Environmental Pollution 231 (2017) 134–142

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Effects of microcystins contamination on soil enzyme activities and microbial community in two typical lakeside soils☆

Qing Cao [a, b], Alan D. Steinman [c], Xiaomei Su [a, b], Liqiang Xie [a, *]

[a] State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, China

[b] University of Chinese Academy of Sciences, Beijing 100049, China

[c] Annis Water Resources Institute, Grand Valley State University, 740 West Shoreline Drive, Muskegon, MI 49441, USA

A R T I C L E I N F O

Article history:
Received 2 March 2017
Received in revised form
2 August 2017
Accepted 3 August 2017
Available online 8 August 2017

Keywords:
Microcystins
Enzyme activities
Biolog Ecoplates
Ammonia-oxidizing bacteria
Soil quality

A B S T R A C T

A 30-day indoor incubation experiment was conducted to investigate the effects of different concentrations of microcystin (1, 10, 100 and 1000 μg eq. MC-LR L^{-1}) on soil enzyme activity, soil respiration, physiological profiles, potential nitrification, and microbial abundance (total bacteria, total fungi, ammonia-oxidizing bacteria and archaea) in two lakeside soils in China (Soil A from the lakeside of Lake Poyanghu at Jiujiang; Soil B from the lakeside of Lake Taihu at Suzhou). Of the enzymes tested, only phenol oxidase activity was negatively affected by microcystin application. In contrast, dehydrogenase activity was stimulated in the 1000 μg treatment, and a stimulatory effect also occurred with soil respiration in contaminated soil. The metabolic profiles of the microbial communities indicated that overall carbon metabolic activity in the soils treated with high microcystin concentrations was inhibited, and high concentrations of microcystin also led to different patterns of potential carbon utilization. High microcystin concentrations (100, 1000 μg eq. MC-LR L^{-1} in Soil A; 10, 100 1000 μg eq. MC-LR L^{-1} in Soil B) significantly decreased soil potential nitrification rate. Furthermore, the decrease in soil potential nitrification rate was positively correlated with the decrease of the amoA gene abundance, which corresponds to the ammonia-oxidizing bacterial community. We conclude that application of microcystin-enriched irrigation water can significantly impact soil microbial community structure and function.

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1. Introduction

In eutrophic waters, the monocyclic heptapeptides microcystins (MCs) are produced worldwide by several cyanobacterial genera, mainly *Microcystis* sp. and *Anabaena*. More than 100 different structural analogues of MCs, with a range of molecular weights from 900 to 1100 Da, have been identified from cyanobacterial blooms and cultures (Rantala et al., 2004; Zastepa et al., 2015). Microcystin-LR is found to be the most common and potent analogue, followed by microcystin-RR and microcystin-YR (Chen et al., 2016). With the increasingly severe environmental problems caused by cyanobacteria blooms, more attention is being paid to impacts caused by MCs. MCs can be brought into contact with soils by irrigation source water that contains cyanobacteria; in addition, cyanobacterial blooms can be applied directly to soil as organic fertilizer after being removed from lakes to reduce contamination (Liu et al., 2008; Saqrane et al., 2009; Chen et al., 2010, 2012). Once toxic cyanobacteria come in contact with soil, MCs can be released and infiltrate from the soil surface to deeper layers. Therefore, there is a possibility that soils adjacent to lakes and reservoirs may be contaminated by MCs. Although a prior study (Chen et al., 2006a) showed that the half-life of MCs in soils is relatively short compared to other organic pollutants (e.g., 6.0–17.1 days for MC-LR, compared to 49–610 days for the compound tebuconazole (Strickland et al., 2004).), regions receiving repeated applications of cyanobacterial-contaminated irrigation water may be threatened with microcystin exposure.

Previous studies of MCs in soils mainly focused on terrestrial vegetation. Some studies have shown that MCs can adversely affect plants. Plant seed germination and root growth inhibition, lateral roots reduction, leaf necrosis and alteration of antioxidant systems were observed after exposure to MCs (Chen et al., 2010; Pichardo and Pflugmacher, 2011; Chen et al., 2012; Azevedo et al., 2014).

☆ This paper has been recommended for acceptance by Klaus Kummerer.

* Corresponding author.
E-mail address: lqxie@niglas.ac.cn (L. Xie).

http://dx.doi.org/10.1016/j.envpol.2017.08.013
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Accumulation of MCs in plants has also been reported (Saqrane et al., 2009; Gutiérrez-Praena et al., 2014; Corbel et al., 2016; Cordeiro-Araújo et al., 2016). However, the impact of MCs on the soil biota itself has received much less attention. Early studies showed an application of both cyanobacterial extracts and pure microcystins had an inhibitory effect on the growth of cultured bacteria (*E. coli* and *Streptoverticillium* sp.) (Valdor and Aboal, 2007). During culture, cyanobacteria can release antibacterial substances (Oufdou et al., 1998; Ostensvik et al., 1998). The permeability of cell membranes was affected by MCs and damage was caused after macromolecular compounds entered the cell (Dixon et al., 2004). These studies indicate that MCs can pose a potential threat to soil health, but additional studies are needed to assess MCs' impact on soil microbial structure and function.

Soil microorganisms, important regulators in soil processes, are vital to nutrient cycling and structure formation (Liu et al., 2016). The activity of soil microbial communities and soil microbial diversity are very useful in predicting the impact of contaminants on soil health (Mijangos et al., 2006, 2009). Microbial indicators including soil respiration, potential nitrification, enzymatic activity and community-level physiological profiles, are commonly used to assess the impact of organic contaminants on soil quality (Winding et al., 2005). Moreover, the gene abundance of important functional groups in soil could serve as a robust indicator of the impact of contaminants on the soil microbial community (Chen et al., 2015).

Cyanobacterial blooms have become more frequent in recent years in many large freshwater lakes and reservoirs in China, especially Lake Taihu (Qin et al., 2007). Consequently, it is suspected that farmland regions adjacent to large lakes in China have become contaminated by irrigation water containing cyanobacteria blooms. Therefore, the aim of the present study was to assess the effect of MCs on soil microorganisms in two specific areas of China. We hypothesized that application of MCs-contaminated irrigation water will induce shifts in soil microbial activity, community and functionality structure. To test this hypothesis, we evaluated the effect of different concentrations of MC on: soil respiration and microbial enzymatic activities (dehydrogenase, urease, β-glucosidase, phenol oxidase, acid phosphatase, alkaline phosphatase); physiological diversity (BIOLOG system); soil potential nitrification; and microbial biomass (qPCR).

2. Materials and methods

2.1. Soil collection and characterization

Two kinds of soils (0–15 cm) were collected from the lakeside of Lake Poyanghu at Jiujiang (Soil A) and Lake Taihu at Suzhou (Soil B); neither soil had any known history of MCs application. Some of their physicochemical properties are as follows: Soil A: 56.2% sand, 27.3% silt, 16.6% clay, pH 6.15, 16.2 g organic matter kg^{-1} soil, 1.10 g total nitrogen kg^{-1} soil, 52.8 mg available P kg^{-1} soil, 52.5 mg available K kg^{-1} soil; Soil B: 27.9% sand, 31.0% silt, 41.2% clay, pH 7.46, 9.91 g organic matter kg^{-1} soil, 0.78 g total nitrogen kg^{-1} soil, 15.4 mg available P kg^{-1} soil, 150 mg available K kg^{-1} soil. After being taken back to the laboratory, the soil samples were homogenized and passed through a 2-mm sieve to sift out roots and other large debris. The soil samples were used for physico-chemical analysis after air-dried at 25 °C in 48 h (Sparks et al., 1996). The remainders were kept fresh at 4 °C for incubation experiments. To allow for temperature days adaptation of soil microbes, the soil was pre-incubated at 22 °C for 7 days before initiating the experiment (Petersen and Klug, 1994).

2.2. Extraction, purification and analysis of microcystin variants

In the present study, we used a natural cyanobacterial bloom (mainly *Microcystis aeruginosa*) extract to mimic natural toxic conditions with multiple MCs. The method of extraction and purification was modified from Harada et al. (1988). Cyanobacterial cells (~1 g DW) were homogenized with 25 mL of 5% (v/v) aqueous acetic acid after freeze-drying. The homogenate was subjected to an ultrasonic bath for 5 min, and then centrifuged at 10,000 r min^{-1} at 4 °C for 15 min. After re-extracting the residue twice more, all the supernatant was collected and then applied to 5 g of Oasis HLB extraction cartridge (Waters). The column with toxin was rinsed with 50 mL of 5% (v/v) aqueous methanol. Subsequently, the column was eluted with 100 mL 100% aqueous methanol. The eluant was evaporated to dryness, and then deionized water was used to dissolve the toxin. Combined toxin-containing solutions were stored at −40 °C before use. Following Corbel et al. (2015b), microcystin variants were analyzed by ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC/MS), using a Waters Acquity UPLC system coupled to a triple-quadruple mass spectrometer (TQD, Waters, France) via an electrospray ionization (ESI) interface. The MC variants were identified by their relative LC-retention times, as well as by their pseudo-molecular and fragment ions. The evaluation of MC percentages was performed using the ion current of the sum of major ions. MC-LR, MC-YR and MC-RR were found in the extract after the analysis. Total MCs accounted for 41% of all the extracts. The toxin extract was diluted as needed for subsequent use.

2.3. Experimental design

A 30-day incubation study was conducted. Fifteen glass beakers (500 mL) were prepared, and each of them was filled with 300 g pre-incubated soil to create a set of five treatments each with three replicates. Daily dosing (mimic of irrigation) with deionized water containing different concentrations of MC (0 [control], 1, 10, 100 and 1000 μg eq. MC-LR L^{-1}, which corresponded to treatments of control, T1, T10, T00 and T1000, respectively) was conducted. During the experiment, the total MCs amount added in each treatment was 0, 0.5, 5, 50 and 500 μg eq. MC-LR kg^{-1} dry soil. An equivalent amount of deionized water only was added to the control treatment. The moisture content of soil was brought to 60% of the maximum water holding capacity (WHC). Soil samples were incubated at 22 ± 1 °C for 30 d in the dark. To avoid excessive water evaporation from soil, beakers were covered with porous plastic film. After 30 d of exposure, the soil was collected from each glass beaker and homogenized, and fresh aliquots were immediately used for enzymatic activities, potential nitrification and biolog determinations; the remaining soils for MC content analysis, nucleic acid extraction and analysis were stored at −80 °C.

2.4. Analysis of MCs in soils

The soil was lyophilized after collection at the end of the incubation. MCs in soils were determined as described by Chen et al. (2006b). Briefly, lyophilized soil samples were extracted 3 times with 30 mL of 0.1 M EDTA-0.1 M $Na_4P_2O_7$ with a 10 min ultrasonic bath after being ground with a mortar. The homogenate was then centrifuged at 4000 g for 10 min. After modifying the pH of the supernatant to 3 with TFA, the solution was centrifuged again under the same conditions. The aqueous extractions were then applied to a Sep-pak ODS cartridge (500 mg, 6 mL, Waters). The cartridge with toxin was rinsed with 15 mL of 20% (v/v) aqueous methanol, and eluted with 10 mL 90% aqueous methanol. The eluant was evaporated to dryness and the residue was dissolved in

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