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Optimization of an effective extraction procedure for the analysis of microcystins in soils and lake sediments

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Efficiency of extraction of microcystins from soil and sediment was greatly increased.

Abstract

Microcystin analysis in sediments and soils is considered very difficult due to low recovery for extraction. This is the primary limiting factor for understanding the fate of toxins in the interface between water and sediment in both the aquatic ecosystem as well as in soils. In the present study, a wide range of extraction solvents were evaluated over a wide range of pH, extraction approaches and equilibration time to optimize an effective extraction procedure for the analysis of microcystins in soils and lake sediments. The number of extractions required and acids in extraction solutions were also studied. In this procedure, EDTA–sodium pyrophosphate solution was selected as an extraction solvent based on the adsorption mechanism study. The optimized procedure proved to be highly efficient and achieved over 90% recovery. Finally, the developed procedure was applied to field soil and sediment sample collected from Chinese lakes during bloom seasons and microcystins were determined in six of ten samples.

Keywords: Microcystin; Extraction method; Soils; Lake sediments; EDTA; Sodium pyrophosphate; Optimization

1. Introduction

Microcystins are a family of hepatotoxins produced mainly by several species of commonly occurring cyanobacteria such as *Microcystis*, *Anabaena* and *Oscillatoria* (Carmichael, 1992, 1994; Aguete et al., 2003; de Figueiredo et al., 2004). These hepatotoxins were involved in many cases of animal poisoning and human health problem in several countries worldwide (Codd et al., 1997; Fastner et al., 1998; Yu, 1989; Sivonen and Jones, 1999). In China, many freshwater lakes and reservoirs are contaminated with heavy cyanobacterial blooms during warm seasons, and most of the blooms produce a high concentration of microcystins (Li et al., 2001; Song et al., 1998), including microcystin-RR (RR), microcystin-LR (LR), and [Dha^7]microcystin-LR ([Dha^7]LR). Bloom biomass harvesting is one of the most commonly used strategies for bloom control. In many developing countries, harvested toxic algal biomass may be directly discharged as organic fertilizer and thus pose a potential threat for groundwater contamination.

In order to evaluate the environmental risk of microcystin and assess its public health implications, it is important to trace the fate of microcystins in the aquatic environment (Sivonen and Jones, 1999; Tsuji et al., 2001). There have already been a number of studies on the fate of microcystins in water columns and aquatic organisms (Lahti et al., 1997; Pflugmacher et al., 1998; Ishii et al., 2004). However, only a few studies on the fate of microcystins in lake sediment and soils are available (Tsuji et al., 2001; Holst et al., 2003). In fact, it is imperative to broaden our knowledge on the fate and moving behavior of toxins in the interface between water and sediment in both the aquatic environment as well as in soils (Tsuji et al., 2001). However, this learning progress

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has been impeded due to lack of an efficient analytic procedure for toxin determination in sediments and soils.

Microcystin is readily absorbed by sediments and soils (Morris et al., 2000; Miller et al., 2001), but the adsorption mechanism is still unknown (Rapala et al., 1994; Morris et al., 2000; Tsuji et al., 2001). Conventional solvents for toxin extraction such as methanol and 5% acetic acid solution (Barco et al., 2005) have proved to be ineffective. Currently, the MMPB (2-methyl-3-methoxy-4-phenylbutyric acid) method is widely considered as the only effective analytical procedure (Tsuji et al., 2001). This method converts microcystin to MMPB first and then to MMPB ester for indirect detection of total toxins (Kaya and Sano, 1999; Tsuji et al., 2001). As a result, toxin structures are destroyed and cannot be analyzed by conventional HPLC, PPIA (protein phosphatase inhibition assay) and ELISA methods. In other words, GC/MS is the only choice. Furthermore, in most cases, especially in the case of toxicological studies, both total toxin concentration and individual concentration of microcystin variants are required. Thus, the development of an effective, rapid, and inexpensive procedure for toxin determination in sediments and soils is urgently needed.

This present paper is primarily focused on the identification of an effective extraction solvent and the establishment of optimal extraction conditions for determining toxins in sediment and soil samples.

2. Materials and methods

2.1. Toxin and other reagents

Microcystin-RR, LR, and [Dha^7]LR were isolated from a laboratory mass culture of *Microcystis aeruginosa* PCC 7806 and cyanobacterial blooms collected from Lake Dianchi using an improved Ramanan method (Ramanan et al., 2000; Chen et al., 2004). In this method, the extraction of *Microcystis* cells was sequentially applied to an ODS column, preparative HPLC (Waters 590), thin-layer chromatogram (TLC), and Sep-pak plus PS-2 cartridges. The content of purified microcystins was over 90% and determined by HPLC-DAD, LC/MS, and HPTLC (Pelander et al., 2000; Chen et al., 2004). ODS and PS-2 Sep-pak cartridges were manufactured by Waters (Part No. Wat051910 and JJAN20131). Microcystin standards for HPLC analysis were obtained from Kanto Reagents, Japan. ODS silica gel and TLC plates were purchased from Merck.

2.2. Soils and sediments

Soils and sediments for the recovery experiments were collected from GuanQiao field experimental ponds in Lake Donghu, and their properties are listed in Table 1. Sediment samples for toxin analysis were collected both from GuanQiao ponds (E-3 and J-4) and Lake Taihu during the bloom period. Soil samples for toxin analysis were from the shore of Lake Taihu. All soil and sediment samples used in the experiments were lyophilized by a freeze dryer (Yamato, Japan) and stored at −20 °C before use.

2.3. Toxin analysis

Identification and quantification of microcystins were performed by HPLC using a Shimadzu LC-10A system with two LC-10A pumps and a UV detector, and also a Shimadzu shim-pack (CLO-ODS 6.0 × 150) column in a run with 57% of solution A (100% methanol) and 43% solution B (0.05 mol/L KH_2PO_4, pH 3) over 25 min. Total flow was 1 ml/min. Column temperature was maintained at 40 °C and injection volume was 10 μl (Lawton et al., 1994; Chen et al, 2004).

Table 1
Physical and chemical properties of the soil and sediment employed in this study

Items	Soil	Sediment
Organic matter (g/kg)	22.31	55.82
pH (1:5 H_2O)	6.6	7.8
Particle size fraction (g/kg)		
Sand	325	705
Slit	423	255
Clay	252	40

2.4. Preparation of soil and sediment sample

In a preliminary test, from which the results are not presented here, sorption kinetics of microcystins in soil and sediment were studied. There were minor differences between the amounts adsorbed after 4 and 24 h, and thus, 24 h was determined to be a sufficient equilibration time. In addition, the added toxins were recovered using acidified Methanol (0.1% TFA, v/v) after equilibration for 0, 4 and 24 h to optimize the equilibration time. Based on the above results, all samples used in the recovery experiments were prepared as following. Ten micrograms of microcystin-RR, LR and [Dha^7]LR were added to 2 g of lyophilized soil and sediment, respectively. Each sample was added with 10 ml Milli-Q (Millipore, UK) water. After stirring using a magnetic stirrer, these samples were set aside for 24 h at 4 °C to facilitate adequate adsorption of microcystins before lyophilization by a freeze dryer. Freeze-dried soil and sediment samples were extracted under different conditions in order to determine the optimal condition for toxin extraction.

2.5. Optimization of extraction conditions

2.5.1. Optimization of the extraction solvent

Freeze-dried soil and sediment samples were extracted three times with 25 ml of solvents for 10 min under ultrasonication (400 W) at 0 °C. Experiments were performed in triplicate. The solvents evaluated were 0.1 M EDTA−0.1 M $Na_4P_2O_7$, 5% acetic acid, methanol, acidified methanol (0.1% TFA, v/v) and EDTA (0.1 M)−methanol (6:4, v/v). After centrifugation at 4000 × *g*, the aqueous extractions were passed through a Sep-pak ODS cartridge (500 mg, 6 ml, Waters), and the eluted solution was evaporated and analyzed by HPLC.

2.5.2. Optimization of the extraction pH

0.1 M EDTA−0.1 M $Na_4P_2O_7$ at different pH values, ranging from pH 1 to 13, were used following the experimental procedure detailed above in order to optimize the extraction pH.

2.5.3. Optimization of the acid in the extraction solvent

Freeze-dried soil and sediment samples were extracted with 0.1 M EDTA−0.1 M $Na_4P_2O_7$ under ultrasonication. To optimize the acid in the extraction solvent, HCl and TFA were added to the extraction system to adjust the extraction pH to ∼3.

2.5.4. Optimization of the extraction approaches

Freeze-dried soil and sediment samples were extracted three times with 30 ml of 0.1 M EDTA−0.1 M $Na_4P_2O_7$ as detailed above under different procedures. The approaches evaluated were Ultrasonication, for 10 min, Stir with magnetic stirrer for 20 min, Horizontal shaking for 2 h and column leaching.

2.5.5. Number of extractions

Freeze-dried soil and sediment samples were extracted three times by sonication for 10 min with 25 ml 0.1 M EDTA−0.1 M $Na_4P_2O_7$ (pH ∼3, acidified with TFA). Each experiment was carried out in triplicate. Extracts from each extraction step were analyzed separately.