

OWRC FY 2021 Water Resources Research Institute Program (104b) Final Report: Cyanotoxin measurement in water treatment residuals

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Abstract

To protect public and environmental health, cyanobacteria and their toxins produced during harmful algal blooms must be managed comprehensively during and after drinking water treatment. We optimized extraction and quantification of microcystins in drinking water treatment plant (WTP) residuals from different unit processes and treatment plants to inform beneficial reuse applications. Although methods have been optimized for extracting and quantifying cyanotoxins in water samples, similar studies have not been done for residuals. Therefore, the prevalence of algal toxins in WTP residuals is largely unknown, leading to boundaries to implementing their safe beneficial reuse. We performed this research through collaboration between The Ohio State University, and Hazen and Sawyer and their WTP clients throughout Ohio and the US. To verify presence and accurately quantify of microcystins in various WTP residuals, we focused on two objectives. **Objective 1:** Focusing on settling residuals and dissolved air flotation residuals from one WTP in Ohio, we translated and optimized existing methods for microcystin measurements in water to be applicable for residuals. **Objective 2:** We compared these methods to verify presence of microcystins in residuals samples from other WTPs and compare impacts of variable residuals properties on microcystin measurement. We also measured impacts of storage and treatments on residuals microcystins concentrations. This research will demonstrate widespread occurrence of microcystins in WTP residuals, will inform best practices for quantifying cyanotoxins in residuals necessary for sustainable HAB residuals management, and will inform future interlaboratory validation of an optimized residuals quantification method.

Problem

Cyanobacteria Cause Harmful Algal Blooms

Cyanotoxins are toxic metabolites produced by some species of the photosynthetic heterotrophic organisms, cyanobacteria. Both are becoming an increasingly prevalent issue in source waters, recreational waters, and water treatment plants. There are several categories of cyanotoxins such as skin irritants, hepatotoxins, and neurotoxins all of which can present a serious human and environmental health hazards. Microcystins, a class of hepatotoxic cyanotoxins, are the most studied, most associated with toxicity-related events, and most monitored in water quality ¹.

The potential human and environmental health effects from exposure to cyanotoxins produced by cyanobacteria during harmful algal blooms (HABs) pose a serious local, regional, and national water quality. Locally and regionally, HABs and their toxins impact the Great Lakes which supply much of the nation's freshwater², especially near Ohio State where most of this proposed work will take place. For example, in 2014 the city of Toledo, OH had to warn nearly 500,000 residents not to drink/boil municipal water due to a bloom, and in 2015 the Ohio River was contaminated by a HAB that stretched hundreds of miles. Throughout the nation, microcystins are the most commonly detected class of cyanotoxins³, and this class contains at least 250 structurally related congeners (including microcystin-LR)⁴. In a

national survey of freshwater, at least one microcystin congener was detectable ($> 0.010 \mu\text{g/L}$ by LC/MS/MS) in 52% of samples.³ HABs globally affect both fresh and marine waters⁵.

Aside from being geographically widespread, threats from HABs and costs of their impacts are increasing. HABs result from population growth and land use changes that increase nutrient loads to water supplies leading to eutrophication, and climate change impacts including rising temperatures, changes in water flows, and changes in ocean currents, which in turn may increase the magnitude and frequency of blooms⁶. Some cyanobacteria species that cause HABs in fresh water produce cyanotoxins that are directly toxic to humans, animals, and the environment, and indirectly toxic through changes to freshwater chemistry and food webs⁷. Many algal species and toxins contribute to widespread human and animal illness across the US which cost millions of dollars annually in direct healthcare costs, and millions (or even billions⁸) of dollars annually in economic impact due to fishing industry losses, recreation and tourism industry losses, and increased costs of water monitoring and treatment⁹. Both outputs of drinking water treatment plants are regulated for cyanotoxins (**Figure 1**): the US EPA recommends <1 ppb of select cyanotoxins in drinking water¹⁰ and the Ohio EPA regulates microcystins in treatment residuals to < 20 ppb for land application (OAC Chapter 3745-599).

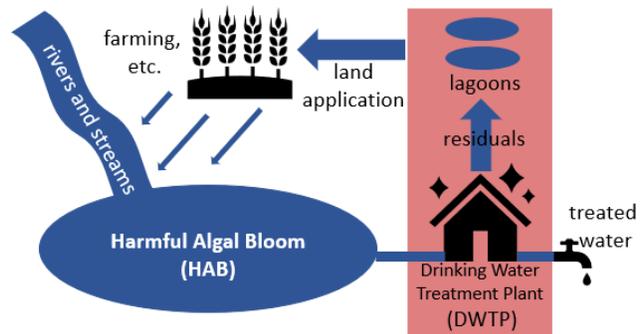


Figure 1 To protect public and environmental health, cyanobacteria and their toxins must be managed comprehensively through the interconnected system including surface water, DWTP intakes, residuals (the focus of this proposal), and treated water.

HAB Impacted Water Treatment Residuals May Contain Cyanotoxins

Residuals accumulate during water treatment processes including after coagulation, flocculation, and sedimentation (CFS residuals), and the float from dissolved air flotation (DAF residuals, also known as DAF float) (**Figure 2**). Residuals have low water content and when accumulated during toxin-producing HABs, they contain cyanobacteria, their toxins, and a variety of other microorganisms and (in)organic constituents.

One complication of residuals management is that current methods for extracting and quantifying cyanotoxins in residuals were developed for water samples rather than residuals. The Ohio EPA (OEPA) method¹¹ for quantifying microcystins in water may not be appropriate for water treatment plant residuals. The current OEPA microcystins method separates solids from liquid through $0.45 \mu\text{m}$ vacuum filtration and quantifies microcystins in the filtrate. Microcystins are hydrophobic toxins that preferentially adhere to solids rather than dissolving in water, based on their low octanol-water partitioning coefficient. Microcystins likely to adhere to lipids in organisms and organic carbons present in the sample¹². Microcystin extraction relies on

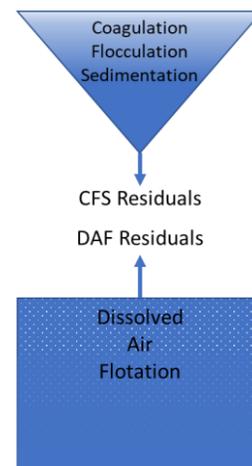


Figure 2 Water treatment residuals can be produced during settling (CFS) and floatation (DAF) processes.

freeze-thaw cycles to release intracellular cyanotoxins, and then discards solids retained on a 0.45 um filter. Because these solids may contain sorbed MC-LR, this extraction method may underestimate cyanotoxin concentrations. The OEPA method also requires antibody-based ELISA quantification, which has varying cross-reactivity to microcystin congeners that can lead to either underestimation or overestimation depending on the congener, and may overestimate concentrations of degradation products. ELISA quantifies the general ADDA moiety of microcystins. Conversely, chromatographic quantification methods can more easily differentiate microcystin congeners and products likely to be found in environmental and biodegraded samples, but have much higher capital costs and have various other interferences and interpretation concerns depending on the method used. Chromatographic quantification is also limited to commercially available microcystin standards for comparison. Accurate extraction and quantification of cyanotoxins is essential for comprehensive management of residuals from water treatment plants battling HABs.

Because of these methodological uncertainties, occurrence of microcystins in residuals is largely unknown. While some work has been done to assess MC's in solid matrices^{13,14}, there are no investigations on assessment of MC's in residuals, outside of applying US EPA water methods. As seen in McCord et al. (2018), many MC variants are pH dependent in partitioning thus could be heavily impacted by the varying constituents found in WTP residuals. MC content in residuals can limit a WTP's participation in beneficial reuse programs.

Another problem with residuals management is that treatment is currently limited to unsustainable solutions including landfills (estimated at 40% of all water treatment residuals¹⁵), long-term storage in lagoons that must be excavated when full, and incineration. A promising alternative residual management strategy is beneficial reuse through land application¹⁵, if toxins can be reduced to meet regulatory limits before land application to prevent leaching into water supplies or accumulating in crops (e.g., regulation OAC Chapter 3745-599). Bench scale findings have demonstrated microcystin degradation by various indigenous surface water bacteria in Ohio¹⁶ and other areas¹⁷. However, these bench-scale findings have not yet translated into industry pilot tests that can inform municipal implementation, where many water treatment plants such as Celina face the persistent or annual challenge of managing harmful algal blooms.

Without accurate quantitation methods and studies of treatment at bench and larger scale, the fate of cyanotoxins from water treatment residuals and their risk to cannot be fully understood and minimized by researchers, design engineers, or water treatment plant operators. To protect environmental health while allowing land application of HAB-impacted drinking water treatment residuals, alternative and sustainable residuals monitoring and management methods are needed.

Accurate quantification of cyanotoxins in residuals will inform sustainable management

Through a collaboration between researchers at Ohio State, and engineers at Hazen and Sawyer (Hazen), we investigated accurate extraction and quantification of microcystins in a variety of residuals. We used bench scale studies to generate mechanistic understanding of factors impacting MC-LR extraction and quantification from water treatment residuals using existing and modified methods. We performed bench testing (Ohio State, under guidance of PI Natalie Hull), pilot testing (at Celina Utilities Water Treatment Plant in OH through partnership with Hazen under guidance of Co-I Elizabeth Crafton-Nelson and full time engineer / part time MS student Emma van Dommelen), and lagoon testing (Hazen/Celina) to generate mechanistic and quantitative understanding of factors impacting MC-LR

(bio)degradation in CFS and DAF residuals in bioreactors and lagoons. We also tested extraction and quantification of residuals from other utilities throughout the country with different source water concerns and unit treatment processes and therefore residuals properties. This research will not only bring attention to the widespread prevalence of microcystins in water treatment residuals, but will also inform best practices for quantifying cyanotoxins in residuals and will help utilities manage their HAB residuals sustainably to minimize risk to humans and the environment.

Research Objectives

Our proposed research combines bench scale, pilot scale, and field scale testing with the goal to determine the best way to quantify microcystin concentrations in water treatment residuals, and to also determine the impacts of residuals properties and pre-treatments on microcystin concentrations. To support this goal, our objectives included:

Objective 1: Focusing on settling residuals and dissolved air flotation residuals from one WTP in Ohio, we translated and optimized existing methods for microcystin measurements in water to be applicable for residuals. **Objective 2:** We compared these methods to verify presence of microcystins in residuals samples from other WTPs, and compared impacts of variable residuals properties on microcystin measurement. We also measured impacts of storage and treatments on residuals microcystins concentrations.

Methods

For both objectives, we quantified bacteria, toxins, and other parameters while following proper chemical and biosafety precautions (PI Hull approved biosafety level 2 protocol 2018R00000109 and approved algal toxin technology control plan TCP035. All residuals samples were collected in glass or PETG bottles and transported or stored on ice or at 4°C. Physical and chemical properties were recorded upon receipt including pH, conductivity, dissolved oxygen, and percent solids.

Objective 1. Modify and/or validate for residual samples the existing extraction and quantification methods of MC-LR in water treatment residuals

We obtained most residuals for Objective 1 from Celina, OH where the water utility has a persistent microcystin-producing *Planktothrix* bloom in their source water, the shallow and hypereutrophic Grand Lake (see **Figure 3**). To improve treated drinking water quality, Celina contracted Hazen to

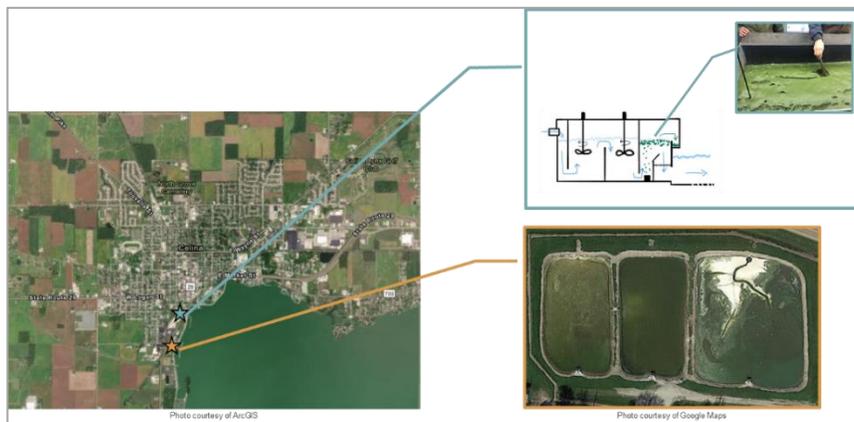


Figure 3 The water treatment plant in Celina, OH (left) currently stores CFS residuals in lagoons (bottom right), which must be excavated when full. Celina contracted Hazen to design and construct a dissolved air flotation (DAF) processes (top right) to combat the year-round HAB impacting Grand Lake source water.

design and construct a DAF system to improve removal of cyanobacteria and reduce cyanotoxins over current performance by CFS. In this research, we used various Celina residuals settling and DAF processes and from their lagoons. Residuals samples were split and stored at -80°C until analysis of different lysis methods, solvents, and quantification method on microcystin concentrations. Based on US EPA Methods 544¹⁸ and 546¹⁹ for quantifying microcystins by LC/MS/MS or by ELISA respectively, a procedure developed for soil samples¹³, and ongoing work for Water Research Foundation project 4716 focused on methods for quantifying microcystins in water²⁰, we tested a suite of extraction methods alone or in combination, including incorporating microwaving to release intracellular and sorbed toxins, and solvent-assisted solids extraction with solid phase extraction (SPE). The basis of comparison for our tests (bottom of **Figure 4**) is the current OEPA method¹¹ (top of **Figure 4**), which relies on freeze-thaw cycles to release intracellular cyanotoxins, and then discards solids retained on a $0.45\ \mu\text{m}$ filter to analyze MC-LR by ELISA in the filtrate only.

Lysis. Each bottle was thawed in a 35°C water bath (Fischer Scientific). The freeze thaw sample was then placed back in the -80°C freezer, which occurred a total of three times. The microwave sample was microwaved (Amana Commercial - 120V) in intervals of 30 seconds, allowing the bottle and sample to cool to room temperature on the bench top between each microwaving interval. This process was repeated for a total of three times. The sonication sample was put into a sonicating bath (Branson) and sonicated for an interval of 20 minutes, with a 5-minute resting period in between. The sonication process was repeated a total of three times.

Solvents. After lysis, each sample was then split up into 20 mL aliquots in 100 mL glass bottles. Therefore, each lysis method was divided so to correlate with the four solvents. Lysed samples were diluted with a 2:1 ratio of solvents. 40mL of each Deionized (DI) Water, Methanol 80% (Fisher Brand), PBS 1x and PBS 10x (Fisher Brand), were added to the 20mL of sample.

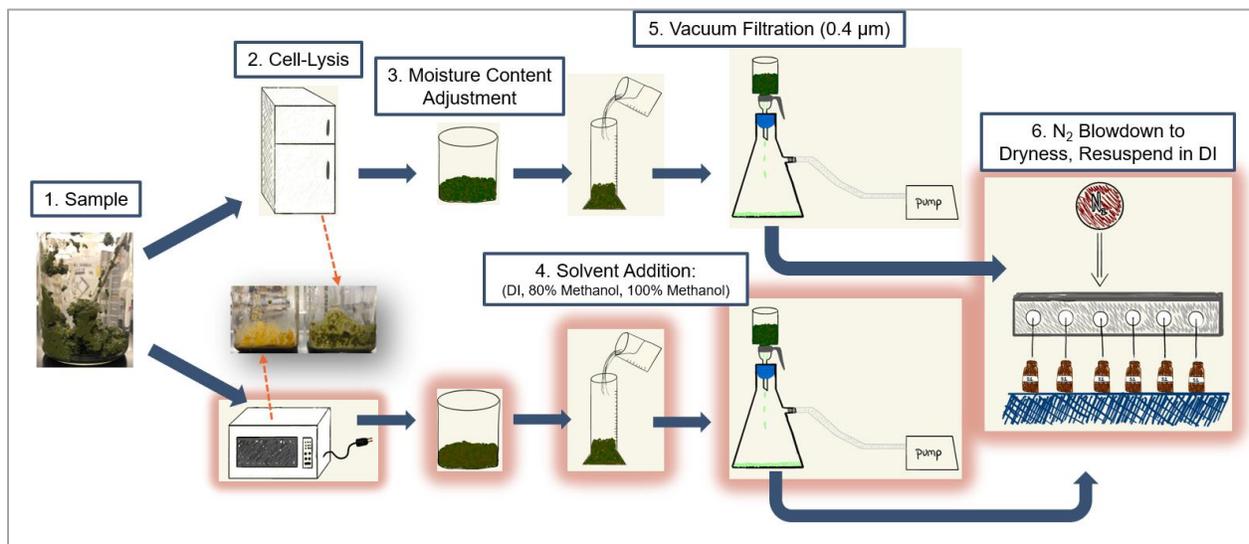


Figure 4 We varied cell lysis method, residuals moisture content, solvent type and concentration, and inclusion of eluate from filter retentate to maximize MC-LR recovery from various residuals.

Filtering. A vacuum filtration method was performed to filter the lysed and solvent-added samples. The vacuum filter was set up using a Buchner funnel, pedestal, clamp, vacuum flask (all Sigma Aldrich) and 0.45 μm membrane filter paper (Whatman, 47 mm). The assembled apparatus was then connected to a vacuum pump. To condition the filter, the vacuum ran while the pedestal was thoroughly rinsed with DI. Next, a membrane filter paper was placed on the pedestal and conditioned with DI. After the filtrate from conditioning was discarded, each sample was filtered and filtrate was aliquoted into replicates before N_2 evaporation to dryness and resuspension in DI. To clean the apparatus, the funnel, pedestal, and vacuum flask was thoroughly rinsed with DI three times after removing filter paper.

Quantification. Two methods of MC quantification were utilized for this study; ADDA-ELISA (Eurofins Abraxis, PN 520011) and Ultra Performance Liquid Chromatography (UPLC) coupled with a Photo Diode Array (PDA) detector. ADDA-ELISA was completed according to the manufacturer's instructions for every sample set. The provided calibration standard solutions (0, 0.15, 0.40, 1.0, 2.0, and 5.0 $\mu\text{g/L}$) were pipetted into the 96-well plate in addition to the diluted filtered samples. Dilutions were made the provided dilutant:sample at a ratio of 50:1. Samples were analyzed in triplicate. After completing the assay according to the provided instructions, the UV absorbance was read at 450nm on a plate reader (Synergy|HTX). The UPLC-PDA method used detected absorbance at the peak wavelength, which is thought to represent primarily MCLR and not other congeners. It was performed with a BEH C18 column (1.7 μm , 2.1 x 50mm, Waters) and a 0.3 mL/min flow rate. Over a 5-minute period, the mobile phase gradient moved from 75:25 water (with TFA, 0.05% v/v):acetonitrile (with TFA, 0.05% v/v) to 50:50 water (with TFA, 0.05% v/v): acetonitrile (with TFA, 0.05% v/v) detecting MCLR at 238 nm (Spoof et al., 2009). Samples were analyzed by duplicate injections.

Objective 2. Determine impact of varied residuals properties and pretreatments on microcystin concentrations

Samples were obtained from utilities that reported microcystin concentrations in their source waters or reported microcystin producing species of cyanobacteria in their source waters. Reports were obtained from the current advisories found in United States Environmental Protection Agency's Tracking CyanoHABs mapping tool. Samples were received from four total utilities in northwest Ohio (Celina), northeast Ohio (Akron), Florida (Tampa) and South Carolina (Gaffney). We also performed bench scale testing of bioreactors to determine impacts of environmental parameters (temperature, light/dark cycles, mixing speed, nutrient additions, aging) or pretreatments (chlorine, permanganate, etc) on MC-LR concentrations. Bench testing allowed us to vary parameters at a small scale with increased replication (**Figure 5**). We also conducted pilot testing of bioreactors and field monitoring of lagoons to understand realistic performance.

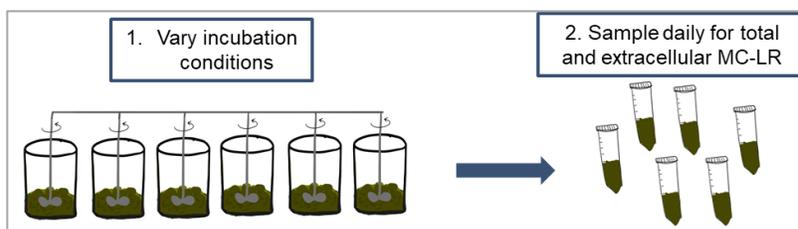


Figure 5 Bench scale bioreactors enable testing impact of multiple variables on MC-LR concentrations.

Results

In initial method development with two batches of Celina DAF samples during parts of the year that were highly impacted and less impacted by bloom conditions, the sample preparation variable that had

the biggest impact on MC-LR measured concentrations was solvent (particularly methanol) concentration (**Figure 6**).

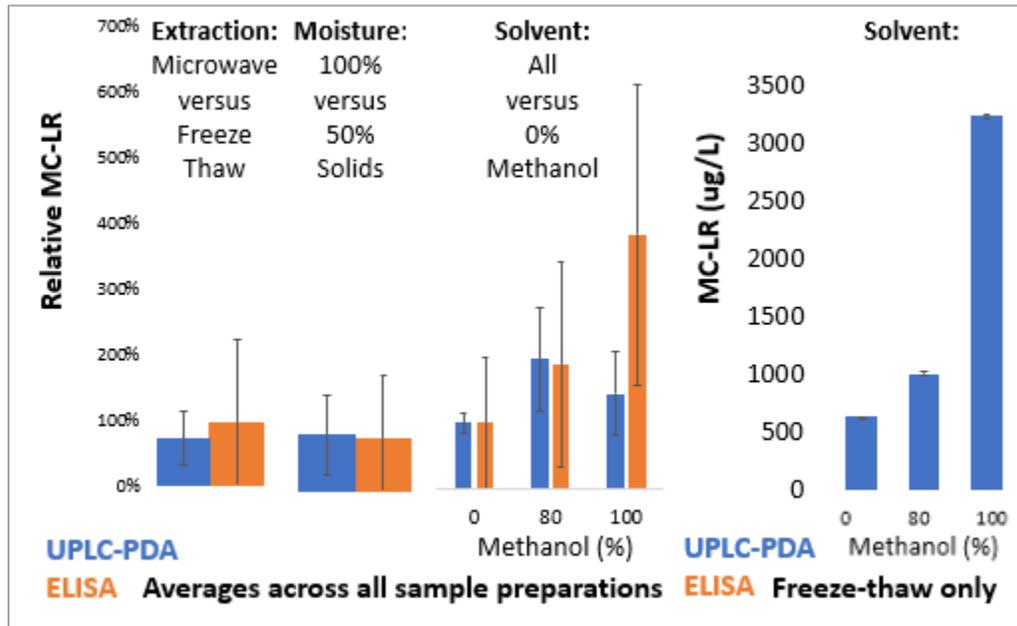


Figure 6 Increasing methanol concentration increased MC-LR concentrations (UPLC-PDA and ELISA), but neither sample extraction method nor solids moisture content nor analysis method impacted MC-LR concentrations in either DAF residuals or lagoon residuals from Celina.

Impacts of storage and biological / chemical treatments on residuals MC-LR concentrations

Pilot-scale bioreactor experiment demonstrated that without any nutrient additions or spiking of any other microorganisms, degradation of MC-LR occurred fairly rapidly (**Figure 7**). Additionally, our MC-LR concentrations in various regions of lagoons (with varying residual age) used to store CFS residuals at Celina demonstrated lower MC-LR concentrations with higher residual age (**Figure 8**). The MC-LR was even below detection in several samples with the greatest residual age, indicating that natural (bio)degradation effectively decreased MC-LR concentrations as residuals were stored in lagoons. In bench scale bioreactors aimed at accelerating biological degradation of MC-LR, the extracellular MC-LR increased over time with increased temperature despite no increase in cell numbers (**Figure 9**). In other bench scale bioreactors, total and extracellular MC-LR varied over time after chemical pretreatment as cell numbers increased and confounded MC-LR quantification (**Figure 10**). Although these bench scale bioreactor studies provide conflicting results for these different batches of DAF residuals (which could not be continued in this study due to COVID impacts), biological degradation and chemical pretreatment deserve further investigation. With additional optimized biological or possibly chemical pretreatment, utilities may be able to decrease the required lagoon holding time or forgo the need for lagoon storage of residuals to proceed directly to land application.

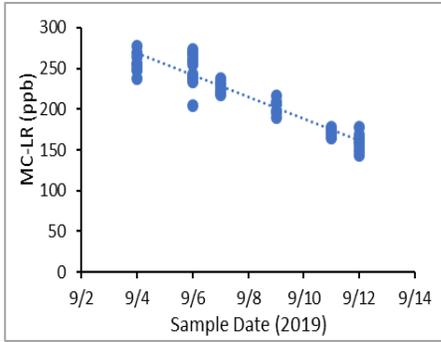


Figure 7 MC-LR (UPLC -PDA) in Celina DAF residuals decreased over time in a pilot scale bioreactor mixed at 1300 rpm.

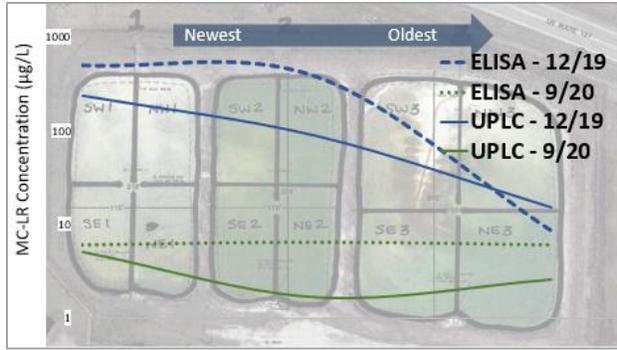


Figure 8 MC-LR (UPLC-PDA and ELISA) decreased with residual age in Celina lagoons.

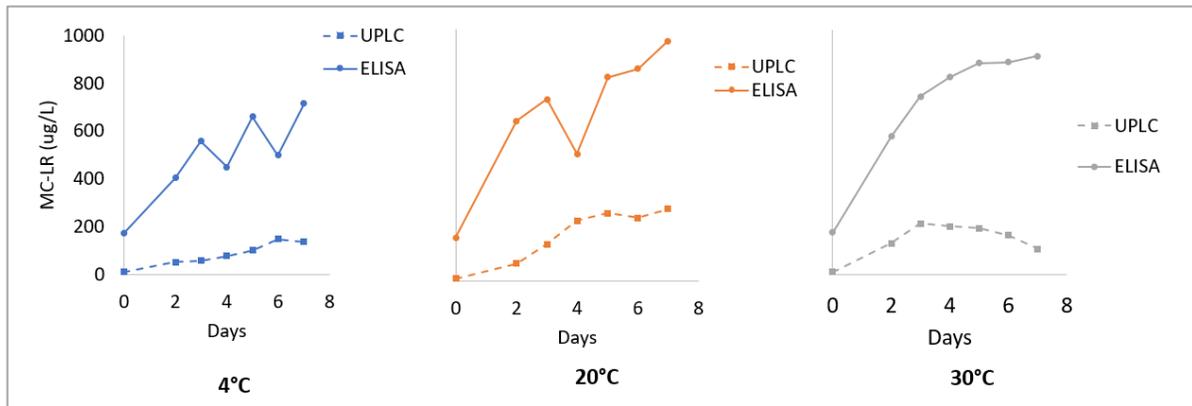


Figure 9 Extracellular MC-LR concentration in bioreactors increased with temperature over time for Celina DAF residuals in 700mL jar testing setup mixed at 100RPM, despite no appreciable changes in cell numbers assessed using optical density measurements and heterotrophic plate counts (not shown).

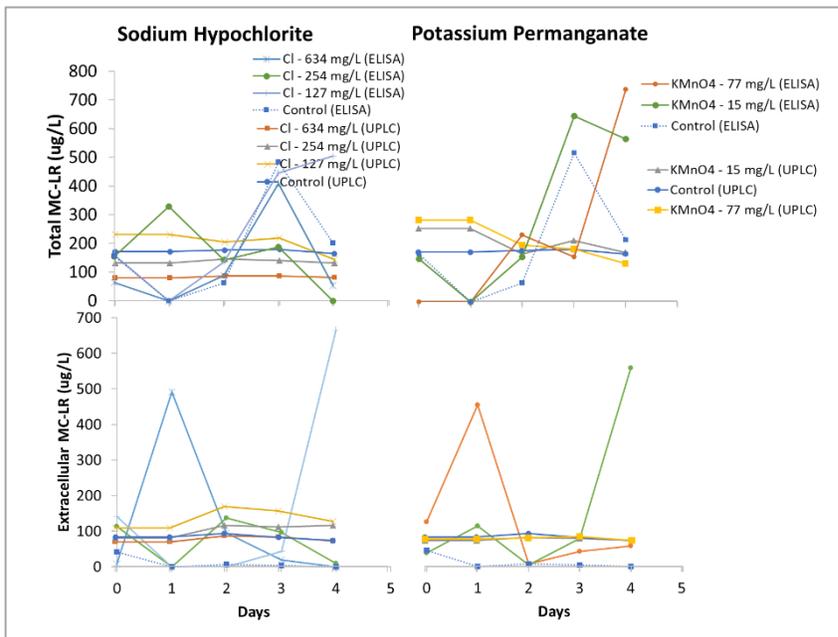


Figure 10 Total and extracellular MC-LR concentration (ELISA or UPLC-PDA) in bioreactors incubated at 25C containing Celina DAF residuals and pretreated with varying concentrations of sodium hypochlorite or potassium permanganate was confounded because cell numbers increased as indicated by increasing optical density measurements (not shown). Bioreactors were decommissioned and not restarted after peak COVID lab impacts.

MC-LR in residuals from various water treatment plants and treatment processes

Gaffney, SC lagoon residuals were from a treatment train that employed sodium permanganate, a carbon slurry, anthracite filtration, before coagulation and addition of polymers. MC-LR at an overall average of 0.43 ug/L (ELISA) was observed across all solvents (DI water, methanol (MeOH), and different concentrations of phosphate buffered saline (PBS)) and cell lysis methods (freeze-thaw, microwave, and sonication) tested (**Figure 11**). Tampa, FL residuals consisted of dewatered residuals in lagoons and their treatment process relied on ferric as a coagulant. Tamps residuals MC-LR averaged 7.29 ug/L (ELISA) across all solvents and cell lysis methods tested. Lagoon samples from Akron, OH had an average MC-LR concentration (ELISA) of 4.69 ug/L, and freeze thaw resulted in the highest overall MC-LR concentration among cell lysis methods while methanol resulted in the highest overall MC-LR concentration among solvents. Methanol also resulted in the highest recovery of MC-LR spiked into Akron residuals among the tested solvents. Three more sample sets from Celina, OH DAF residuals and lagoons remain to be analyzed by ELISA or UPLC-PDA. All these samples remain to be analyzed by UPLC-PDA. Tests for statistical significance between cell lysis methods, solvents, and quantification method are ongoing, but trends can be seen in the averages across the two plots for Gaffney, SC and Akron, OH.

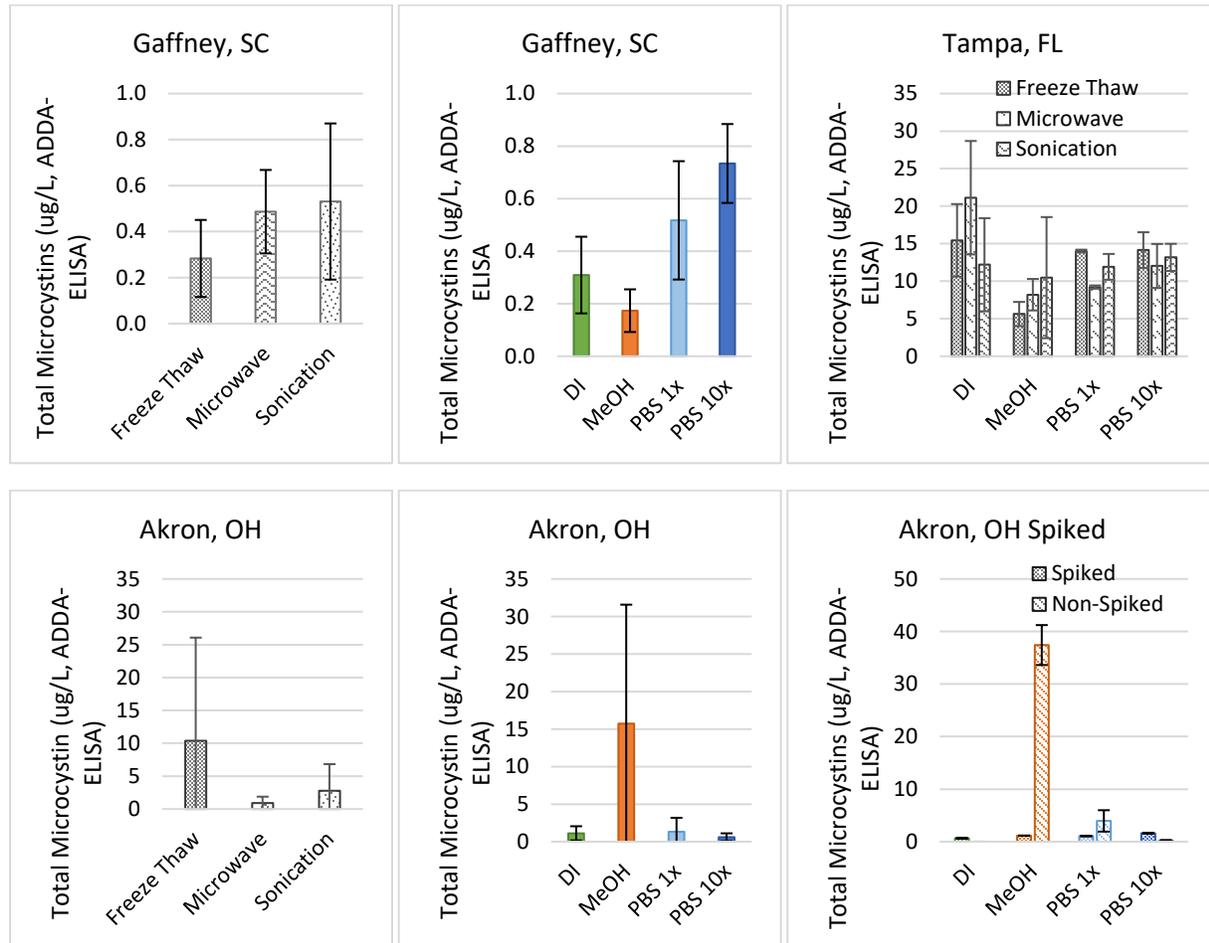


Figure 11 MC-LR concentrations in residuals samples from water treatment plants throughout the United States. MC-LR concentrations in residuals from these utilities can be compared to concentrations in other residuals samples from Celina, OH in this report.

Significance

This study presents the first survey of microcystins in residuals from drinking water treatment plants throughout the country, demonstrating that they are in fact prevalent. This study demonstrates the impacts of cell lysis method, extraction solvent, and quantification method on quantifying microcystins in residuals from several samples collected over time from one treatment plant, and also among one batch of samples collected from the same and other treatment plants. This study also preliminarily demonstrates impacts of storing or biologically/chemically treating residuals on microcystins concentrations. These data highlight the importance of cyanotoxins as one of the contaminants that should be considered in responsible and sustainable management of drinking water treatment residuals, including for beneficial reuse applications to minimize landfilling and incineration. These data can be used with further inter-lab and inter-utility testing to develop recommendations to extract and quantify microcystins in DWT residuals across a variety of residuals.

Through this combination of bench- and pilot-scale testing and real-world sampling, our research was able to synergize with ongoing consulting engineering efforts by Hazen to immediately apply our findings at Celina to help combat their persistent bloom. MS student Emma van Dommelen won an award for her presentation of this translated work at OAWWA in 2021. This is an example of transferring university research from the bench to the field directly to the stakeholders of water treatment plant operators and engineers battling HABs. This project addresses how quantification of algal toxins in water treatment is affected by complexity of residuals samples, and this information will enable utilities to analyze and optimize microcystins in various residuals to levels suitable for land application (<20 ppb²¹), while also considering storage and treatment options to help achieve land application goals.

This project focused on residuals complements other ongoing research areas in Hull's lab. For one, we are studying wavelength specific optimization of treating cyanotoxins and cyanobacteria with novel UV technology and advanced analysis of degradation products, involves collaboration with USGS chemists, and was recently funded by HABRI. This project also complements a recently completed Ohio Department of Transportation funded project to Hull and Ohio State PI Dr. Lisa Burris focusing on using environmental sources of bacteria for biomineralization and self-healing of cracks in concrete. In the future, water treatment residuals may be a source of bacteria and/or nutrients for the concrete that would enable another beneficial reuse of residuals besides land application. All levels of this project engaged women, an underrepresented group in engineering, across consulting and academia and across both graduate and undergraduate students.

Information has been presented by the graduate student at regional (e.g., OAWWA, One Water) and national (e.g., AWWA) conferences and incorporated into various class lectures taught by the PI (Bioremediation of groundwater and soils, and Bioprocesses, technical electives) and in various conference presentations by the PI. The results are being finalized and integrated into Emma's MS thesis and a manuscript that we will submit for consideration of publication in a peer-reviewed journal such as *ES&T*, *Water Research*, or *STOTEN*.

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