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Analysis of intracellular and extracellular microcystin variants in sediments and pore waters by accelerated solvent extraction and high performance liquid chromatography-tandem mass spectrometry

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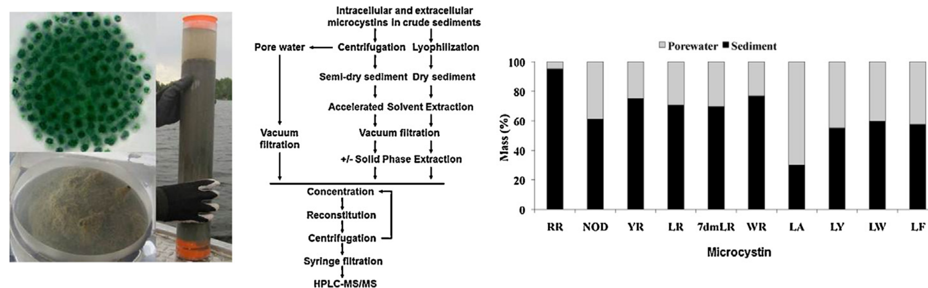
HIGHLIGHTS

- First analytical method for intracellular microcystins (MCs) in sediment.
- Includes a suite of variants (LR, ^{7dm}LR, RR, YR, WR, LA, LF, LY, LW) and nodularin.
- Reports the first measurements of MCs in sediment pore waters.
- MCs detected in >100 year old lake sediments suggesting long-term preservation.
- Sediment-pore water distribution (K_d) differed between variants suggesting differences in environmental fate.

GRAPHICAL ABSTRACT



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ABSTRACT

The fate and persistence of microcystin cyanotoxins in aquatic ecosystems remains poorly understood in part due to the lack of analytical methods for microcystins in sediments. Existing methods have been limited to the extraction of a few extracellular microcystins of similar chemistry. We developed a single analytical method, consisting of accelerated solvent extraction, hydrophilic–lipophilic balance solid phase extraction, and reversed phase high performance liquid chromatography-tandem mass spectrometry, suitable for the extraction and quantitation of both intracellular and extracellular cyanotoxins in sediments as well as pore waters. Recoveries of nine microcystins, representing the chemical diversity of microcystins, and nodularin (a marine analogue) ranged between 75 and 98% with one, microcystin-RR (MC-RR), at 50%. Chromatographic separation of these analytes was achieved within 7.5 min and the method detection limits were between 1.1 and 2.5 ng g^{-1} dry weight (dw). The robustness of the method was demonstrated on sediment cores collected from seven Canadian lakes of diverse geography and trophic states. Individual microcystin variants reached a maximum concentration of 829 ng g^{-1} dw on sediment particles and 132 ng mL^{-1} in pore waters and could be detected in sediments as deep as 41 cm (>100 years in age). MC-LR, -RR, and -LA were more often detected while MC-YR, -LY, -LF, and -LW were less common. The analytical method enabled us to estimate sediment-pore water distribution coefficients (K_d), MC-RR had the highest affinity for sediment particles (log K_d = 1.3) while MC-LA had the lowest affinity (log K_d = −0.4), partitioning mainly into pore waters. Our findings confirm that sediments serve as a reservoir for microcystins but suggest that some variants may diffuse into overlying water thereby constituting a new route of exposure

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following the dissipation of toxic blooms. The method is well suited to determine the fate and persistence of different microcystins in aquatic systems.

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1. Introduction

Microcystins are a diverse class of cyclic heptapeptides produced by cyanobacteria in freshwaters [1]. They are the most prevalent cyanotoxins and can reach toxic concentrations in surface waters, which can affect human and wildlife health [1]. During algal blooms, microcystins are typically found within living cells and on suspended particles, but dissolved microcystins can arise as blooms dissipate [2,3]. Microcystins can subsequently deposit into surficial sediments by adsorption to particulates [4–6] and within intact cyanobacterial cells [7–10] that settle to the lake bottom. Intracellular microcystins have also been found in benthic cyanobacteria growing on the surface sediments of shallow lakes [11–13] as well as in viable cyanobacterial colonies from deep sediments [14]. These reports highlight the need for an extraction method capable of rupturing the cells within sediments to recover intracellular toxins in addition to extracellular microcystins adsorbed to particles or dissolved within sediment pore waters.

Some progress has been made in the extraction and quantitation of extracellular microcystins from sediments. However, only four variants of similar chemistry have been tested thus far [15–18] and laboratory and field studies suggest differences in the adsorption properties of different microcystins [3,19,20]. Furthermore, no method has yet demonstrated the recovery of microcystins from intact cyanobacterial cells in sediments despite increasing evidence of the significance of this microcystin pool [7–14].

Hence, the goal of the present study was to develop and validate an analytical method to extract and quantify nine chemically diverse microcystins from freshwater sediments. Accelerated solvent extraction (ASE) followed by SPE were used to recover both intra- and extracellular microcystins from sediment and, separately, from pore water. Triple quadrupole mass spectrometry in MRM mode was used for the detection and quantitation of microcystins in sediment and pore water extracts at environmentally relevant concentrations. The method enabled us to estimate the sediment-pore water distribution coefficients distribution coefficients (K_d) and investigate the fate and persistence of individual microcystins in aquatic ecosystems.

2. Experimental

2.1. Cyanotoxin standards

Certified standards were obtained from Cedarlane (Burlington, ON, Canada) and the National Research Council (Halifax, NS, Canada). A standard mix of MC-LR (CAS: 101043-37-2), -^{7dm}LR (134842-07-2), -RR (111755-37-4), -YR (101064-48-6), -WR (138234-58-9), -LA (96180-79-9), -LF (154037-70-4), -LY (123304-10-9), -LW (157622-02-1), and nodularin (NOD) (118399-22-7) (Fig. 1) was prepared for the development of the RP-HPLC-MS/MS separation and quantitation method as well as for spiking sediments in order to determine the efficiency and precision of various extraction and post-extraction procedures. Purity of each standard was reported as >99% (determined by flash chromatography followed by RP-HPLC-UV).

2.2. Sediment sampling and preparation

Table 1 lists seven lakes from which sediment cores were collected in duplicate using a modified gravity corer [22]. Cores were sectioned on site at 0.5 cm intervals using a vertical extruder [22], sediments samples were collected in pre-labeled Whirl-Pak® bags, and transported on ice in a dark cooler. Prior to storage in dark at −20 °C, crude sediment aliquots of 10 g were centrifuged (1200 × g at 4 °C for 25 min) to obtain semi-dry sediment and pore water (Fig. 2). Cores were dated using lead-210 as described by Appleby [23]. For recovery experiments, sediment (2 kg wet) was collected from Constance Lake (0–10 cm homogenized), which has a history of very low surface water concentrations of microcystins (average 0.08 $\mu g\,L^{-1}$) [24] and no detectable concentrations in surface sediments.

To determine recoveries of extracellular microcystins, semi-dry sediment was spiked with standard mix to 0.5 $\mu g\,g^{-1}$ dw of each cyanotoxin. Equilibrium was reached after 4 h at 4 °C, which was consistent with that found by Chen et al. [16]. Semi-dry sediment was extracted directly or first lyophilized (referred to as dry sediment) in order to allow direct comparison to current extraction methods [15–17,25,26]. Recoveries were compared by Student's *t*-test analysis at $p < 0.05$.

2.3. Extraction of cyanotoxins from sediments

ASE conditions, originally developed for the analysis of cyanotoxins in culture [27], were used for microcystins in sediments. Each stainless-steel extraction cell (33 mL) was lined with a cellulose filter and packed with sediment mixed with Hydromatrix™ (Varian, Mississauga, ON, Canada). Extractions (two cycles) were performed with either water or methanol:water (3:1) (HPLC grade, J.T. Baker, Center Valley, PA, USA) using an ASE 200 (Dionex, Oakville, ON, Canada) operated at 1900 psi and 80 °C. Each cycle included a pre-heating (1 min), heating (5 min), and static (5 min) phase.

Extracts (40 mL) were collected in amber glass vials and immediately vacuum filtered (0.7 μm, 25 mm GF/F) to remove precipitating matrix components (Fig. 2). Extracts in water were concentrated by lyophilization while extracts in methanol:water (3:1) by nitrogen- and heat-assisted evaporation in a TurboVap LV (Zymark Corp., Hopkinton, MA, USA). Resulting residues were reconstituted with methanol:water (1:1) and centrifuged (13,000 × g at 4 °C for 10 min). The concentration–reconstitution–centrifugation cycle was repeated with the supernatant (concentration by TurboVap LV). The resulting supernatant (0.2 mL) was syringe-filtered (0.45 μm, 4 mm LH-PTFE, Millipore, Montreal, Canada) and stored at −20 °C until analysis. To improve the recovery of microcystins, the ASE extracts were loaded onto a hydrophilic (*N*-vinyl pyrrolidone)-lipophilic (divinyl benzene) balance (HLB) SPE polymeric sorbent cartridge (500 mg/6 mL, 33 μm, Strata®-X, Phenomenex, Torrance, CA, USA) after vacuum filtration (Fig. 2). Methanol was evaporated from the methanol:water (3:1) extracts by TurboVap LV and replaced with equal volume of water prior to SPE application. Cartridges were washed with two volumes of methanol:water (1:4) and eluted with 6 mL of methanol.

2.4. Extraction of intracellular cyanotoxins from sediments

In order to develop an extraction method for intracellular microcystins, sediments were spiked with a well-characterized culture of *Microcystis sp.* and the recovery of each intracellular microcystin from sediments was compared to that from the culture alone. Semi-dry sediment was spiked with *Microcystis sp.* CPCC

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