Spring 2022

Adsorption of Microcystins to Sediment Affects Their Bioavailability and Remediation

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ADSORPTION OF MICROCYSTINS TO SEDIMENT AFFECTS THEIR BIOAVAILABILITY AND REMEDIATION

by

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Bachelor of Science
University of Florida, 2019

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Chemistry

College of Arts and Sciences

University of South Carolina

2022

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ABSTRACT

The freshwater harmful algae *Microcystis spp* produces a family of toxins called microcystins[1]. These species of algae are known to release microcystins from their cells directly into the surrounding water[2]. This creates an array of human health risks depending on the fate and transport of the toxins in the water column[3,4]. Risk modelling and previous literature has shown that sediment is a sink for free microcystin, microcystin can be stably adsorbed to sediment for hundreds of years, and its adsorption to sediment is determined by the sediment adsorption partitioning coefficient (K_d)[5,6,7]. In this work it is hypothesized that the sediment phase is only a metastable reservoir for microcystin given that varying water conditions can affect K_d values of microcystin[8,9]. In particular, studying how sudden environmental disruptions that alter water quality such as flooding, storm surges, industrial chemical release, fire suppression runoff, etc. can result in the destabilization of adsorbed microcystin with an accompanying release into the water column on a relatively rapid timescale. Although many surface waters are monitored for microcystin, the monitoring systems are triggered by visible algae in the water, implying that unmonitored toxins could enter drinking water supplies or the food chain. Work supported includes experiments that measure the functional aspects of K_d for the various microcystins with the goal of developing predictive models for the potential release of historically adsorbed microcystin reservoirs. Where possible, strategies to degrade or stabilize adsorbed microcystin to inhibit future release will also be pursued.

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CHAPTER 1

INTRODUCTION

Harmful algal blooms are commonly described as the rapid growth of algae or cyanobacteria in a body of water. These blooms are influenced most predominantly by the abundance of nutrients such as nitrogen and phosphate, defined as eutrophication. The rise in concentration of the water's limiting nutrients paves the way for simple, highly competitive algal cells to quickly dominate the area.

The U.S. Environmental Protection Agency released a national data summary on assessed waters of the United States in 2017. The summary contained information that showed 52.9% of assessed rivers/steams and 70.9% of assessed lakes/reservoirs/ponds were impaired based on exceeding total maximum daily loads (TMDLs) of various contaminants[10]. Approximately 10-15% of the impairments were directly linked to excessive nutrient loads, translating to 118,831 miles of river/streams and 3,943,395 acres of lakes/reservoirs/ponds in the United States that are experiencing eutrophication[10]. This survey conveys how substantial the spatial impacts of harmful algal blooms could potentially reach in the future[11].

Besides the known ecological and economical effects of a harmful algal bloom (HAB), a major concern is from various species of cyanobacteria that are known to produce toxins. While not all HABs produce toxins, the frequency and duration of harmful algal blooms have been increasing worldwide, pushing the toxin producing HABs further into the algal research spotlight[12]. The various toxins that algal cells can produce account for a

wide array of human exposure risks including drinking water contamination, recreational contact, and potential to bioaccumulate into later-consumed seafood. While recreational contact and seafood contamination with algal toxins are usually more acute with milder symptoms such as skin irritations, gastroenteritis, amnesia, muscle cramps, and disorientation, drinking water contamination unfortunately offers longer-term, chronic exposure routes due to more routine doses[4,12,13]. Chronic exposure to algal toxins can lead to respiratory paralysis, organ damage, cirrhosis, severe neurotoxicity, development of cancers, and even death in a few notable cases[4,13].

A well-studied toxin producing freshwater cyanobacteria is *Microcystis aeruginosa*, which produces a family of toxins known as microcystins (MC)[1]. There are over 200 identified congeners of microcystin, however, there are six most widely studied variants that have reference analytical standards accessible. The six are known by their abbreviated names; MC-LR, MC-YR, MC-RR, MC-LF, MC-LA, and MC-LY. The two letter code following the microcystin (MC) identifies which amino acids are present at two different locations on the molecule by their one letter codes.

While all of the congeners are produced and contained within the cell, these toxins can be released into the surrounding water either as a cell defense mechanism or when the cell lyses. Once freely in the water column, the concerns over possible human microcystin exposure become more critical. Microcystins are hepatotoxins (toxins that specifically bind to liver cells) and have been linked to severe human health exposure effects including acute hepatotoxicosis, chronic liver cirrhosis, and possibly liver cancers[13,14,15]. Understanding the fate and transport of free microcystin in the water column becomes a crucial part of trying to limit human exposure to these toxins.

The reductionist approach of fugacity in environmental chemistry can be used as a tool to recognize how contaminants such as microcystin will move within a modelled environment. A unit world model (figure 1.1) is made up of five compartments, each with phase interfaces with each other[25]. Fugacity is simply the tendency for the analyte to move from its current phase to another. The actual mathematical model is a series of equations that connect the different compartments with the contaminant's physico-chemical properties to ultimately predict the fate of the contaminant.

Based on preliminary work done with the fugacity of microcystin, an environmental sink for microcystin that enters the water column is being adsorbed to soil/sediments. While the precise adsorption mechanism is widely unknown, multiple researchers have documented that microcystin is very stable on sediments and it has been shown that microcystin can be detected over 200 years later from sediment cores[6,16,17]. This data on microcystin stability seemingly contradicts experiments that show degradation of microcystin-LR (MC-LR), as well as MC-LF, begins after three days and complete removal from the system by eighteen days[18]. This leads to the questions of not only how microcystin is bonding to sediments, but also how its adsorption to sediments prevents this natural, biological degradation pathway.

Furthermore, the temporal accumulation of microcystin on sediments may pose problems of its own. The free toxin in the water can be adsorbed onto sediment based on the sediment adsorption partitioning coefficient (K_d). The K_d value is described as the equilibrium ratio of concentrations adsorbed to the sediment versus remaining in the aqueous phase. Therefore, as K_d changes, the concentrations in each phase would change.

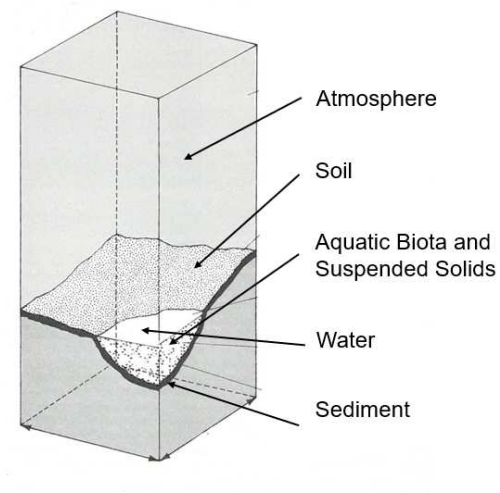

Figure 1.1: The unit world model representing the various compartments (phases) that a contaminant can partition through[25]

An alteration in aqueous phase conditions such as salinity and pH are known to be able to effect K_d values of microcystin[8,9]. The concern is that aqueous conditions could change in a way that K_d values decrease, which could release hundreds of years of stored microcystin back into the water. While this scenario may seem unlikely, the fact is these conditions could arise from common natural phenomena like a saline storm surge flooding, seasonal overturn of a eutrophic water column after a drought, or through human agency like industrial accidents such as coal ash pond collapses or fire suppression runoff. As toxin monitoring programs rely on visible algal blooms to trigger water testing, this would then decouple the association of microcystin toxicity in the water supply with a current algal bloom, making water monitoring programs unaware of possible current toxin exposure.

In an attempt to prevent the release of microcystins stored in sediments, degradation of the microcystin while still adsorbed to sediments should be explored. Previously studied methods to degrade microcystin include free-radical formation, gamma irritation,

photodegradation, and photocatalytic degradation[20,21,22,23]. It is important to note that the vast majority of prior microcystin degradation research is of free toxin, not toxin already adsorbed to sediments. Trying to degrade a contaminant that is securely bound to sediment particles where reactions may be more difficult to occur delivers a level of complexity to securing a sediment remediation strategy of microcystins. However, at the same time, the possibility of the requirement of a remediation reagent to be co-adsorbed to the sediment in order to degrade microcystin may offer a unique level of selectivity of the target molecule that is rarely seen in remediation strategies.

CHAPTER 2

EXPERIMENTAL METHODS

2.1 Using fugacity approach to model microcystin fate

The fugacity approach to modelling the movement of contaminants within the unit world stems from the assumption that all of the compartments within the model are at equilibrium and therefore thermodynamics can be used to understand a contaminant's partitioning between the phases[25]. The mathematical model is a set of equations derived from "Finding Fugacity Feasible" that can be inputted into a Microsoft Excel workbook for easier calculations[25]. Fugacity (f) itself is the tendency of a component to move out of its current phase (compartment) and into another one[25]. The fugacity capacity (Z) can be thought of as quantifying the thermodynamic stability of the contaminant in a given phase. Therefore, fugacity is a single value given to a contaminant whereas fugacity capacity is a value given to each compartment type. The initial step in determining a contaminant's environmental fate is inputting the number of moles of contaminant that is going to enter the system. From there, relative volumes (V) of each compartment, the equation for fugacity (f), and how to calculate each compartment's fugacity capacity (Z) are well defined in the paper. Then using the expression [(Z*V)/f] at each compartment calculates the number of moles of contaminant in each compartment. With this information, one can then find the percentage of contaminant, out of the total moles inputted into the system to begin with, in each compartment at equilibrium. These ending values will provide information on the distribution of the contaminant across the different phases at equilibrium conditions.

2.2 Microcystin working solution and extraction

To perform phase equilibrium and degradation studies with microcystin, a reliable and inexpensive source of microcystin was needed. While purified standards are available for six of the microcystin congeners (including MC-LR), the concentrations and volumes needed for experiments made extraction from a natural source more feasible. A working solution of microcystin was extracted from a sample of freeze dried *Microcystis*[28]. Extraction procedures included using 10mL of 75% methanol per 50mg of freeze-dried algae. This solution was sonicated for 20 minutes in a glass test tube and then cellular debris was spun down with centrifugation for 15 minutes. The supernatant containing the microcystins was collected, filtered, and stored at -4 ^{o}C until use.

2.3 Microcystin quantification

A sensitive method for microcystin quantification was needed in order to detect small differences in microcystin concentrations for equilibrium studies. EPA method 544 quantifies six microcystin congeners, including MC-LR, using liquid chromatography/tandem mass spectrometry (LC/MS/MS)[29]. For each congener, the EPA 544 method defines a single selected reaction monitoring (SRM) transition to identify the microcystin congener[29]. This method was adapted to the Waters Acquity ultra performance liquid chromatography (UPLC) instrument connected with a Xevo triple quadrupole (TQ) mass spectrometer using positive electrospray ionization (+ESI), however, sensitivity for quantification was lower than expected when initial standards were run. This EPA 544 method is still used for qualification purposes of the six most commonly studied microcystin congeners, but a new method was adjusted specifically for MC-LR detection that optimized parameters such as cone voltage and collision energy based off of peak signal from the microcystin-LR pure standard. MC-LR was chosen because literature shows

it is usually the most abundant (as well as most toxic) congener in environmental microcystin samples and this was confirmed in the algal sample used based on the preliminary results from the EPA 544 method (figure 3.2)[24]. Therefore, MC-LR was used a representative congener for these experiments and more a sensitive method was set up for its detection.

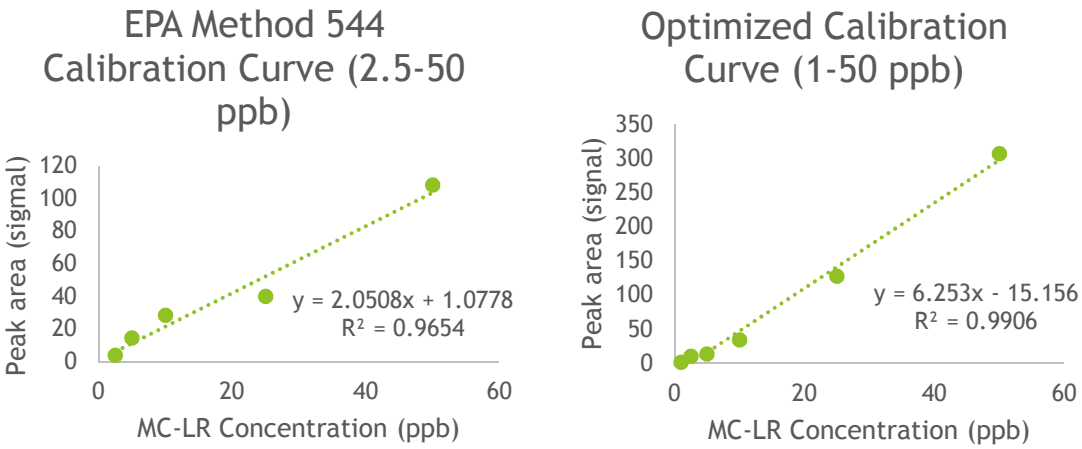

Figure 2.1: The initial calibration curve generated using EPA method 544 parameters (left) and the calibration curve generated from optimized parameters (right)

Figure 2.1 shows the comparisons of sensitivity of the EPA method 544 and our optimized method. The limit of detection was lowered to 1ppb in the optimized method compared to 2.5ppb in the EPA method. Signals increased by about a factor of 3 at each standard calibration point. The optimized method utilized 20mM ammonium formate as the aqueous phase and 100% methanol for the organic phase. The nine-minute C_{18} column run time included a gradient elution that started at 90/10 aqueous:organic, ramped down to 10/90, then back up to 90/10 by the end of the run. The optimized cone voltage was 90 V and collision energy was 68 eV. The transition studied for quantification was from the parent ion (m/z=995.64) to the daughter ion fragment (m/z=135.11). The retention time for MC-LR was approximately 4.77 minutes.

A calibration curve was made to quantify microcystins in unknown samples. The stock solution of pure 10,000 ppb MC-LR standard in 100% methanol was diluted to concentrations ranging from 1 to 300 ppb with the final calibration solution matrix containing less than 3% methanol as experimental samples did.

2.4 Preparation of sediment for experiments

The sediment used in these studies was natural material collected from Lake Wateree in South Carolina. It was collected from the lake floor at a depth of less than 0.25 meters, in an area with no visible algae. The sediment was bagged and stored on ice during transport back to the lab. Then sediment was rinsed using a 0.45 micrometer sieve and larger non-sediment debris was also removed. The experimental sediment used was the portion greater than 0.45 micrometers, as this made the sediment easier to work with during experiments. Finally, sediment was dried in an oven at 55 ^{o}C for two days until its mass remained constant then stored at -4 ^{o}C until needed.

2.5 Equilibrium experiments to determine K_d

To find K_d values, the equilibrium concentrations of microcystin adsorbed to sediment and in the aqueous phase are needed[7]. The overview of this process is dividing the capacity of the sediment by the concentration in the aqueous phase at equilibrium.

$$K_d = \frac{q}{Ce} = \frac{capacity\ of\ sediment\ (\frac{mg\ MC-LR}{kg\ sediment})}{[MC-LR] in\ Aqueous\ Phase\ (\frac{mg}{L})}$$

The experimental setup was as follows: 15 mL screw cap glass test tubes were used to hold 2 grams of dried sediment and 10mL of MilliQ water spiked with 100 ppb microcystin-LR. The test tubes were rocked continuously on a tray for 4 hours to ensure equilibrium was reached. Then the aqueous phase was filtered into a 2mL vial and stored at -4 ^{o}C until analysis. The change in concentration from the initial 100 ppb to the aqueous concentration was attributed to adsorption onto sediment, using the mass balance approach. The capacity of microcystin onto the

sediment was determined, in mg microcystin per kg sediment. Then this capacity value was divided by the aqueous phase equilibrium concentration to attain the final K_d values[7].

The starting aqueous portion can be manipulated to test a water condition variable's effect on K_d values, such as salinity and pH, while using the same procedures above. For the salinity variation experiment, 250 mL of starting 100 ppb microcystin solution was diluted from the working solution. The 100 ppb solution was split into five 50mL portions and each of these was adjusted to a varying salinity: 0, 8.75, 17.5, 26.25, and 35 parts per thousand using Instant Ocean salt mix. This variation represents a range from the freshwater salinities to pure saltwater salinity. Three experimental sets of the 5 different salinity values were made in the test tubes, each test tube containing 10mL of the MC-LR spiked/salinity adjusted aqueous solution and 2 grams of dried sediment. Then three control sets of the 5 different salinity values were also made as blanks without sediment to ensure the microcystin was not degrading during the course of the experiment. The experimental parameters and data analysis from here are the same as described previously in section 2.5.

2.6 Salinity matrix effects

Given that the ending aqueous solutions that were to be run through the mass spectrometer were of varying salinities, an experiment was designed to test for any matrix effects from salinity. Working solution was diluted to a 200 ppb microcystin solution and portions were split and varied to the five salinity points. Therefore, each salinity point vial was nominally 200 ppb, but goal was to see if the instrument would also detect 200 ppb at each salinity given a possible salt content matrix effect with electrospray ionization. Three trials of this experiment were performed to attain an understanding of possible signal adjustments.

2.7 Further Analysis of Sediments

During the analysis of experimental data, it was concluded that more needed to be known about the possible molecular interactions at work as microcystin adsorbs to sediment. A series of experiments were designed to try and fill in this knowledge gap that has not been described in previous literature. This included insight on the interaction between the sediment and microcystin when adsorbed, stability of microcystins on sediment particles, and desorption mechanisms.

2*.7.1 Preliminary Adsorption-desorption experiment*

The set up for this experiment included a screw cap 15 mL glass test tube containing 2 grams of dried sediment and 10 mL of 45 ppb microcystin-LR solution. These were allowed to reach equilibrium over a course of four hours then the remaining solution was decanted off the sediment. Three rinse stages were done to ensure non-adsorbed microcystin would be rinsed off. Each rinse was 10mL of water, the test tube was shaken for 15 minutes, and the rinse solution was decanted off for analysis. Then 75% MeOH was used to try and remove the previously adsorbed microcystin, as an extraction solvent for the microcystin on the sediment. Data analysis was done to see the mass of microcystin adsorbed, mass rinsed off with water, and mass desorbed with the extraction solution.

2.7.2 Comparing adsorption-desorption to a control

After initial results were analyzed of the preliminary experiment, a set of experiments were designed to test the microcystin adsorption-desorption parameters and methods against a control substitute for sediment. Silica gel was used as an invariable control adsorbate to compare to the natural environmental sample of sediment used. A total of four experiments were done, two sets of side by side experiments that were meant to better understand the results of the first

preliminary experiment. The first set was done the same way as the preliminary experiment but tested the method on sediment adsorption-desorption by comparing extending out the rinse cycles to eight (from three) before using the extraction solution to ridding all the rinse cycles and immediately using the extraction solution. The second set of experiments then switched out silica get as a control adsorbent for the sediment in the first set of experiments. This gave a total of four experiments of equal conditions to compare various ways.

Table 2.1: Showing the two factor experiment where both factors could be considered separately

	Eight rinses then extraction solution	Extraction solution only
Sediment	A	C
Silica Gel	B	D

2.8 Degradation of free toxin with the Fenton Reaction

The Fenton reaction is a radical forming reaction initiated by combining hydrogen peroxide with iron (II) sulfate[20]. Before degradation of microcystin adsorbed to sediments can be studied, a baseline degradation capability of free toxin with the Fenton reaction was pursued. The experiment started with 10mL of diluted working solution of microcystin solution that was quantified at 36 ppb MC-LR. 20uL of 0.16mM hydrogen peroxide solution and 100uL of 0.54mM iron (II) sulfate solution was added to the microcystin solution[20]. The reaction was contained in a flask, stirred constantly, and left to react in dark conditions for 2 hours. After the reaction time, the radical formation processes were quenched with 200uL of 0.1M sodium

sulfite[20]. Before and after Fenton reaction MC-LR concentrations were compared in order to show if microcystin was successfully degraded.

2.9 In-situ remediation

To test if this degradation would be effective once microcystin was adsorbed to sediment, an experimental and blank method were set up. First, sediment was fully saturated with microcystin. This was achieved by adding 20 grams of dried sediment to 50mL of approximately 100 ppb microcystin solution. The solution was allowed to reach equilibrium after 4 hours of stirring and starting/ending concentrations were taken. The capacity of the sediment was determined to be 4.43 mg microcystin per kg sediment (4.43 mg/kg). Then experimental procedures split into two pathways. A 2 gram portion of the retained saturated sediment was exposed to the same Fenton reaction procedures as described above. A concurrent blank experiment had a 2 gram sediment portion exposed to the 10mL of MilliQ water instead of the Fenton reagents. After the 2 hour reaction times, both the experimental and blank sediment samples were extracted for microcystin using 10mL of 0.1M Sodium pyrophosphate/EDTA solution. Comparison of the two will show if less microcystin was extracted after the Fenton reaction exposure than the blank extraction to demonstrate that the reaction did degrade microcystin adsorbed to sediments.

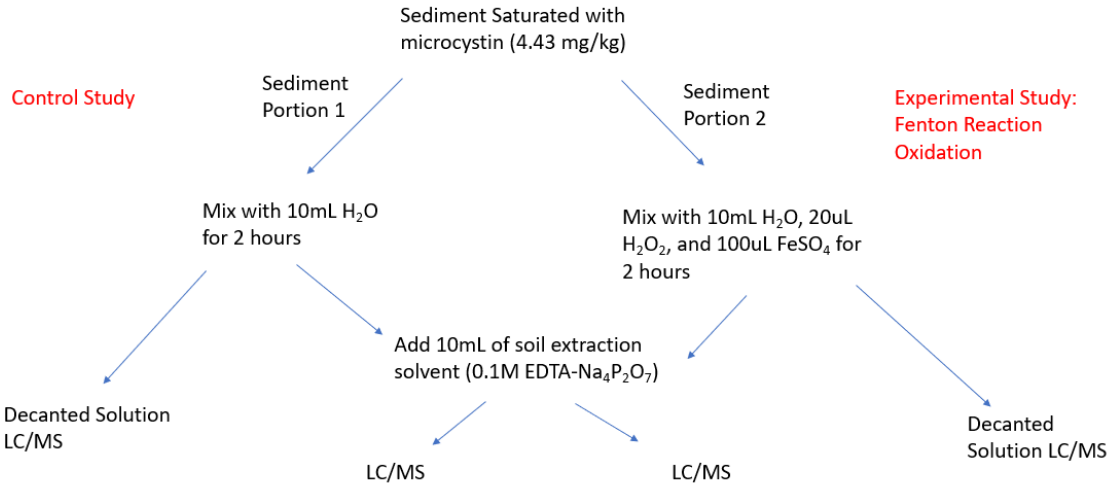

Figure 2.2: Experimental schematic of testing the Fenton reaction as a means of in-situ remediation of microcystin adsorbed to sediment.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Fugacity

Using the necessary physio-chemical properties of microcystin-LR and the fugacity modelling equations derived from 'Finding Fugacity Feasible", it was predicted that approximately 9.1% of introduced MC-LR into the system would end up adsorbed to sediments and 91% would be adsorbed to soil (see cells F5 and F7 of excel data).

A caveat of this modelling system is it cannot account for which compartment the contaminant is introduced. For example, microcystin can only enter the system through the water compartment as it is an algal cellular product, however, soil is not a phase that as readily interfaces with water as sediment would. Therefore, movement between these phases in a true environment is different than the constraints governed in the unit world model. Given this, soil and sediment values are combined to make a the "sorbed phase" in which over 99% of introduced microcystin-LR would end up.

These results would not include any natural degradation in the water column. While it is understandably likely that before 99% of introduced microcystin could be adsorbed to sediments a percentage would degrade, however, the model is still useful in demonstrating that microcystin has an overwhelming tendency to adsorb to sediments when possible and solidifies the need to further study sediments as microcystins sinks.

	A	B	C	D	E	F
1	Compartments	Z	V	Z times V	mols	%
2	Air	4.1E+01	1.0E+10	4.1E+11	1.0E-39	1.00547E-40
3	Water	6.6E+36	1.0E+06	6.6E+42	1.6E-08	1.62912E-09
4	Suspended solids (water)	1.8E+43	1.0E+06	1.8E+49	4.5E-02	4.54525E-03
5	Sediments	3.7E+48	1.0E+04	3.7E+52	9.1E+01	9.09050E+00
6	Biota	1.6E+36	1.0E+06	1.6E+42	4.0E-09	3.99902E-10
7	Soil	3.7E+48	1.0E+05	3.7E+53	9.1E+02	9.09050E+01
8	SUM		1.0E+10	4.1E+53	1.0E+03	1.00000E+02
9						
10	moles	1.0E+03				
11	fugacity	2.5E-51				

Figure 3.1: An excel spreadsheet showing the setup of the compartments, fugacity related variables, and finally the percentage of introduced microcystin-LR that would be found in each phase.

3.2 Toxin work up on Microcystis sample

The EPA method 544 for the six most common microcystin congener's chromatograms are shown in figure 3.2. Based off this data, the microcystis samples used for this project contain MC-LR, MC-LF, MC-YR, and MC-RR. The intensity is highest for MC-LR and the same trend is seen when signal is translated to concentrations based on the MC-LR pure standard calibration curve. MC-LR is present in the sample at 0.19 ug toxin per mg of freeze-dried algae, MC-RR at 0.06 and MC-YR at 0.05. While these toxin values were interpreted using the less sensitive method, they can still be used to compare the relative amounts of different toxins. These results demonstrate that using MC-LR as a representative congener is appropriate.

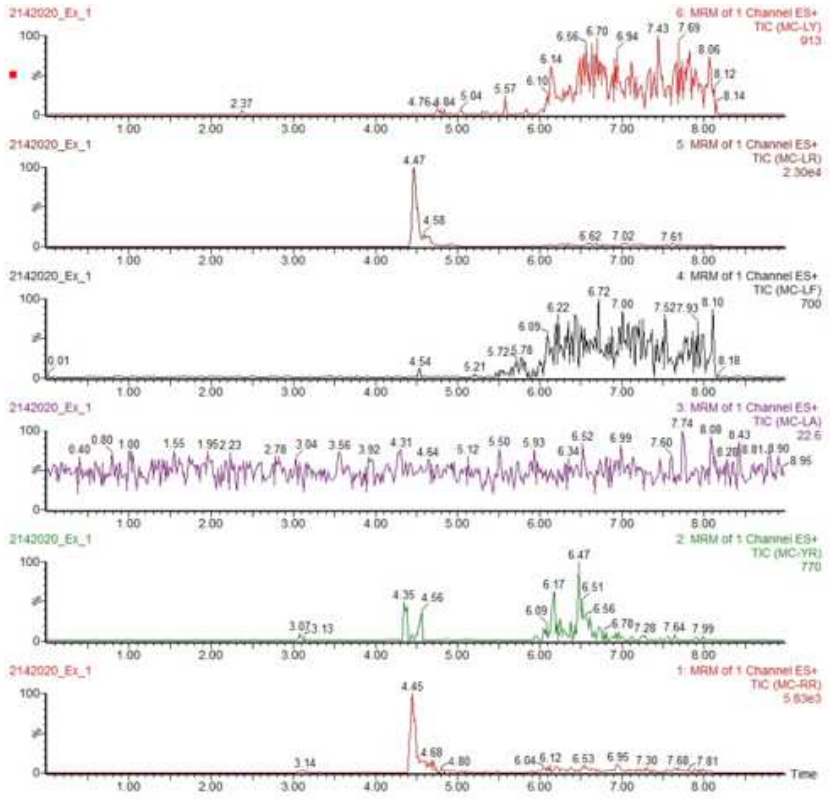

Figure 3.2: Chromatographs for each of the six congeners from EPA method 544 (from top to bottom: MC-LY, MC-LR, MC-LF, MC-LA, MC-YR, MC-RR).

3.3 MC-LR calibration curves

The dynamic range of concentrations needed for this project is large in order to encompass the different experiments planned to include high concentrations for quantifying working solutions to the lower concentrations expected in the degradation studies. Therefore, two separate calibrations curves were made to make each curve for accurate with its smaller dynamic range.

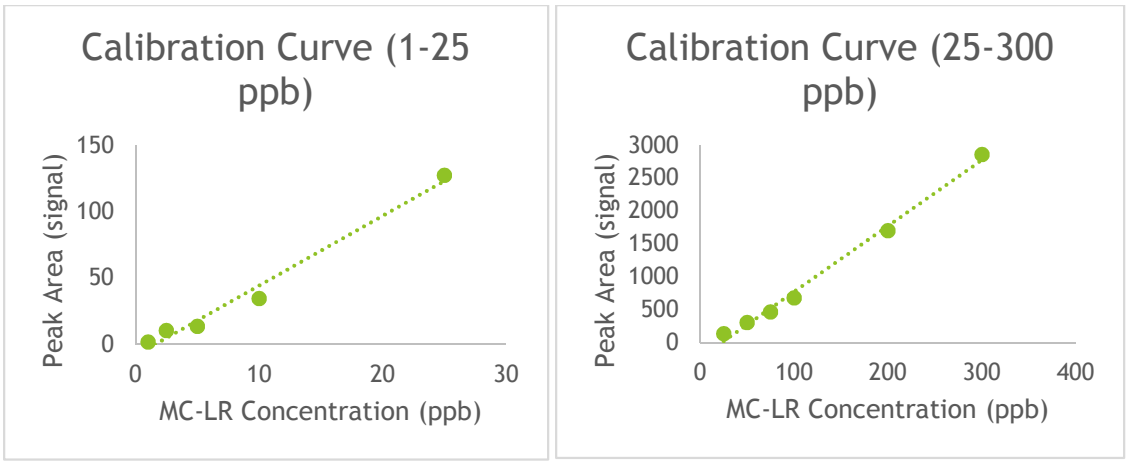

Figure 3.3: Calibration curves for 1-25 ppb MC-LR (left) and for 25-300 ppb MC-LR (right)

3.4 Salinity matrix effect

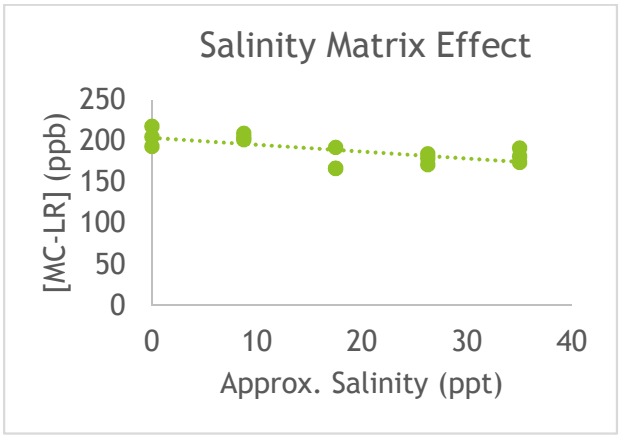

Figure 3.4: A graph showing the change of quantified concentration from instrument response versus the salinity of the sample

Based on the data shown, increased matrix salinity is suppressing the signal of MC-LR. Each salinity point is nominally 200 ppb however the calculated concentration based off of instrument response decreased as salinity increased. As salts in the matrix increase and compete for ionization against the analyte, the ionization rate of the analyte would decrease. Given that mass spectrometry can only detect ionized molecules as signal, the

signal would decrease leading to a lower quantified concentration as matrix salinity increased.

Figure 3.4 was then used as a calibration curve to correct for the effects of salinity on the instrument response. Correction factors at each salinity value were made from the linear regression equation and these correction factors were used to adjust experimental signal values for samples based on salinity.

Table 3.1: Correction factors used at each of the five different salinity points measured

Salinity (ppt)	Correction Factor
0	0.981
8.75	1.017
17.5	1.056
26.25	1.099
35	1.145

3.5 K_d vs. salinity experiment

The K_d values at varied salinity experiment's results are represented in figure 3.5. Based off the present data there does not seem to be a trend between K_d and salinity. However, a change in salinity is showing at least an impact on the K_d values across the board. This is somewhat minimized due to the error shown in the graph, as natural variation in experimental parameters such as sediment is expected making experimental errors indisputably higher.

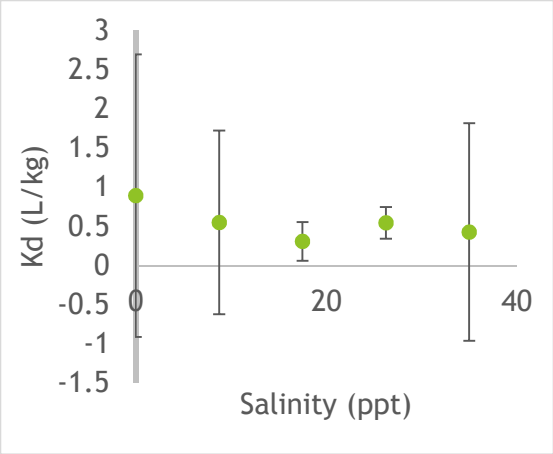

Figure 3.5: Experimental results of investigating how the change of matrix salinity affects the K_d value.

Traditional factor-response studies involve varying a single variable at a time and measuring its change of response, much like the K_d vs. salinity work presented. While various single factor response experiments could be performed each relating salinity, pH, and temperature etc. to K_d independently, there is a oversimplification with this approach. It does not allow the consideration that there are synergistic combinations between multiple variables that can push the response further than any single variable could. In a true environmental system, there is a continuous flux of factors that can both change independently or interact with one another. Therefore, future exploration of how to predict a change in K_d that impacts how microcystins are released from sediments needs to be based off a multivariate analysis of the influencing factors.

The ultimate goal is to end up with an equation similar to $K_df = a_1x + a_2y + a_3z + b$ where K_df is the functional K_d, x,y, and z are the studied influencing factors, a is the slope (contribution of factor to K_df), and b is the equation intercept. The variables salinity, temperature, and pH should be initially studied to understand how the

combination of these parameters is expected to modify K_d, although the list of variables is expected to grow to include different sediment properties and other water quality conditions.

3.6 Additional adsorption/desorption studies

3.6.1 Preliminary adsorption-desorption experiment

Table 3.2: Preliminary adsorption-desorption experiment rinse data

Trial	Rinse 1 (ug)	Rinse 2 (ug)	Rinse 3 (ug)
1	0.117	0.105	0.104
2	0.112	0.106	0.106
3	0.113	0.108	0.105
Average	0.114	0.106	0.105

Table 3.3: Preliminary adsorption-desorption experiment complete data

Trial	Total adsorbed (ug)	Total Rinsed (ug)	Total desorbed (ug)
1	0.335	0.326	0.043
2	0.330	0.324	0.040
3	0.339	0.326	0.048
Average	0.335	0.325	0.043

It was expected that the concentration (and therefore mass of microcystin) in the rinse solution would decrease by about a factor of 10 each time, given that the rinse volume was 10 mL and the sediment slurry was about 1 mL liquid. Each of the three rinse cycles (Table 3.2) all removed about the same mass of microcystin off the sediment suggesting microcystin is repeatedly being desorbed from the sediment by solely a water rinse, but that microcystin was adsorbed initially rather than being in the sediment slurry.

Looking at the complete data set for this preliminary experiment (Table 3.3), at the point the extraction solution was used to desorb the microcystin, the water rinses had

already removed at least 95% of the initially adsorbed microcystin. This minimized the ability to investigate the efficiency of the extraction solution as there was little microcystin left to desorb.

The point of this experiment was to start to understand how microcystin is adsorbed by looking at how it comes back off the sediment. The takeaways from this initial experiment were the need to increase the number of rinses to look at the continuality of rinsing microcystin, increase capacity of sediment by increasing the starting concentration of microcystin, and to add a comparative test showing desorption with no rinses. Also, it was clear that an adsorbent with no natural variation was needed (silica gel) to repeat the new parameters with a type of reference control experiment.

3.6.2 Additional comparative experiments

Table 3.4: Experiments alphabetically labeled within the two factor comparative experiment

Adsorbent	Eight rinses then extraction	Extraction only
Sediment	A	C
Silica Gel	B	D

Table 3.5: Experiment A rinse data- Using sediment as the absorbent and having 8 rinse cycles before extraction

Trial	Rinse 1 (ug)	Rinse 2 (ug)	Rinse 3 (ug)	Rinse 4 (ug)	Rinse 5 (ug)	Rinse 6 (ug)	Rinse 7 (ug)	Rinse 8 (ug)
1	0.176	0.166	0.134	0.170	0.114	0.114	0.107	0.107
2	0.197	0.163	0.149	0.166	0.142	0.117	0.109	0.109
3	0.157	0.164	0.143	0.152	0.140	0.120	0.115	0.107
Average	0.177	0.164	0.142	0.162	0.132	0.117	0.110	0.108

Table 3.6: Experiment A complete data- Using sediment as the absorbent and having 8 rinse cycles before extraction

Trial	Adsorbed (ug)	Total Rinsed (ug)	Total desorbed (ug)
1	1.421	1.087	0.052
2	1.397	1.152	0.048
3	1.397	1.098	0.051
Average	1.405	1.113	0.050

For experiment A, extending the rinses out to eight cycles continued to desorb microcystin in the same trend as the three rinses in section 3.6. While they decreased by a factor of about 2 overall over the eight rinses, this is far from the expected 10 fold dilution at each rinse cycle. Again, at the point of using the extraction solution, much of the microcystin had already been desorbed with the rinses. However, in this experiment there was still about 30% left and only 10% of what was left was desorbed with the extraction solution. This allows the differentiation between the extraction solution not working because of too little microcystin available and the efficiency of the extraction solvent itself. The results show that even when there is microcystin able to be desorbed, the 75% MeOH is not very effective at desorbing it. While 75% MeOH as an extraction solvent for microcystin on sediment has been successfully studied in previous literature[8], experiment A is showing the overall method used is not to par.

Table 3.7: Experiment B complete data- Using silica gel as the absorbent and having 8 rinse cycles before extraction

Trial	Adsorbed (ug)	Total Rinsed (ug)	Total desorbed (ug)
1	1.272	1.011	0.193
2	1.233	1.171	0.211
3	1.199	1.158	0.218
Average	1.235	1.114	0.207

Table 3.8: Experiment B rinse data- Using silica gel as the absorbent and having 8 rinse cycles before extraction

Trial	Rinse 1 (ug)	Rinse 2 (ug)	Rinse 3 (ug)	Rinse 4 (ug)	Rinse 5 (ug)	Rinse 6 (ug)	Rinse 7 (ug)	Rinse 8 (ug)
1	0.179	0.146	0.145	0.132	0.109	0.101	0.099	0.100
2	0.200	0.196	0.165	0.173	0.123	0.109	0.102	0.104
3	0.194	0.179	0.176	0.170	0.125	0.107	0.105	0.102
Average	0.191	0.174	0.162	0.158	0.119	0.106	0.102	0.102

Experiment B, substituting in silica gel for sediment and continuing with eight rinses, was completed to compare against experiment A. Here the microcystin was also rinsed off dramatically with the rinse cycles, against the intended 10 fold dilution trend. However, the 75% MeOH seems to have removed near 100% of the microcystin left behind after the rinses. This is much different than the 10% extraction efficiency seen with the sediment in experiment A. It can be noticed in the average value that the amount adsorbed in less than what was removed between the rinsed and desorbed. This is attributed to method and instrumental error that becomes more noticeable as you approach 100% additive values. It is concluded with experiment B that while water is still taking off microcystin against what is expected, the extraction solution was able to remove microcystin from the silica gel.

Table 3.9: Experiment C complete data- using sediment as the adsorbent and having no rinse cycles before extraction

Trial	MC adsorbed (ug)	MC desorbed (ug)
1	1.393	0.153
2	1.387	0.158
3	1.357	0.144
Average	1.379	0.151

Experiment C data showed that immediately following the adsorption with the extraction solution yielded about a 10% removal efficiency for microcystin on sediment. Although there is error in this value due to non-adsorbed microcystin leftover in sediment slurry solution that gets accounted for in this experimental method, this experiment was meant to be compared to experiment A. Given that the extraction solution was no better at removing microcystin than the first water rinse in experiment A, it is concluded that the experimental conditions are not optimal to use 75% methanol as an extraction method.

Table 3.10: Experiment D complete data- using silica gel as the adsorbent and having no rinse cycles before extraction

Trial	MC adsorbed (ug)	MC desorbed (ug)
1	1.191	0.559
2	1.146	0.746
3	1.100	0.676
Average	1.146	0.660

Experiment D is showing the extraction solution to be about 50% efficient for removing microcystin adsorbed to silica gel. This is about the same removal efficiency cited in literature for 75% methanol rinse to remove microcystin from sediment.

In conclusion on the study of microcystin adsorption to sediments, the composite of results show that fugacity may not be an appropriate modelling method for microcystin fate and transport. With an initial prediction of 99% of microcystin adsorbing to sediment, the K_d value would be approximately 100. However, the experimental K_d values (figure 3.5) only ranged from 0 to 1 L/kg. The mechanism of adsorption for microcystin to sediments may not be simply nonpolar interactions and associations (as fugacity assumes) but instead ion exchange or metal-ligand complexes between the

microcystin and components of the sediment. This is also supported by the MeOH rinse not removing adsorbed microcystin because MeOH would be expected to disrupt hydrophobic interactions between the microcystin and sediment. Then, further strengthened by the contrast of removal efficiency when microcystin was adsorbed to silica gel. Gaining insight on the best supported adsorption mechanism of microcystin to sediments will be crucial in investigating the effects of temporal microcystin accumulation and single release events.

Future work will also involve correlating the results produced to sediment characteristics such as cation exchange capacity and organic content. The pH should be investigated in the future and the starting aqueous solution should be more basic to match natural systems. The pH difference may not be allowing microcystin to adsorb to sediment as it would in nature and how previous literature shows microcystin highly stable on sediments.

3.7 Fenton reaction degradation

Degrading free toxin in solution proved successful by the Fenton reaction with more than 90% removal under all tested conditions. The concentrations progressed from 36 ppb MC-LR in the starting solution to all less than or equal to approximately 4 ppb in all trials.

Since future experiments to test potential remediation strategies will rely on understanding how to ensure chemical degradation outpaces the natural degradation of toxin adsorbed to sediments, it would be useful to establish a baseline of natural degradation in laboratory conditions. This would entail setting up parallel experiments to

test the change in concentration over time of free toxin in solution compared to the change in concentration over time of toxin adsorbed to sediments.

Table 3.11: Shows the change in concentration of MC-LR in each of the four trials when exposed to the Fenton reaction reagents

Trial	Starting Concentration	Ending Concentration
1	36 ppb	<1 ppb
2	36 ppb	4 ppb
3	36 ppb	<1 ppb
4	36 ppb	1.5 ppb

3.8 Testing degradation on sediment

Table 3.12: Results of the control and experiment tests assessing if the Fenton reaction is degrading microcystin adsorbed to sediments.

	Fenton Reagents Added		Blank experiment	
Trial	10 mL of reagents	Extraction Solution	10 mL blank	Extraction Solution
1	<1ppb	<1ppb	<1ppb	<1ppb
2	<1ppb	<1ppb	<1ppb	<1ppb

Based on this data it was concluded that the sediment extraction technique of using 0.1 M sodium pyrophosphate/EDTA was ineffective even though it had performed exceptionally well against other extraction solvents studied in the published paper. This conclusion comes from knowing the sediment has 4.43 mg toxin adsorbed per kg

sediment and even the blank extraction did not show any microcystin being pulled off the sediment. The 10 mL reagent and blank solutions were originally saved to ensure mass balance was preserved, but these also came back as less than 1ppb demonstrating that microcystin did not come off early during the experiment. Work should be done to test new extraction solvents to desorb microcystin back off of sediments. This will complement as the extraction solvent needed for additional adsorption-desorption work as discussed in section 3.6.

While the Fenton reaction showed potential to degrade microcystin, the application to natural sediments would be difficult given there are two liquid reagents that must be combined to form the reaction. Also, this is a free radical producing reaction meaning further propagation would rely on reagents staying in proximity and in a real system these would likely be quickly quenched. Therefore, future degradation work will focus on using MnO_2 to oxidize and degrade microcystin adsorbed to sediments. A solid could more easily be distributed onto and within contaminated sediments plus MnO_2 has the potential to be co-adsorbed onto the sediment, possibly being highly selective in degrading microcystin as mentioned in the introduction. The same initial experiments used with the Fenton reaction will be adapted to MnO_2 use, first ensuring the solid can degrade free toxin and then moving to degradation of adsorbed toxin.

REFERENCES

(1) Jang, M.-H.; Ha, K.; Jung, J.-M.; Lee, Y.-J.; Takamura, N. Increased Microcystin Production of Microcystis aeruginosa by Indirect Exposure of Nontoxic Cyanobacteria: Potential Role in the Development of Microcystis Bloom. *Bulletin of Environmental Contamination and Toxicology* **2006**, *76* (6), 957–962.

(2) Chen, W.; Song, L.; Peng, L.; Wan, N.; Zhang, X.; Gan, N. Reduction in microcystin concentrations in large and shallow lakes: Water and sediment-interface contributions. *Water Research* **2008**, *42* (3), 763–773.

(3) de Figueiredo, D. R.; Azeiteiro, U. M.; Esteves, S. M.; Gonçalves, F. J. M.; Pereira, M. J. Microcystin-producing blooms—a serious global public health issue. *Ecotoxicology and Environmental Safety* **2004**, *59* (2), 151–163.

(4) Meneely, J. P.; Elliott, C. T. Microcystins: Measuring human exposure and the impact on human health. *Biomarkers* **2013**, *18* (8), 639–649.

(5) Waters, M. N. A 4700-Year history of CYANOBACTERIA Toxin production in a Shallow Subtropical lake. *Ecosystems* **2015**, *19* (3), 426–436.

(6) Zastepa, A.; Taranu, Z. E.; Kimpe, L. E.; Blais, J. M.; Gregory-Eaves, I.; Zurawell, R. W.; Pick, F. R. Reconstructing a long-term record of microcystins from the analysis of lake sediments. *Science of The Total Environment* **2017**, *579*, 893–901.

(7) Miller, M. J.; Critchley, M. M.; Hutson, J.; Fallowfield, H. J. The adsorption of cyanobacterial hepatotoxins from water onto soil during batch experiments. *Water Research* **2001**, *35* (6), 1461–1468.

(8) Wu, X.; Xiao, B.; Li, R.; Wang, C.; Huang, J.; Wang, Z. Mechanisms and Factors Affecting Sorption of Microcystins onto Natural Sediments. *Environmental Science & Technology* **2011**, *45* (7), 2641–2647.

(9) Liu, G.; Qian, Y.; Dai, S.; Feng, N. Adsorption of Microcystin LR and LW on Suspended Particulate Matter (SPM) at Different pH. *Water, Air, and Soil Pollution* **2008**, *192* (1-4), 67–76.

(10) Total assessed waters. U.S. EPA 2017 https://ofmpub.epa.gov/waters10/attains_nation_cy.control#total_assessed_waters

(11) USEPA, National lakes assessment: A collaborative survey of the nation's lakes. In USEPA, Ed. Office of Water and Office of Research and Development: Washington DC, 2009, Vol. EPA 841-R-09-001.

(12) Dolah, F. M.; Roelke, D.; Greene, R. M. Health and ecological impacts of harmful algal blooms: Risk assessment needs. *Human and Ecological Risk Assessment: An International Journal* **2001**, *7* (5), 1329–1345.

(13) Falconer, I. R. An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. *Environmental Toxicology* **1999**, *14* (1), 5–12.

(14) Carmichael, W. W. Health effects of toxin-producing cyanobacteria: "the cyanohabs." *Human and Ecological Risk Assessment: An International Journal* **2001**, *7* (5), 1393–1407.

(15) A. de la Cruz, A.; G. Antoniou, M.; Hiskia, A.; Pelaez, M.; Song, W.; E. O'Shea, K.; He, X.; D. Dionysiou, D. Can we Effectively Degrade MICROCYSTINS? -

implications on human health. *Anti-Cancer Agents in Medicinal Chemistry* **2011**, *11* (1), 19–37.

(16) Chen, W.; Song, L.; Peng, L.; Wan, N.; Zhang, X.; Gan, N. Reduction in microcystin concentrations in large and shallow lakes: Water and sediment-interface contributions. *Water Research* **2008**, *42* (3), 763–773.

(17) Song, H.; Coggins, L.; Reichwaldt, E.; Ghadouani, A. The importance of lake sediments as a pathway for MICROCYSTIN dynamics in Shallow EUTROPHIC LAKES. *Toxins* **2015**, *7* (3), 900–918.

(18) Edwards, C.; Graham, D.; Fowler, N.; Lawton, L. A. Biodegradation of microcystins and nodularin in freshwaters. *Chemosphere* **2008**, *73* (8), 1315–1321.

(19) Paerl, H. W. 2016. Impacts of Climate Change on Cyanobacteria in Aquatic Environments. Caister Academic Press: Wymondham, p 5-22.

(20) Bandala, E. R.; Martínez Dolores; Martínez Evaristo; Dionysiou, D. D. Degradation of Microcystin-lr toxin by Fenton and Photo-Fenton processes. *Toxicon* **2004**, *43* (7), 829–832.

(21) Zhang, J. B.; Zheng, Z.; Yang, G. J.; Zhao, Y. F. Degradation of microcystin by gamma irradiation. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* **2007**, *580* (1), 687–689.

(22) Qiao, R.-P.; Li, N.; Qi, X.-H.; Wang, Q.-S.; Zhuang, Y.-Y. Degradation of microcystin-rr by uv radiation in the presence of hydrogen peroxide. *Toxicon* **2005**, *45* (6), 745–752.

(23) Wang, Y.; Cao, Y.; Li, H.; Gong, A.; Han, J.; Qian, Z.; Chao, W. Removal of mcs by bi2o2co3: Adsorption and the potential of photocatalytic degradation. *Environmental Science and Pollution Research* **2018**, *25* (12), 11867–11874.

(24) Gupta, N.; Pant, S. C.; Vijayaraghavan, R.; Rao, P. V. L. Comparative toxicity evaluation Of CYANOBACTERIAL cyclic peptide toxin MICROCYSTIN variants (LR, RR, yr) in mice. *Toxicology* **2003**, *188* (2-3), 285–296.

(25) Mackay, D. Finding fugacity feasible. *Environmental Science & Technology* **1979**, *13* (10), 1218–1223.

(26) MEHLICH, A. Determination of cation- and anion-exchange properties of soils. *Soil Science* **1948**, *66* (6), 429–446.

(27) Barančíková, G.; Makovníková, J. COMPARISON OF TWO METHODS OF SOIL ORGANIC CARBON DETERMINATION. *Polish Journal of Soil Science* **2016**, *48* (1), 47.

(28) Peter Moeller, personal communication.

(29) Shoemaker, J., Dan Tettenhorst, and A. Delacruz. METHOD 544. DETERMINATION OF MICROCYSTINS AND NODULARIN IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS). U.S. Environmental Protection Agency, Washington, DC, 2015.