0.002$, $\alpha = 0.05$). The most significant effect occurred in the treatments

containing the highest concentration of MC-LR (500 ppb) with 100 ppm of LMB ($P = 0.006$, $\alpha = 0.05$). There was no significant difference in reduction of MC-LR among the different LMB concentrations.

The most efficient removal of MC-LR from the water occurred at high MC-LR concentrations, regardless of LMB application rates. Comparatively, application of LMB at any rate only reduced MC-LR by up to 37.05% when the MC-LR concentration was 100 ppb or lower. These results indicate that there is a significant interaction between LMB application and the concentration of dissolved MC-LR, and that LMB can be applied in the removal of MC-LR when concentrations are relevant.

Fear of toxin release often restricts implementation of reactive management such as an algaecide treatment. However, chronic natural toxin release and potential large pulse releases with bloom senescence suggest that water resource managers concerned with free toxin will need to address this regardless of other management. This research provides information regarding a commercially available and highly studied technology commonly used in surface waters throughout the world. Though specifically designed for P mitigation, a periphery benefit of this LMB formulation was found to sorb dissolved MC. This was more efficient at higher MC levels as may be observed in large bloom accumulations, which often pose increased risks to humans and wildlife. Ultimate transfer of toxins out of the water column is predicted to have less exposure risks to organisms such as fish and zooplankton in the water and on external organisms, such as livestock. Additionally, decreased exposure risks to humans is predicted as fewer toxins can enter irrigation or potable water systems or become aerosolized. Persistence of toxin in sediments is possible, though many sediment-associated organisms have been found to biodegrade the toxin, with degradation rates up to > 80% within 1 d in anoxic and nitrate-amended sediments (Holst et al. 2003, Manage et al. 2009).

This study demonstrates that there is an effect of LMB application on the concentration of MC-LR. Although the effect of LMB was not significant on MC-LR at lower concentrations (50 and 100 ppb), it was demonstrated that with higher concentrations of MC-LR (500 ppb), regardless of LMB concentration, MC-LR can be scrubbed from the water column. This is especially important to note, as field concentrations of MCs may reach levels far beyond those tested here, especially in highly accumulated surface scums (Chorus and Bartram 1999).

Future trials are aimed at using higher concentrations of both MC-LR and LMB and testing *in situ*. These experiments can reflect the naturally occurring and diverse toxin levels in the field, and further elucidate the effects of LMB on sorbing MC-LR and other toxic bioactive compounds. Additional work is needed to clarify the affinity of LMB among cyanobacterial cells, toxins, and/or P.

SOURCES OF MATERIALS

¹Phoslock® SePRO Corporation 11550 North Meridian St. Suite 600, Carmel, IN 46032 (Phoslock is a registered trademark of Phoslock Environmental Technologies, Ltd.).

²Microcystins/Nodularins enzyme-linked immunosorbent assay (ELISA) Kit, PN 520011, Abraxis, Inc. Warminster, PA 18974.

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