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Harmful Algal Blooms: Dominance in Lakes and Risk for Cyanotoxin Exposure in Food Crops

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HARMFUL ALGAL BLOOMS: DOMINANCE IN LAKES AND RISK FOR CYANOTOXIN EXPOSURE IN FOOD CROPS

by

Austin D. Bartos

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ABSTRACT

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Cyanotoxin Exposure in Food Crops

by

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Utah State University, 2020

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Harmful algal blooms (HABs) producing cyanotoxins have increased worldwide in the past decade and threaten human and ecosystem health. HABs are stimulated by climate and anthropogenic changes to the environment, which are projected to worsen over time. We combined meta-analyses of existing data and experimental trials to evaluate the changes in HAB frequency and the potential risk to humans through novel exposure pathways. In 2012, nearly 45% of lakes in the United States experienced a HAB that produced quantifiable cyanotoxins - an increase of over 12% from 2007. Total nitrogen and phosphorus concentrations were the most important drivers of HAB frequency and toxicity in nearly every region of the United States. As lake waters are often used for the irrigation of food crops, increased HABs intensifies the risk of exposure to cyanotoxins via accumulation in plants. Our soil study showed that soils can render some of the cyanotoxins temporarily unavailable for plant uptake through sorption promoted by various physical and chemical soil characteristics and subsequent

degradation. We tested eight soils for their sorption capacity and soil-water partition coefficients (K_d) ranged from 1.35 to 10.73 L kg^{-1} for neurotoxic *beta*-Methylamino-L-alanine (BMAA) and 1.59 to 5.03 L kg^{-1} for hepatoxic microcystin-LR. A greenhouse experiment tested the capacity for cyanotoxin uptake into lettuce by irrigating with one of three cyanotoxins, microcystin-LR, nodularin, and BMAA at three environmentally relevant concentrations. We were unable to quantify exact cyanotoxin concentrations in our lettuce due to analytical limitations. A review of previous literature revealed a strong correlation ($R^2 = 0.70$) between irrigation water toxin concentration and the concentration in fruit and grains. Cyanotoxin concentrations in edible roots and leaves of crops had stronger relationships with toxin concentration ($R^2 = 0.19 – 0.23$) than their inedible counterparts ($R^2 = 0.04$), which suggests differing uptake mechanisms between plant parts. Eutrophication mitigation strategies and the monitoring of irrigation waters for cyanotoxins are management actions that help reduce the risk of HABs and cyanotoxin exposure to humans through food crops. However, more research on analytical methods development is required to fully understand the uptake and translocation mechanisms within plants for all relevant cyanotoxins.

(100 pages)

PUBLIC ABSTRACT

Harmful Algal Blooms: Dominance in Lakes and Risk for Cyanotoxin Exposure in Food Crops

Austin D. Bartos

Climate change and human activities are promoting the dominance of a photosynthetic family of aquatic bacteria, cyanobacteria. Blooms of cyanobacteria are not only a visual nuisance but can produce a variety of cyanotoxins than can harm the liver, skin, and nervous system of animals and humans. We analyzed lakes in the contiguous United States and found that between 2007 and 2012, the number of lakes that produced measurable quantities of cyanotoxins increased from 33% to 45%. Nitrogen and phosphorus pollution were the main drivers of cyanobacteria blooms and toxin production between these years. Many of these lakes and reservoirs are used for crop irrigation and more frequent and toxic cyanobacteria blooms intensifies the risk of human and animal exposure to cyanotoxins through the consumption of toxic plants. We assessed how three cyanotoxins are distributed between soil, irrigation water, and lettuce plants to evaluate the exposure risk that cyanotoxins in food pose to human health. We found soil to sorb between 12 to 52% of two cyanotoxins from water, which could temporarily prevent the toxins from being taken up by plant roots and deposited into edible tissue. Also, we grew lettuce plants in a greenhouse and irrigated them with cyanotoxins. Cyanotoxins did not affect plant growth, however, we were unable to quantify the concentration of the toxins in the lettuce due to analytical limitations or that the plants were unable to sorb the toxins. Lastly, we analyzed the results from 14 published research studies on cyanotoxins in food irrigated with contaminated water. We

found significant relationships between cyanotoxin concentrations in the irrigation water and those measured in plant tissues. Generally, the more cyanotoxins in the irrigation water the more cyanotoxins are measured in plants. The increase in cyanotoxin producing blooms needs to be mitigated to reduce associated health and economic risks. Management and policies should be implemented that not only mitigate the drivers of cyanobacteria and their toxins but also places limits on the acceptable concentrations in irrigation water.

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Austin D. Bartos

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Lakes around the world have experienced an increase in harmful algal blooms (HABs) in recent decades. Many HABs produce cyanotoxins, which have toxic effects on both the ecosystem in which they exist and humans who encounter them via drinking water, recreation, or in food irrigated with cyanotoxin laden water. Effects of climate change and human activity drive the occurrence of HABs; ranging from increased temperatures and precipitation to greater nutrient loading into watersheds. The symptoms associated with climate change and human activities are only expected to worsen in the coming years, exacerbating the problems HABs impose on both water quality and human health. To mitigate the risks associated with greater HAB occurrence in lakes around the world, policies and management actions should be taken to curtail the drivers of HABs. However, the most influential drivers need to be identified to maximize the effectiveness of any proposed policy or management decision. To do this, both individual and collective waterbodies must be studied to allow for a comprehensive assessment of the mechanisms affecting HABs in these systems. Assessing individual lakes is both time consuming and expensive yet gives high-resolution data on their HABs. Adding together many singular lakes into a large dataset then provides a means to make broad conclusions about the relationship between a variety of environmental variables, whether they be chemical or physical, on HAB frequency and severity. The second chapter of this thesis leverages the United States Environmental Protection Agency's National Lakes Assessment from 2007 and 2012 to establish such large-scale relationships. The ability to target the most important factors that promote HABs on both a large and temporal scale

established a framework that can be built on with additional years of data and observations. This framework can then inform management and policy decisions aimed at reducing HABs on both national, regional, and individual scales.

Chapter 3 addresses a human's risk of exposure to cyanotoxins in food that was irrigated with water that contains the toxins. Because plants can accumulate cyanotoxins in their edible tissue by transporting from roots in contact with contaminated water they can contain enough cyanotoxins to pose a chronic health risk. The World Health Organization has set a total daily intake limit of 0.04 micrograms of a common cyanotoxin, microcystin-LR, per kilogram of the human's body weight. Exceeding this limit puts a person at risk for chronic liver damage. Therefore, determining the quantity of cyanotoxins various plants can accumulate from different concentrations in irrigation water is valuable information for food safety. With less than 30 studies in the past 3 decades, cyanotoxins in food are heavily understudied. Furthermore, at least 9 individual cyanotoxins are known to exist, with some having dozens of congeners. Chapter 3 adds to this body of research and highlights further knowledge gaps that need to be filled to accurately account for the risks cyanotoxins pose in a world with more and more HABs. When combined, both chapters in this thesis can help to inform policymakers and land managers on creating legislation and management decisions that can reduce the risk HABs and their toxins pose to the environment, economy, and human health.

CHAPTER 2

CHANGES IN ENVIRONMENTAL CONDITIONS AND THE IMPACT ON HARMFUL ALGAL BLOOMS ACROSS U.S. LAKES AND RESERVOIRS

Abstract

The frequency and severity of harmful algal blooms (HABs) have increased globally in the last decade and is expected to continue. At small spatial scales, principal drivers of HABs have been linked to climate and anthropogenic changes to the environment. However, few studies have identified the relationship between these mechanisms on a large or temporal scale. Even fewer studies separate by lake origin (natural or man-made) or ecoregion. The U.S. EPA National Lake Assessments (NLA) from 2007 and 2012 were combined to determine which environmental variables related to climate change or human influence were correlated to the HAB indicators: phytoplankton abundance, cyanobacteria biovolume, and bloom toxicity. A statistical model selection approach was used to generate linear models to explain the variance in these HAB indictors. Large differences were found in model strength between two lake origins and nine ecoregions when compared across all NLA lakes. Across both lake origins and nearly all ecoregions, HAB abundance and density have increased, but microcystin-LR concentration did not. Increases in HABs were more related to indicators of nutrient pollution, particularly nitrogen concentration, than by temperature. Generally, ecoregion and lake origin conditions associated with HABs showed decreases in the total nitrogen to phosphorus ratio ($p < 0.001$) and increases in epilimnion depth ($p = 0.003$). Further, man-made lakes experienced a greater increase in cyanobacteria biovolume and

HABs that produced ≥ 0.1 *u*g L^{-1} of microcystin-LR than their natural counterparts. Finally, the Xeric and Western Mountain ecoregions experienced the most drastic increases in HABs and the conditions that promote them. The widespread rise in HABs highlights the need for management to curtail HABs in many regions.

Introduction

The prevalence of harmful algal blooms (HABs) and their associated cyanotoxins in lakes is an increasing global issue that poses an environmental and human health hazard (Huisman et al. 2018; Ho et al. 2019). Cyanobacteria are a diverse assemblage of aquatic bacteria that can occur in blooms large enough to produce harmful quantities of cyanotoxins. These compounds can be moderately to severely toxic to humans and wildlife (Chen et al. 2016) leading to both acute and chronic neurotoxic and hepatoxic effects. Shallow water, warmer temperatures, and eutrophication promote toxin production (Huisman et al. 2018), however, HABs still occur in large deep lakes during periods of stratification of the water column.

Already, large and essential water sources have been affected by algal blooms. *Microcystis aeruginosa* blooms in the western basin of Lake Erie shut down the city of Toledo's water supply for three days (Steffen et al. 2014) and recurring *Dolichospermum circinale* (formerly *Anabaena)* and *Microcystis aeruginosa* blooms in Utah Lake degrade recreation water and may contaminate irrigation water (Wurtsbaugh and Marcarelli 2006). Not only can cyanobacteria blooms cause toxic drinking and irrigation water that may impact food crops, but blooms can decrease US recreation income and property values of waterfront land by approximately $2.2 billion annually (Dodds et al. 2009).

Increased bloom frequency has been linked to eutrophication and increases in lake temperature (O'Neil et al. 2012; Verspagen et al. 2014; Sandrini et al. 2016; Scholz et al. 2017; Huisman et al. 2018). Current estimates indicate that global mean surface temperatures have increased between 0.8 and 1.2 ℃ above pre-industrial levels and may be influencing harmful algal bloom frequency and intensity through several mechanisms (IPCC 2018). Cyanobacteria like *Microcystis* have a higher temperature optimum than most phytoplankton, allowing them to dominate reproduction in waters warmer than ~25 °C (Jöhnk et al. 2008). As water temperatures increase, stratification intensifies in the water column, which lengthens the optimal growing season for cyanobacteria. Additionally, some cyanobacteria can use stratification to shade out competitors by forming gas vesicles to float to the surface (Huisman and Hulot 2005; Jöhnk et al. 2008). IPCC climate models predict a further 0.3°C to 0.7°C increase in global mean surface temperatures between 2030 and 2052 which are likely to exacerbate bloom-forming conditions (IPCC 2018).

Cyanobacteria also outcompete other aquatic photosynthesizers in nutrient-rich waters due to several adaptations including rapid reproduction rates, the ability to fix nitrogen and acquire and sequester phosphorus (e.g. *Nodularin spumigena* and *Dolichospermum circinale*) (Elliott et al. 2006; Schindler et al. 2008; Beversdorf et al. 2013). Many HAB lakes are nitrogen-limited and the nitrogen-fixing ability of some cyanobacteria gives them a competitive advantage to outcompete other algae (Schindler 1974; Chaffin et al. 2011; Yang et al. 2017). In systems with low total nitrogen to total phosphorus mass ratios (< 29 grams nitrogen per gram phosphorus) cyanobacteria are more likely to dominate (Smith 1983; Havens et al. 2003; Huisman and Hulot 2005;

Orihel et al. 2012). Finally, some cyanobacteria can outcompete other aquatic photosynthesizers by utilizing and concentrating an additional carbon source, bicarbonate, in a specialized protein microbody, a carboxysome, where it is catalyzed into carbon dioxide for use in photosynthesis (Badger and Price 2003). Atmospheric carbon dioxide levels have increased to over 400 ppm, potentially supporting HABs (Sandrini et al. 2016).

Climate change will lead to increased air temperatures and intensified drought in many regions while increasing precipitation in others (IPCC 2013). In areas with increased precipitation frequency and intensity, more nutrient runoff from agriculture and urban landscapes is washed into lakes, promoting algal blooms (Trenberth 2011). Furthermore, if strong precipitation events that increase nutrient runoff are followed by decreased precipitation, the residence time of the nutrients increases, favoring cyanobacteria growth (Paerl and Huisman 2009; Michalak et al. 2013).

An assessment of past data to discover patterns in bloom occurrence and environmental variables can provide a greater understanding of how cyanobacteria blooms may increase in future climate and anthropogenic conditions, both globally and on a landscape level. Previous authors have used various large lake datasets to determine explanatory variables for cyanobacteria biovolume, biomass species composition, and toxin concentrations. However, most of these studies looked at only one year of observations and often used dependent variables in their HAB linear models, such as chlorophyll *a* content (Beaulieu et al. 2013; Rigosi et al. 2014; Yuan et al. 2014). The first study to combine two years of observations found nutrients (total phosphorus and nitrogen) and mean spring and summer air temperatures to have the strongest

relationships with the indicators of HABs (chlorophyll *a* concentration, cyanobacteria biovolume, and the concentration of microcystin-LR) (Ho and Michalak 2019). However, Ho & Michalak selected lakes sampled between June and August and for lakes that had measurable microcystin-LR concentrations (> 0.1 μg L^{-1}). To date, no studies have leveraged the importance of lake origin (natural and man-made) or ecoregion to better explain HAB distribution and severity. Natural and man-made lakes differ in location and basin characteristics, which can influence the lake's physical characteristics (e.g. size and depth) and chemical composition (e.g. nutrient inputs from local runoff) as well as their hydrology (e.g. timing of drawdown and hypolimnetic release). Also, different ecoregions can have varying climate effects and local land-use and management strategies that can affect HABs. Because different ecoregions will respond to climate and anthropogenic pressures differently, we examined the relationship between HAB indicators according to both lake origin and lake ecoregion.

The NLA datasets afford the opportunity to examine across space and time the relationship between cyanobacterial blooms and environmental drivers. In this study, we aim to answer the following questions:

1. What is the distribution of toxin-producing algal blooms (TPB) in the United States based on ecoregion and lake origin between years?
2. How have environmental conditions changed between 2007 and 2012 and do they have a greater explanation of variance on different origins of lakes and ecoregions?

3. Which environmental variables best explain HAB indicators: phytoplankton abundance (chlorophyll *a*), cyanobacteria biovolume, and cyanotoxin concentration (microcystin-LR) in the continental U.S.?

Methods

Datasets used

As part of the Natural Aquatic Resources Surveys (NARS), the U.S. Environmental Protection Agency completes the National Lakes Assessment (NLA) of lakes distributed across the United States measuring a variety of physical, cultural, and environmental variables. The assessment occurs every 5 years, with the most recent data available from 2007 and 2012. A total of 2249 lakes were sampled between the two study periods. A subset of lakes was sampled twice in both study periods and are treated as independent samples. A probability-based sample design was used to choose lakes and to ensure a representative set of lakes for the United States. We combined the two years of sampling to increase the statistical power of our models and to compare results to Ho & Michalak 2019. Lakes were defined as either naturally occurring or man-made. Further, lakes were sorted into nine new ecoregions designed specifically for the efforts of the NARS to normalize the quality of waters across the ecoregions (Herlihy et al. 2008). All analyses were completed on a national, lake origin, and ecoregion level (Table 2.1). The total number of lakes was reduced from 2249 to 1914 due to missing data for some variables.

Table 2.1. Lake categories used from the NLA dataset and their abbreviation.

Geographic Region	Abbreviation	*n*
National	U.S.	1914
Natural	NAT	852
Man-made	MAN	1062
Coastal Plains	CPL	219
Northern Appalachian	NAP	180
Northern Plains	NPL	123
Southern Appalachian	SAP	198
Southern Plains	SPL	217
Temperate Plains	TPL	292
Upper Midwest	UMW	297
Western Mountain	WMT	253
Xeric	XER	135

Created variables

Numerous environmental variables that may have a significant effect on the HAB indicators are not directly measured in the NLA data. Therefore, we extracted average air temperature and precipitation for four time periods of increasing resolution for the latitude and longitude of each lake from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) and added them to the NLA dataset (PRISM 2004). We calculated yearly, monthly, and seasonal average air temperatures. Spring temperatures were calculated for March, April, and May, and summer temperatures were calculated for June, July, and August. We also averaged air temperatures for the two weeks before the visit date for each lake and calculated the number of days (7, 14, 30, 50, 60, 75, and 100) before sampling that recorded a maximum temperature greater than 25 °C. The number of days exceeding 25°C before a bloom acted as a measure of temperature intensity to better represent the meteorological conditions immediately leading up to the day the lake was sampled. Due to the importance of the nitrogen to

phosphorus ratio in cyanobacteria dominance, we calculated the mass ratio from the total nitrogen and phosphorus concentrations in the NLA data. To account for the ability for cyanobacteria to thrive in stratified lakes, we calculated epilimnion and hypolimnion temperature, depth (m), volume (m^3), and energy content (calories per m^3). The distance from the water surface to the top of the metalimnion was considered the epilimnion and the distance from the maximum depth to the bottom of the metalimnion was the hypolimnion.

Toxin-producing blooms

To answer question one, a "toxin-producing" bloom (TPB) was defined as a lake with a microcystin-LR concentration of above the 0.1 μg L^{-1} detection limit reported by the EPA. A true or false value was assigned to each lake for each year based on the detection of microcystin-LR. The percentage of TPBs was visualized using point density maps within ArcGIS.

Temporal changes

To determine the changes in environmental variables between sampling years, a Welch Two Sample t-test was used to determine any significant increases or decreases. Temporal changes were determined for the national, lake origin, and ecoregion levels to identify spatial differences. Due to the high variability in some of the environmental variables, *p*-values are reported based on the level of significance. Further, the difference in median values for total phosphorus and nitrogen concentrations were compared to negate the impact of numerous outliers.

Model development

Linear models were developed to determine relationships between HAB indicators and environmental drivers. After combining the 2007 and 2012 NLA observations and the addition of PRISM metalogical data we had 83 variables (including natural log transformations) across 1914 lakes. All data manipulation and analysis were done in R statistical software (RStudio 2020). Due to the large range of values for some of the variables (e.g. total nitrogen and chlorophyll *a* concentration), we performed a natural log transformation of every numeric variable (including the HAB indicators) to increase their explanatory power and added the values to the existing data. A full list of explanatory variables is included in Table A.2.1. Least angle regression (LAR) was employed to reduce the number of explanatory variables for the untransformed and natural logarithmic transformation of the three HAB indicators. Models were created using variables identified by LAR above a set coefficient of significance ($p \leq 0.1$) and subjected to Akaike information criterion (AIC) comparisons. The model with the lowest AIC value was determined to be the best-fitting model. Untransformed and natural log-transformed HAB indicator models were compared with ANOVA and the best-fitting version became the final model.

Results

Distribution of toxin-producing blooms

Separating the NLA lakes by both lake origin and ecoregion exhibits differences in TPB distributions. On a national scale, TPBs between 2007 and 2012 increased from 33% to 45%. When categorized by origin, TPB in natural lakes rose from 40.1% to 45.4% and man-made reservoirs experienced a larger increase from 26.1% to 45.2%.

(Fig. 2.1). Both 2012 percentages for lake origins are nearly identical to the U.S. national percentage, with man-made lakes experiencing a 19% increase in TPB.

By ecoregion, all regions experienced an increase between the sampling years in TPB (Fig. 2.2) except for the Upper Midwest, which decreased by less than a percent. Most regions saw a similar increase in TPB percent as the national average of 12%, with the Southern Plains and Xeric having the largest increases of 36.4 and 23.5% respectively. The Northern Appalachians saw the smallest increase with only 4.4%. Overall, the Temperate Plains had the most lakes with TPB in 2012 (74%) and the Western Mountains had the fewest (16%).

Density plots of TPBs show clustering in the Upper Midwest, Northern Plains, and Temperature Plains in both years (Fig. 2.3). Generally, the density of TPBs is similar in both years as much of the increase is in singular lakes that are widely distributed around each ecoregion.

Temporal changes

Cyanobacteria

Cyanobacteria biovolume and microcystin-LR concentrations were compared between the two sampling years. Both man-made and natural lakes showed significant increases (Fig. 2.4) in cyanobacteria biovolume (210% and 155% respectively). Generally, cyanobacteria biovolume increased across all ecoregions but were only significant in the Temperate, Southern, and Northern Plains (Fig. 2.5).

Microcystin-LR concentrations did not change significantly between any of the lake origins, nor on the national level (Table 2.2). Neither lake origin differed significantly from each other in 2012 for either biovolume or microcystin-LR. The Upper

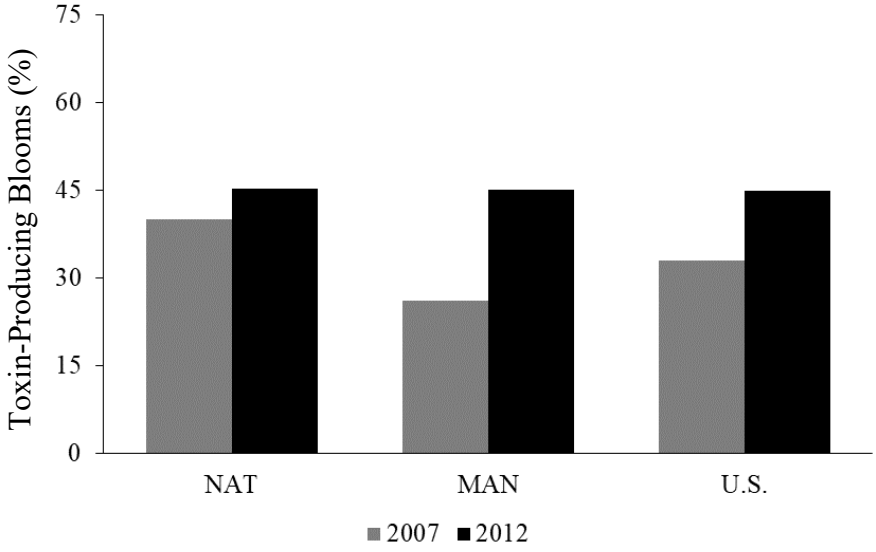

Fig. 2.1. Percent of toxin-producing blooms by lake origin: natural (NAT) and man-made (MAN) compared to the national change (U.S.).

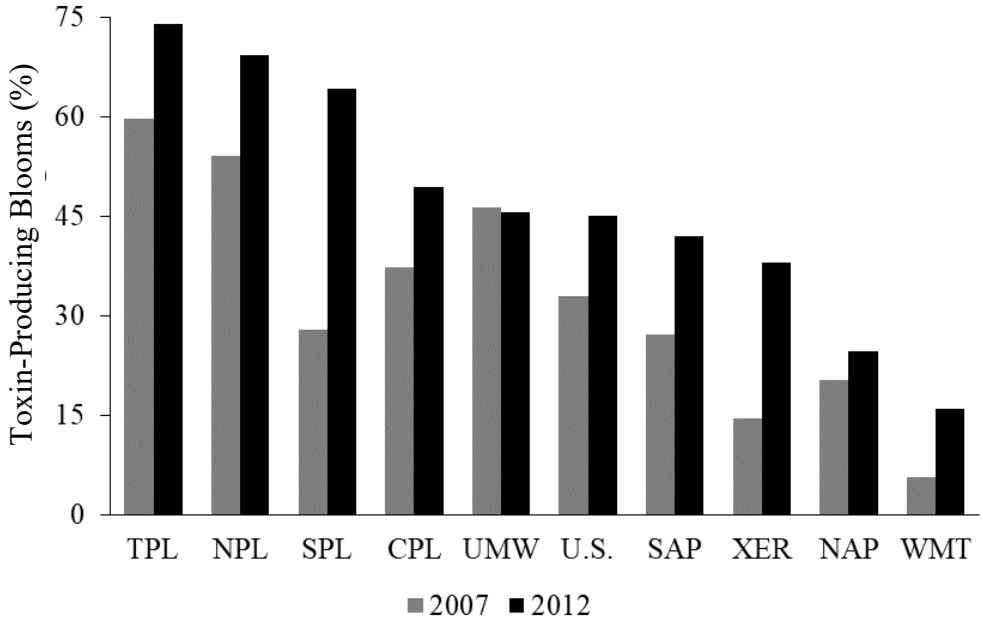

Fig. 2.2. Percent of toxin-producing blooms by ecoregion compared to the national change (U.S.).

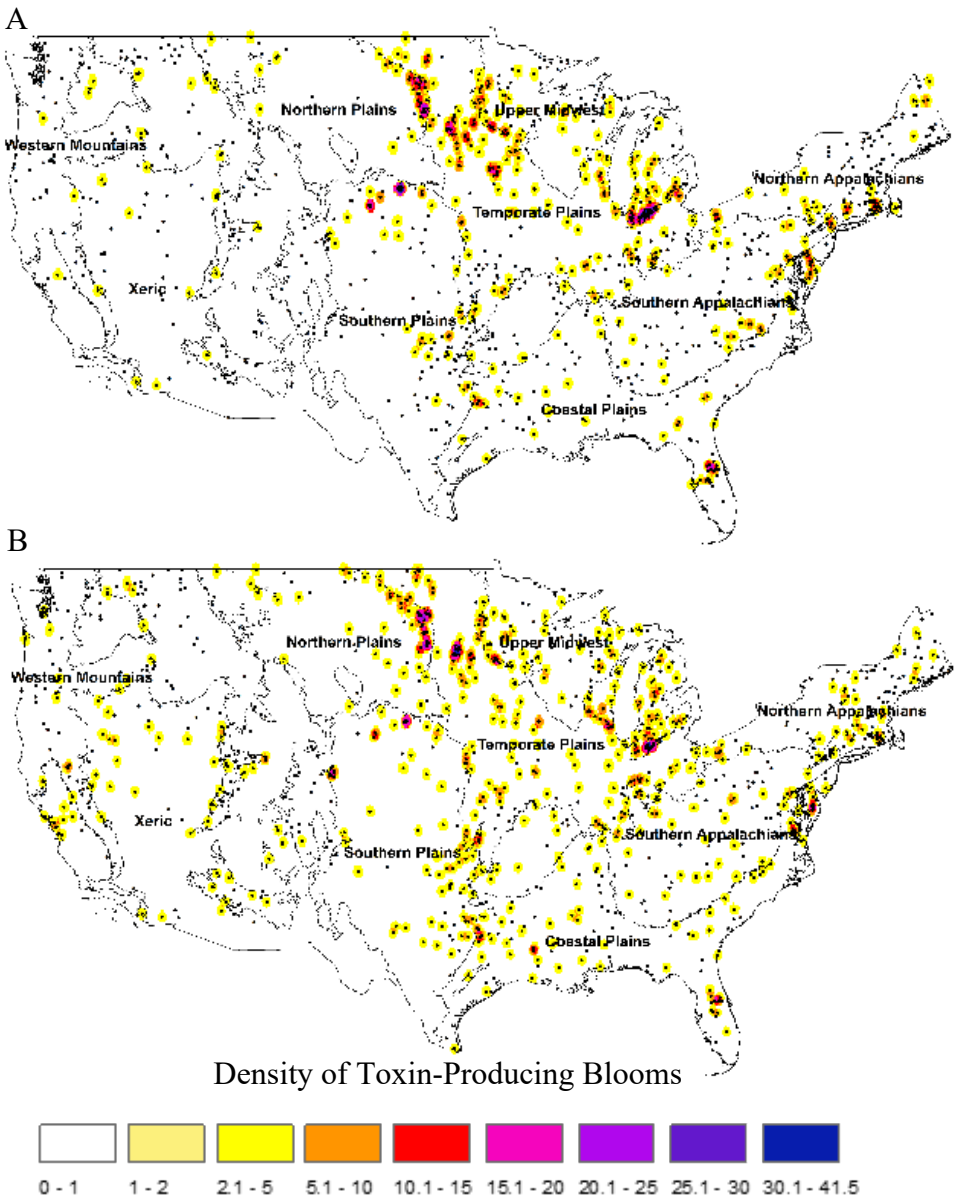

Fig. 2.3. The density of toxin-producing HAB blooms where microcystin-LR concentrations exceed 0.1 μg L^{-1}. (A) is the visualization of 2007 and (B) is 2012.

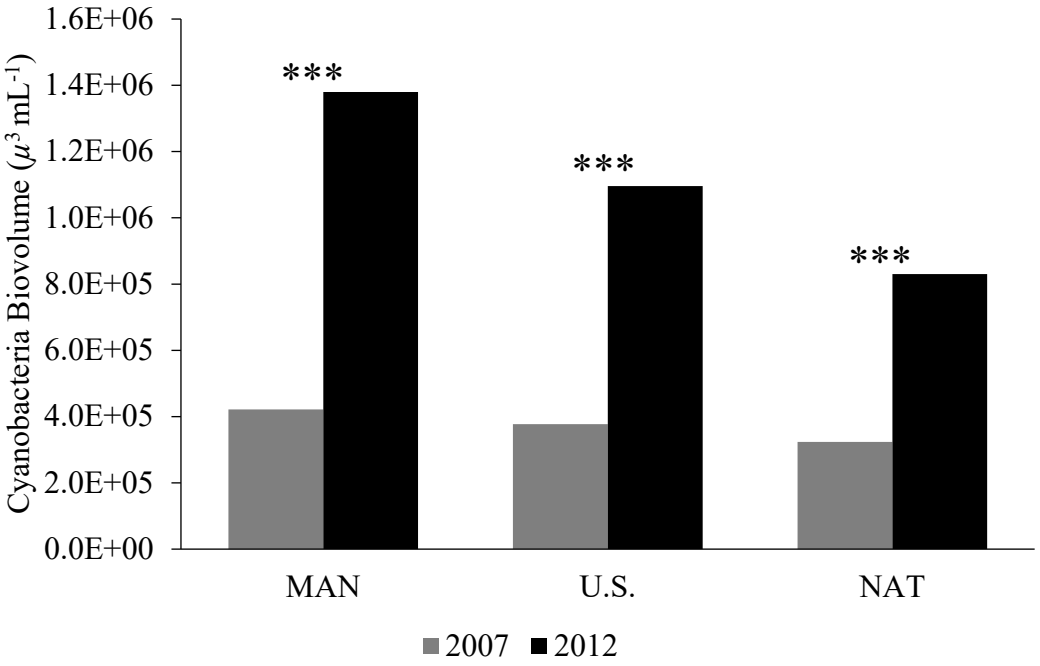

Fig. 2.4. Cyanobacteria biovolume by lake origin. Statistically significant changes in biovolume between 2007 and 2012 are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

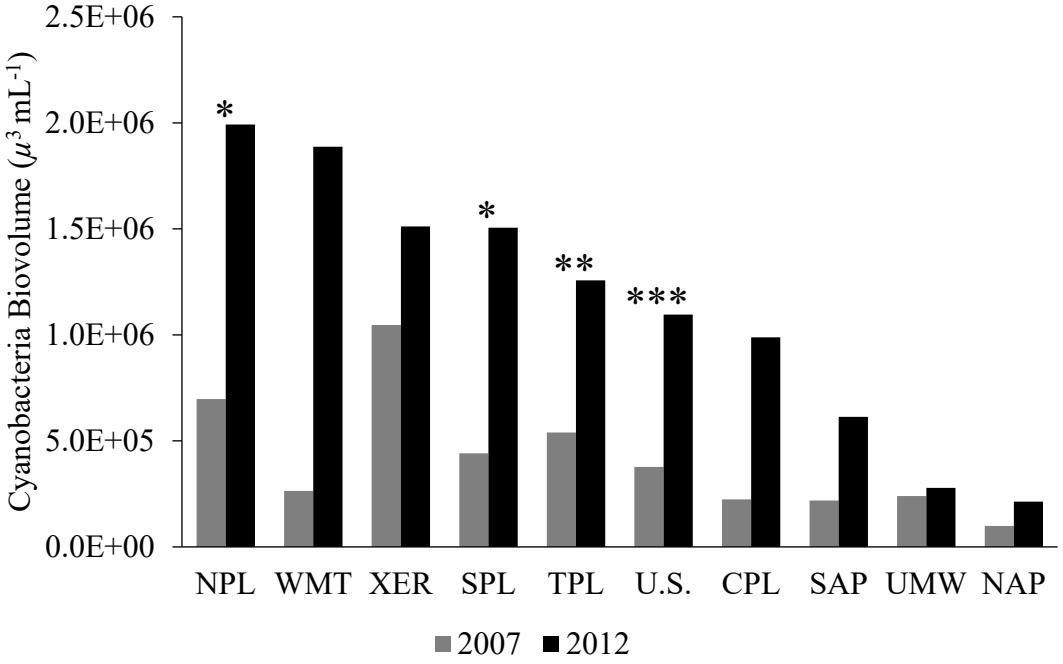

Fig. 2.5. Cyanobacteria biovolume by lake ecoregion. Statistically significant changes in biovolume are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

Table 2.2. Microcystin-LR concentrations between sample years. Standard error included in brackets. Statistically significant changes in concentration are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

Ecoregion	2007 (μg L^{-1})	2012 (μg L^{-1})	Change (μg L^{-1})
U.S.	3.07 (0.85)	1.97 (0.44)	-1.10
MAN	1.52 (0.51)	1.99 (0.72)	0.47
NAT	4.27 (1.5)	1.94 (0.42)	-2.33
XER	2.73 (1.3)	6.70 (5.0)	3.97
NPL	8.76 (5.8)	2.50 (0.96)	-6.26
SPL	8.81 (0.99)	2.35 (5.6)	-6.46
TPL	2.92 (0.70)	2.73 (0.85)	-0.19
SAP	0.63 (0.14)	1.05 (0.68)	0.42
CPL	0.90 (0.19)	0.80 (0.28)	-0.10
WMT	0.76 (0.32)	0.63 (0.19)	-0.13
UMW	1.18 (0.18)	0.62 (0.19)	-0.56*
NAP	0.65 (0.18)	0.35 (0.09)	-0.32

Midwest was the only ecoregion to experience a strong decrease (p < 0.05) in microcystin-LR from 1.18 to 0.62 μg L^{-1}, notably crossing the 1.0 μg L^{-1} drinking water limit. Due to high variability in cyanobacteria biovolume and cyanotoxin concentration, none of the ecoregions differed significantly from each other in 2012.

Water temperature and depth

As a whole and when separated by lake origin, the lakes sampled in the NLA decreased significantly in epilimnion temperature (Fig. 2.6) and increased in epilimnion depth (Table 2.3). The epilimnion in natural lakes were 2.81 °C cooler (p < 0.001) and 1.05 m deeper (p = 0.023) than man-made lakes in 2012.

In five of the ecoregions, Temperate Plains, Southern Appalachian, Upper Midwest, Western Mountains, and Xeric, epilimnion temperatures decreased significantly between the two sampling periods (Fig. 2.7). Each ecoregion that decreased in epilimnion temperature also significantly increased in depth (Table 2.3). The epilimnion volume and

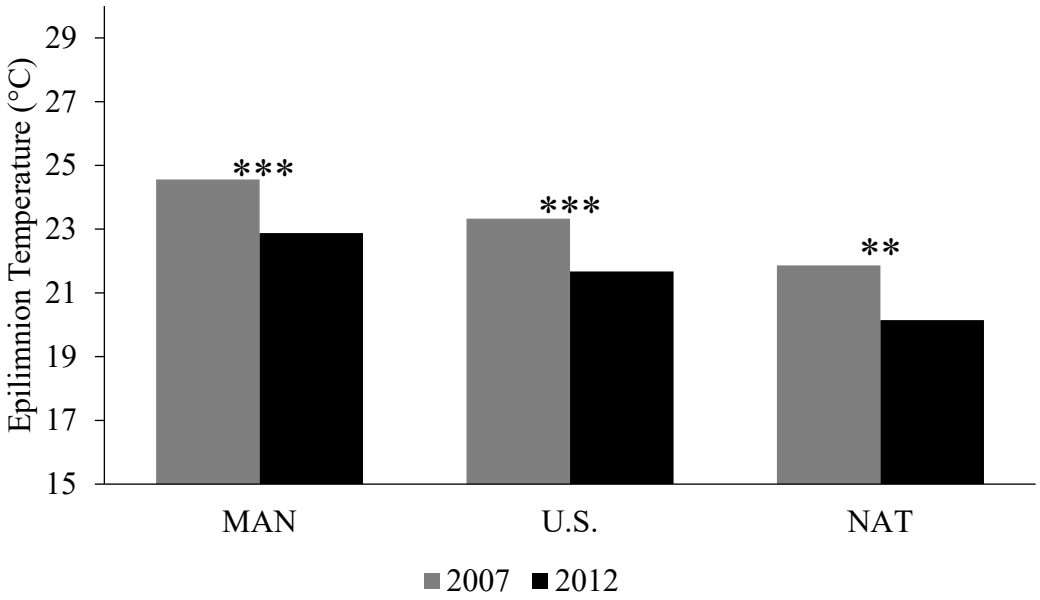

Fig. 2.6. Epilimnion temperature by lake origin. Statistically significant changes in temperature are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

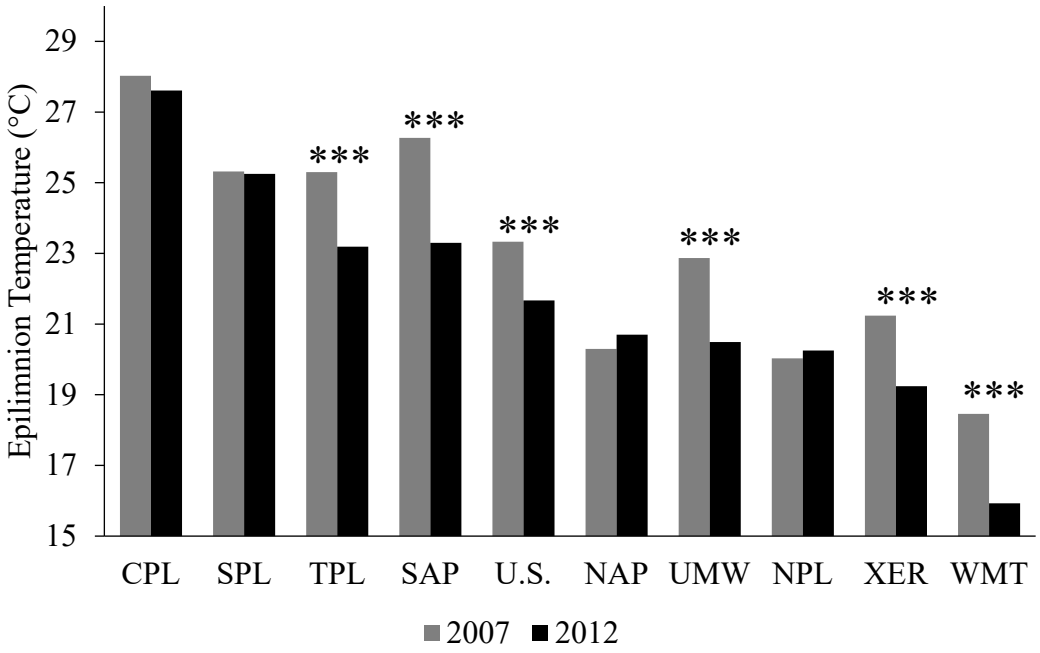

Fig. 2.7. Epilimnion temperature by lake ecoregion. Statistically significant changes in temperature are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

Table 2.3. Epilimnion depth between sample years. Statistically significant changes in depth are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

Region	2007 (m)	2012 (m)	Change (m)
U.S.	5.46	6.40	0.94**
MAN	5.13	5.90	0.77*
NAT	5.86	6.95	1.09*
CPL	3.16	3.08	-0.08
SPL	4.68	3.82	-0.86†
TPL	3.43	4.42	0.99***
SAP	5.00	7.07	2.07***
NAP	9.07	7.48	-1.58
UMW	4.47	6.63	2.16***
NPL	4.73	5.25	0.52
XER	5.71	8.37	2.65*
WMT	8.75	10.45	1.70

energy did not significantly change between years. The average lake area of each ecoregion did not change or decreased slightly but not significantly.

Nitrogen to phosphorus ratio

The total nitrogen (TN) to total phosphorus (TP) ratio for the U.S. and both lake origins decreased below the mass threshold previously identified for cyanobacteria dominance (Huisman and Hulot 2005) (Fig. 2.8). Only man-made lakes were below the threshold in 2007 but in 2012 all lakes fell below the threshold to similar ratios. The average concentration of total nitrogen in man-made lakes increased by 206.5 μg L^{-1} (median = 77 μg L^{-1}; p = 0.037) (Fig. 2.10).

Nearly every ecoregion was below the mass ratio threshold for HAB occurrence in 2012 (Fig. 2.9). Interestingly, the Northern Plains increased in the ratio in 2012 above the threshold. Four of the regions experienced significant decreases in the TN:TP ratio, each of which saw its ratio reduced nearly by half between 2007 and 2012. The shift in

the ratio was driven by a widespread increase in total phosphorus concentrations and a concurrent but smaller increase in total nitrogen concentrations. In contrast to most ecoregions, the Northern Plains instead showed a large decrease in nitrogen leading to greater TN:TP ratios (Fig. 2.10).

Accompanying values located in Table A.2.2. The WMT region had little to no change in total phosphorus between years. Statistically significant changes in the TP, TN, or the TN:TP ratio are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

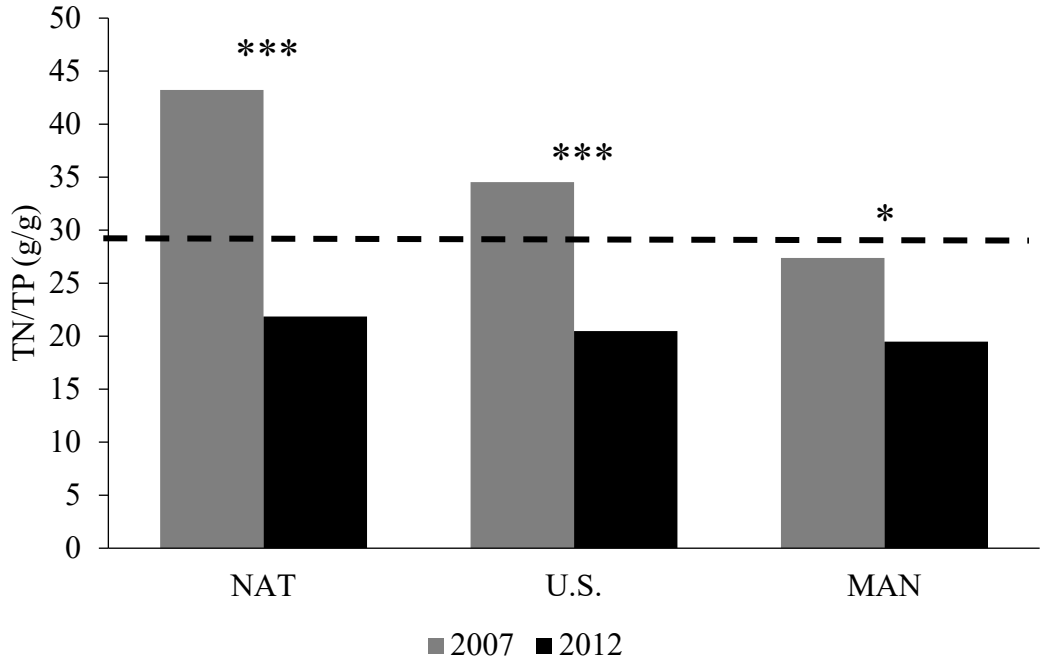

Fig. 2.8. Total nitrogen to phosphorus ratio by lake origin. Statistically significant changes in the ratio are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

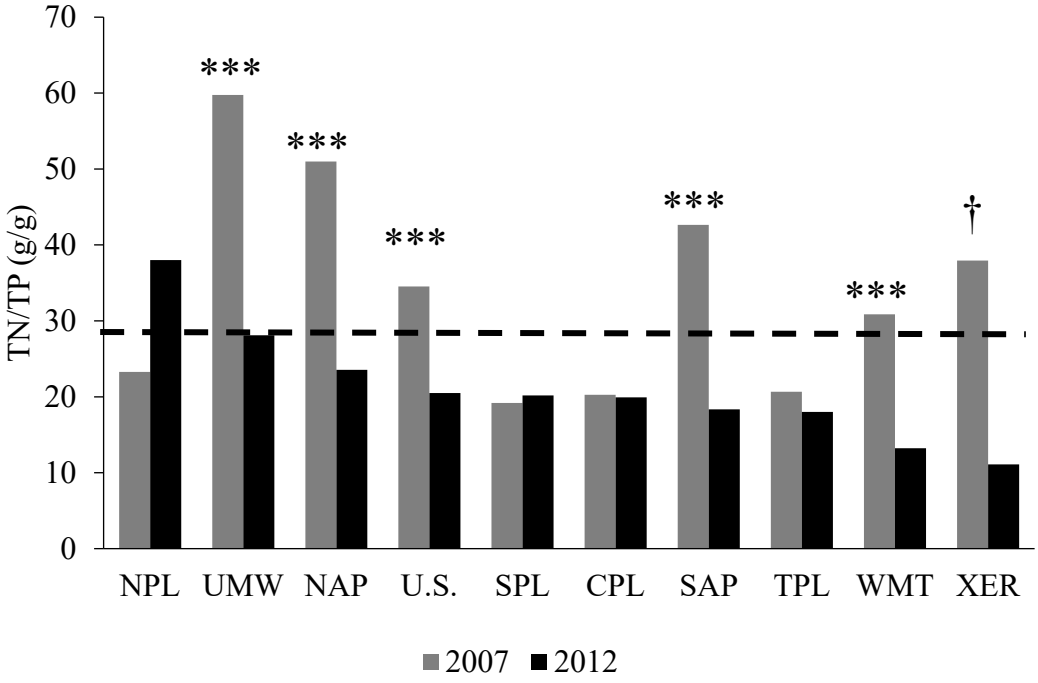

Fig. 2.9. Total nitrogen to phosphorus ratio by lake ecoregion. Statistically significant changes in the ratio are indicated by †p<0.1, *p<0.05, **p<0.01, and ***p<0.001.

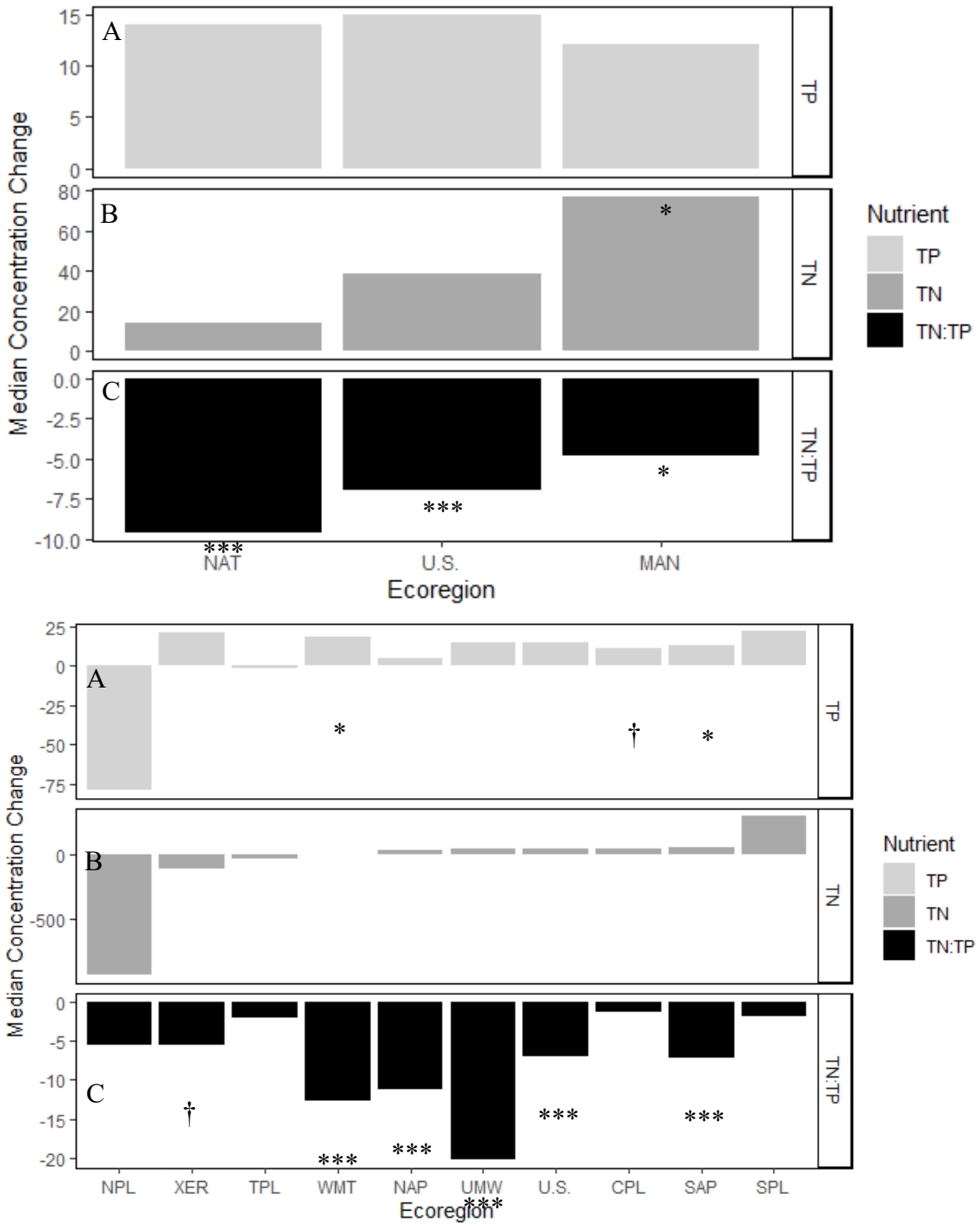

Fig. 2.10. Median changes in (A) total phosphorus (μg L^{-1}), (B) total nitrogen (μg L^{-1}), and their (C) mass ratio between 2007 and 2012 by lake origin and ecoregion.

Model results

National level

Linear models using all sampled lakes in 2007 and 2012 had a high explanation of variance for chlorophyll *a* (R^2 = 0.62) with the fewest number of parameters (Table 2.4). Weaker trends were identified for cyanobacteria biovolume (R^2 = 0.26) and microcystin-LR concentration (R^2 = 0.21). Each of the three models contained nitrogen, phosphorus, and epilimnion temperature as a significant explanatory variable. Nitrogen had the highest model coefficient for both chlorophyll *a* and microcystin-LR. Phosphorus was the most influential variable for cyanobacteria biovolume. Two precipitation metrics, P_{annual} and $P_{summer,}$ also appeared as important variables.

Table 2.4. Best model results for the three natural logarithmic transformed HAB indicators on a national scale: chlorophyll *a* (CHLA), cyanobacteria biovolume (BIOV), and microcystin-LR concentration (MICL). The coefficient of variation (R^2), the number of parameters (*k*), and sample size (*n*) are given for each model. Model variables include total phosphorus (TP), total nitrogen (TN), annual mean precipitation (P_{annual}), epilimnion temperature (EPI.T), Lake Origin (natural or man-made), and dissolved organic carbon (DOC).

	Best model determined by AIC	R^2	*k*	*n*
ln(CHLA)	0.44 ln(TN) + 0.35 ln(TP) + 0.18 EPI.T + 0.09 P_{annual} + 0.02	**0.62**	4	1914
ln(BIOV)	0.21 ln(TN) + 0.21 ln(TP) + 0.16 ln(pH) + 0.1 ln(EPI.T) - 0.06 P_{summer} + 0.01	**0.26**	5	1914
ln(MICL)	0.29 ln(TN) - 0.18 LakeOrigin + 0.15 pH + 0.14 ln(TP) - 0.05 DOC +0.3	**0.21**	5	1914

Lake origin

When separated by lake origin, a greater amount of variance was explained for all HAB indicators for natural lakes when compared to both the national models and only

man-made lakes (Table 2.5). Again, nutrients were the most important variables for almost every model. In natural lakes, TN alone explained 24% of variance microcystin-LR concentrations. ANC was determined to be significant for only the biovolume models.

Table 2.5. Best model results for the three natural logarithmic transformed HAB indicators for each lake origin. Model variables not yet defined in the previous table include mean precipitation two weeks before sample data (P_{prior}) and acid-neutralizing capacity (ANC).

	Best model determined by AIC	R^2	*k*	*n*
ln(CHLA)				
Natural	0.39 ln(TP) + 0.38 ln(TN) + 0.15 ln(EPI.T) - 0.03 DOC –	**0.65**	4	852
Man-	0.02	**0.59**	5	1062
made	0.58 ln(TN) - 0.43 DOC + 0.32 ln(TP) + 0.16 EPI.T + 0.09 $\ln(P_{annual})$ + 0.0			
ln(BIOV)				
Natural	0.29 ln(TP) + 0.28 ln(TN) - 0.04 ANC – 0.02	**0.33**	3	852
Man-	0.34 ln(TN) - 0.26 ANC + 0.18 ln(TP) - 0.06 P_{prior} + 0.05	**0.18**	2	1062
made				
ln(MICL)				
Natural	0.54 ln(TN) + 0.11	**0.24**	1	852
Man-	0.19 ln(TN) + 0.13 pH + 0.07 ln (TP) - 0.12	**0.11**	3	1062
made				

Ecoregion level

Linear models generated for four ecoregions had higher explanations of variance for various HAB indicators than the national model (Table 2.6). Chlorophyll *a* and cyanobacteria biovolume were best explained in Southern and Northern Appalachia. Phosphorus was the most important explanatory variable for both regions for chlorophyll

a and for biovolume in only Southern Appalachia. Alternatively, the acid-neutralizing capacity in Northern Appalachian lakes explained nearly three times the variance as phosphorus. Nitrogen had the strongest relationship with microcystin-LR concentrations for all three ecoregions: Northern Appalachia, Temperate, and Southern Plains.

Table 2.6. Best model results for the three natural logarithmic transformed HAB indicators for lake ecoregions. Only ecoregions that had a higher explanation of variance than the national model were included in this table. All other ecoregion model results are included in Table A.2.3. Model variables not yet defined in the previous tables include mean air temperature two weeks before sample date ($Air.T_{prior}$), mean annual precipitation (P_{annual}), mean summer air temperature ($Air.T_{summer}$)**,** and the number of days the air temperature had a maximum of 25 °C or greater ($Air.T_{25}$).

	Best model determined by AIC	R^2	*k*	*n*
ln(CHLA)				
SAP	$0.52\ \ln(TP) + 0.35\ \ln(TN) + 0.28\ \ln(DOC) + 0.08\ Air.T_{prior}$	**0.70**	4	198
NAP	$+ 0.44$	**0.64**	4	180
	$0.57\ \ln(TP) + 0.3\ \ln(TN) + 0.09\ P_{prior} - 0.06\ Air.T_{prior} + 0.1$			
ln(BIOV)				
NAP	$2.5\ ANC + 0.63\ \ln(TP) + 0.14\ P_{annual} + 0.45$	**0.41**	3	180
SAP	$0.51\ \ln(TP) + 0.32\ \ln(DOC) + 0.29$	**0.32**	2	198
ln(MICL)				
NAP	$0.19\ \ln(TN) + 0.08\ pH + 0.08\ Air.T_{25} + 0.06\ P_{prior} - 0.07$	**0.32**	3	180
TPL	$0.74\ \ln(TN) - 0.22\ Air.T_{summer} - 0.2\ LakeOrigin + 0.35$	**0.25**	3	292
SPL	$0.71\ \ln(TN) + 0.2\ AIR.T_{25} – 0.32$	**0.25**	2	217

Discussion

The United States experienced major temporal changes in HABs between 2007 and 2012 and were correlated to both effects of climate change and anthropogenic activity. Environmental conditions altered by climate change (warmer waters and

intensified stratification) and human activity (increased nutrient loading) that support cyanobacteria growth did not equally increase toxin production. Even though cyanobacteria thrive in warmer temperatures, the dominant strain of the cyanobacteria, either toxic or non-toxic, can shift depending on the exact temperature. For *Microcystis* spp., lower water temperatures result in slower cell growth but greater microcystin synthesis, while higher temperatures increase cell growth but slow toxin production (Peng et al. 2018). 2012 was the warmest year in the U.S. between 1895 and 2020 for every state; nearly 1 °C warmer than 2007 (www.climate.gov, Accessed October 12, 2019). Subsequently, peak summer air temperatures increased by an average of 0.28 °C ($p = 0.08$) across U.S. lakes in 2012. Higher maximum air temperatures could lead to greater lake temperatures and a shift from toxic strains of cyanobacteria to their non-toxic counterparts (Ninio et al. 2020). Higher, more favorable temperatures for *Microcystis aeruginosa* growth also led to the selection of a non-toxic genotype during a bloom, while the toxic genotypes are selected for when environmental conditions are less favorable (Briand et al. 2009). Alternatively, U.S. lake epilimnion temperatures decreased but depth increased, indicating more intense lake stratification. Greater stratification does promote cyanobacteria dominance due to its competitive advantages but no studies have been done on stratification's impact on cyanotoxin production. Rising temperatures will continue to promote HABs that still cause major environmental damage, but toxicity increases may not be linear with cyanobacteria biovolume.

Nutrient pollution had a greater impact on HABs than temperature. Concentrations of total nitrogen and phosphorus increased, resulting in a nearly nationwide decrease in their ratio below the mass threshold established by Smith 1983.

Most of the decrease in the national TN:TP ratio can be attributed to a 60.0% increase in median total phosphorus concentrations followed by a smaller 6.6% increase in total nitrogen. Cyanobacteria commonly dominate systems with high phosphorus concentrations and low TN:TP ratios due to their ability to fix nitrogen (Smith 1983; Tingguang Song and Xiepei Wang 2005; Orihel et al. 2012). The amount of nutrient loading can be more influential on cyanobacteria dominance than the ratio itself (Xu et al. 2010; Paerl et al. 2011). Also, cyanobacteria can dominate in nitrogen-limited systems, but toxin production is often promoted by an abundance of exogenous nitrogen (Watanabe and Oishi 1985; Berman 1999; Gobler et al. 2016; Chaffin et al. 2018; Jankowiak et al. 2019). Increases in total phosphorus were far greater than nitrogen in U.S. lakes, which may explain why cyanobacteria biovolume increased but microcystin-LR concentrations did not. Similar to Ho & Michalak 2019, all three of our linear models for HAB indicators selected total nitrogen as the most influential variable rather than its ratio to phosphorus, further supporting the importance of individual concentrations in cyanobacterial dominance and toxicity. Cyanobacteria biovolume and microcystin-LR concentrations were also unrelated ($R^2 = 0.006$), further indicating conditions that increased cyanobacteria abundance did not increase cyanotoxin production as well. However, a limitation of the NLA dataset is that only microcystin-LR is measured, which is one variant of only one cyanotoxin. Nearly a dozen cyanotoxins exist, and each may be impacted differently by environmental conditions than microcystin-LR. Future studies should include a greater number of cyanotoxins to increase the statistical power of modeling efforts.

Climate change is expected to generally increase air and water temperatures which could increase HABs but would not necessarily promote greater cyanotoxin production. However, nutrient pollution is expected to increase due to increased human activity which could impact both HABs and their toxicity if nitrogen concentrations continue to rise. As shown in this and previous studies, nitrogen and phosphorus are the leading drivers of phytoplankton abundance, cyanobacteria biovolume, and HAB toxicity. Therefore, the best method for reducing HABs can be through limiting the amount of nutrients entering watersheds or removing them before they reach the targeted water body. Currently, numerous policies are attempting to mitigate nutrient pollution in watersheds, such as reductions in agricultural fertilizer use, bans on the spread of manure in winter, limits on nutrients in the effluent from water treatment plants, and the removal of phosphorus from watersheds using wetlands and sorbents (Vohla et al. 2011; Wall et al. 2012; Liu et al. 2018). However, the extent of these efforts is primarily limited to regions that already had frequent HABs (e.g. Wisconsin and Minnesota) (USEPA 2018). The concern is greatest for regions such as the Xeric and Western Mountains that are rapidly increasing in HAB occurrence where such nutrient mitigation policies are non-existent. Moreover, nutrient pollution may result from factors outside the catchment (e.g. dust deposition) and are therefore inherently more difficult to manage (Brahney et al. 2015b; Stoddard et al. 2016). Further, nutrient targets need to be established for individual lakes to effectively manage local nutrient sources and pollution. As of 2019, 27 U.S. states do not have EPA-approved criteria for regulating total nitrogen and phosphorus in any their waterways (USEPA 2018).

A lake's origin and ecoregion differed in the degree to which HABs and environmental conditions changed temporally. Man-made lakes experienced a greater increase in TPBs and cyanobacteria biovolume than their natural counterparts. The change in independent loading of nitrogen in man-made lakes in 2012 was greater than in natural lakes ($p = 0.1$) which could help explain the relatively greater increase in TPBs and cyanobacteria biovolume in man-made than natural lakes. Also, man-made lakes had a higher median total phosphorus concentration of 46 μg L^{-1} in 2012 compared to only 32 μg L^{-1} in natural lakes. Further, the linear models for man-made lakes had nitrogen explaining the most variance in chlorophyll *a* and cyanobacteria biovolume, while natural lakes had nitrogen and phosphorus explained nearly identical amounts of variance. Potential reasons for higher phosphorus loading in man-made lakes are due to different hydrologic and basin conditions than in natural lakes. Man-made lakes are more commonly surrounded by developed land and were shown to have increased summer blooms in some regions of the U.S. (Doubek et al. 2015; Doubek and Carey 2017; Marion et al. 2017). For example, developed land around man-made lakes rose from 8.3% to 10.1% ($p = 0.036$), while natural lakes decreased from 8.3% to 7.15%. More developed land in a lake basin could indicate more anthropogenic sources of nutrients from wastewater treatment plants and sewage emissions, both of which are important point sources for nutrient pollution in surface freshwater (Paerl and Fulton 2006). Similarly, more agricultural land in a watershed is a major non-point source of nutrient pollution. As shorelines become more developed, nutrient loading and HABs will continue to increase unless regulations keep pollution in check. Another effect of increased development is the removal of forested land near shorelines and rivers,

commonly referred to as a riparian zone. Riparian zones act as buffers to rivers, reducing the amount of nutrients entering the water from agricultural runoff (Anbumozhi et al. 2005). Low forest cover has been associated with increased cyanobacteria abundance and cyanotoxin concentrations in lakes (Marion et al. 2017). With forest dieback, nutrient fluxes are known to increase from forested catchments which could then promote HABs in nearby lakes (Berg and McClaugherty 2008; Mikkelson et al. 2013).

Even though nearly every ecoregion increased in TPBs and in the conditions that promote them, the Xeric and Western Mountain regions experienced the most drastic changes between the two sample years. These two ecoregions had the fewest TPBs in 2008 and nearly tripled the number in 2012. The unique combined impact of climate change and human activity could be the reason for the stark increase in lakes that hosted cyanobacteria blooms when compared to other regions. The Xeric and Western Mountain both had increased lake phosphorus concentrations and some of the largest declines in the TN:TP ratio. However, they had some of the lowest percentages of agricultural and developed land of the 9 ecoregions, which are typically associated with HAB occurrence. Therefore, nutrient pollution may instead be coming from the indirect effects of human activities and climate change. Warmer temperatures and more extreme droughts from the effects of climate change in Xeric and Western regions combined with increased human activity (e.g. air pollution) led to the atmospheric deposition of nutrients in alpine lakes and increased soil erosion and acidification (Brahney et al. 2014, 2015a; Stoddard et al. 2016). Even though median nitrogen concentrations did not increase in these ecoregions between sample years, high elevation lakes in the western U.S. have lower critical loads of nitrogen and phosphorus than lakes in the northeast making the productivity of western

lakes particularly vulnerable to any increases in nutrient pollution (Baron et al. 2011; Brahney et al. 2014, 2015a). For example, Utah Lake, a natural Xeric lake that receives the output of numerous wastewater treatment plants, had cyanobacteria counts increase by three orders of magnitude between 2008 and 2016 (Page et al. 2018). Lakes in semi-arid regions of Ethiopia, despite limited riverine inputs of nutrients, had sufficient atmospheric deposition of ammonia and phosphorus to trigger cyanobacteria blooms (Tilahun and Kifle 2020). Likewise, with atmospheric nutrients concentrations, particularly nitrogen, to increase in the future (Galloway et al. 2008) lakes in the semi-arid regions of the Xeric and Western Mountains can experience sustained nutrient pollution despite a lack of agriculture or wastewater effluent

Conclusion

The frequency and severity of HABs in the U.S. and the world are expected to increase due to climate change and anthropogenic activity. Abating the effects of climate change is no longer a viable option for preventing HABs as controlling temperatures and precipitation in large water bodies is unfeasible. However, policies and management can be implemented to reduce the input of nutrients into lakes from point (e.g. water treatment facilities) and some non-point sources (e.g. agricultural runoff). The U.S. is a diverse geographical region, with lakes across the country responding differently to local and global inputs of nutrients and effects of climate change. Immediate action aught be taken to regulate nutrient pollution in the U.S. on a regional scale to prevent the risk HABs present to environmental and human health from becoming more severe.

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CHAPTER 3

QUANTIFYING CYANOTOXIN UPTAKE IN LETTUCE CROPS FROM CONTAMINATED IRRIGATION WATER

ABSTRACT

Toxic harmful algal blooms (HABs) have increased globally over the past few decades in both severity and frequency due to climate change and anthropogenic pollution. Cyanotoxins created from HABs pose a potent threat to human and ecosystem health. For example, crops can become contaminated with cyanotoxins because some waterbodies with HABs are used for irrigation in public gardens and agriculture. To test the potential for soils to sorb cyanotoxins and prevent or slow uptake into vegetables, we determined soil-water partition coefficients for eight soils with different physical and chemical characteristics. The cation exchange capacity of soil was the most significant driver of cyanotoxin sorption (R^2 = 0.26 and 0.44, $p < 0.01$), with soil water partition coefficients ranging from 1.35 to 10.73 L kg^{-1} for *beta*-Methylamino-L-alanine (BMAA) and 1.59 to 5.03 L kg^{-1} for microcystin-LR. We examined the uptake of three different cyanotoxins, liver toxic microcystin-LR, nodularin, and neurotoxic BMAA into lettuce in a greenhouse experiment conducted over six weeks. Though we found a positive relationship between dose and concentration in the plants despite quantification interference using ELISA, LC-MS failed to detect the presence of any cyanotoxins in the plant tissues as concentrations were below the analytical detection limit of the instrument. Using existing relationships between microcystin-LR concentration in plants and the amount in the irrigation water, we predicted the concentrations of toxin needed to exceed the World Health Organization's 0.04 μg kg^{-1} total daily intake limit for edible leaves and

fruit. Irrigation water with a microcystin-LR concentration of 3.39 μg L^{-1} and 42.0 μg L^{-1} would exceed the daily intake limit for lettuce and tomatoes respectively. Due to the importance of cyanotoxin presence and concentrations with vegetables, more research and method development is needed to both measure cyanotoxin concentrations and to better understand the mechanisms of cyanotoxin transport in plants and their bioaccumulation potential.

INTRODUCTION

Cyanobacteria blooms that produce cyanotoxins are increasing in frequency and intensity around the world creating a severe ecological and human health concern (Huisman et al. 2018; Ho et al. 2019). Water conditions that promote cyanobacteria and toxin production, such as increased temperatures, salinity, and nutrient concentrations are becoming more common globally (Paerl and Huisman 2009; Paerl and Paul 2012). The increased dominance of cyanobacteria in lakes and rivers not only decreases water quality and harms other aquatic organisms, but produces a variety of cyanotoxins that can have both acute and chronic health effects on all organisms, including humans (Chen et al. 2016). Cyanotoxins can be hepatoxic (liver), neurotoxic (nervous system), dermatoxic (skin), or cytotoxic (cellular), with varying degrees of toxicity and be present in both freshwater and saltwater. Harmful quantities of cyanotoxins are typically associated with high-density cyanobacteria bloom events. A greater abundance of cyanotoxins elevates the risk of exposure to humans via numerous routes: ingestion, inhalation, dermal contact, and the consumption of contaminated food. However, most toxicity and exposure studies have been performed with one cyanotoxin, hepatoxic microcystin-LR.

Information on other toxins, such as nodularin, anatoxin, and cylindrospermopsin, is severely limited.

Drinking water is the most understood route for human exposure to cyanotoxins and has a regulated limit of 1.6 μg of microcystin-LR per liter in the United States (D'Anglada and Strong 2015). However, given adequate preparation and knowledge of cyanotoxin contamination, water treatment plants can help mitigate this threat by either removing or degrading the toxins below the limit (Hitzfeld et al. 2000; Westrick et al. 2010). Cyanotoxin exposure via inhalation and dermal contact can occur during recreation. The concentration of cyanotoxins during a bloom is low enough that an unrealistic amount of water must be inhaled to pose an acute health threat, however, the health effects of long term chronic exposure are not well understood (Backer et al. 2010; Wood and Dietrich 2011). Further, water bodies experiencing a cyanobacteria bloom are often closed to the public when above the U.S. Environmental Protection Agency 8.0 μg microcystin-LR per liter recreation limit (Environmental Protection Agency 2019) and the green bloom material makes them unattractive to swimming and recreation, further limiting exposure.

Exposure to cyanotoxins via contaminated food is relatively understudied when compared to other exposure routes. The majority of studies assess the concentration of cyanotoxins in aquatic organisms, such as fish and mollusks, commonly harvested as a food source (Ferrão-Filho and Kozlowsky-Suzuki 2011; Mulvenna et al. 2012; Flores et al. 2018). However, exposure to cyanotoxins can occur beyond the geologic confines of a water body. As many lakes and rivers are surrounded by cropland and are commonly used for irrigation, crop plants may be exposed to cyanotoxins from contaminated

irrigation water. Currently, in the U.S., there are no regulations on the amount of cyanobacteria or cyanotoxins that can exist in irrigation water (U.S. FDA 2016). In addition, the only guidance on cyanotoxin exposure from food is for one variant of microcystin, microcystin-LR.

The only metric used to determine if a food item containing cyanotoxins is a health risk is a total daily intake (TDI) limit calculated by the World Health Organization (WHO). The TDI, 0.04 μg toxin per kilogram of human body weight was calculated only for microcystin-LR, again highlighting the lack of toxicity data for other toxins (WHO 2017). However, another hepatoxic cyanotoxin, nodularin, is similar in structure and toxicity to microcystin-LR, and established exposure limits can be applied to both equally (Pearson et al. 2010). A few studies have examined the relationship between contaminated irrigation water and the bioaccumulation of cyanotoxins in soils and plants, however, most only focus on microcystin-LR and only for short periods. Some studies have shown crop plants to exceed the TDI limit of microcystin-LR, including lettuce, cucumber, rice, carrots, and spinach (Saqrane et al. 2009; Bittencourt-Oliveira et al. 2016; Lee et al. 2017; Levizou et al. 2017; Cao, Steinman, Wan, et al. 2018a; Zhu et al. 2018; Cao et al. 2019; Wijewickrama and Manage 2019). Additionally, recent studies show the newly discovered neurotoxin, *beta*-Methylamino-L-alanine (BMAA) can bioaccumulate in crop plants, but conflict exists on whether the protein-bound fraction of the toxin accumulates more than the free fraction and where in a plant they concentrate (Contardo-Jara et al. 2014; Contardo-Jara et al. 2018; Esterhuizen-Londt and Pflugmacher 2019; Li et al. 2019). Protein-bound BMAA is a greater health concern than free BMAA as the protein-bound fraction can transfer between trophic levels and create a

cyanotoxin reservoir within the brain of an organism which then slowly releases cyanotoxins via cerebral protein metabolism (Murch et al. 2004).

Despite the evidence that plants can bioaccumulate cyanotoxins into their edible tissues in dangerous concentrations, knowledge is limited on toxins other than microcystin-LR, the distribution of cyanotoxins between plant parts, and on the relationship between plant uptake and the concentration of toxins in the water and type of irrigation. Two methods of irrigation are commonly used, drip and spray, each providing a unique mechanism of cyanotoxin introduction to a plant and soil system. Many studies on cyanotoxins in food crops use spray irrigation (Codd et al. 1999; Saqrane et al. 2009; Hereman and Bittencourt-Oliveira 2012; Romero-Oliva et al. 2014; Cao et al. 2018b), which cannot discern between translocation of cyanotoxins from root to shoots and cyanotoxin incorporation in the wax cuticle or stomata.

Additionally, the relationship between soil and cyanotoxin bioavailability is not well understood and current literature is limited to only one cyanotoxin, microcystin-LR. Soil can contain microorganisms that can fully degrade microcystin-LR in as little as 18 days, potentially reducing the concentration of toxins below harmful levels before they are taken up by plants if the rate of degradation is greater than the rate of uptake (Chen et al. 2006; Cao, Steinman, Yao, et al. 2018). However, pesticides common in agriculture such as glyphosate and chlorothalonil can slow the degradation of microcystin-LR (by nearly a factor of two), slowing the decrease in exposure concentration (Cao, Steinman, Yao, et al. 2018). The physical properties of some cyanotoxins can also affect the persistence and fate in soil (Table A.3.1). Cyanotoxins such as microcystin-LR become more hydrophilic and available to plants in a soil-plant matrix at pH values greater than 2

(De Maagd et al. 1999; McCord et al. 2018). However, since most crops are grown in the pH range of 6 to 8 (Gale et al. 2001), cyanotoxin soil mobility will be near its maximum in this range. The amount of organic matter (OM) also heavily influences cyanotoxin mobility in soils, with mobility decreasing at less than 8% OM and greater than 8% OM (Wu et al. 2011). Clay content also can bind microcystin-LR by metal chelation (Chen et al. 2006). Therefore, in an agricultural setting, the characteristics of the soil and can play an important role in the partitioning of cyanotoxins between soil, water, and plants.

To expand scientific understanding of the ability of plants to concentrate cyanotoxins in their tissue, we conducted two experiments and a meta-analysis. First, we measured the sorption of microcystin-LR and BMAA by eight soils (Table 3.1) to identify any relationship between sorption and chemical (pH, OM, and cation exchange capacity) and physical (clay, sand, silt) soil characteristics. Second, we tested whether root to shoot transfer of microcystin-LR, nodularin, and BMAA in lettuce crops can occur via contaminated drip irrigation water. Finally, we performed a meta-analysis of current literature that measured cyanotoxin concentration in plants to identify any relationships between toxin concentration in irrigation water and different plant parts. Determining the ability for soil properties to mitigate cyanotoxin uptake and for food crops to accumulate cyanotoxins can help define the risk to humans from crop consumption.

METHODS

Cyanotoxin soil sorption

Soil was acquired from the North American Proficiency Testing Program (NAPT) with varying textures, pHs, and organic matter concentrations from agricultural land

around Utah and the United States. In total, eight soils were measured for cyanotoxin sorption to calculate soil-water partition coefficients (K_d; $\frac{Concentration_{soil}}{Concentration_{water}}$). Because only one concentration of cyanotoxins was used in this experiment, K_d values were not calculated from an isotherm and instead only represent the partitioning for one treatment concentration. Chemical and physical soil characteristics were analyzed by the NAPT following the program protocols defined in Miller et al. 2013.

TABLE 3.1: Physical and chemical soil characteristics measured by the North American Proficiency Testing Program

Soil	Source	pH	SOM-LOI%	CEC	Sand %	Silt %	Clay %
Calcined Clay	Positive control	5.5	0	33.6	60	0	40
Palouse	Union, OR	6.1	6.4	25.5	29	50	20.7
Quindoqua	Sommerset, MD	6.4	3.3	10.2	33	52.3	14
Westmoreland	Monongalia, WV	6.8	4.1	12.7	24	53.9	20.8
Ottawa Sand	Negative control	7	0	1	100	0	0
Mendon	Cache, UT	7.4	5.1	31.1	29.4	44	28.7
Collinston	Cache, UT	7.9	4.9	33.6	17.3	49.6	34
Ivy	Cache, UT	7.9	4.5	20.7	52.2	31.2	17.8
Kidman	Davis, UT	8	1.5	10.8	47.9	37.4	14.9
Clawson	Cache, UT	8.1	2.7	17.3	18	57	24.3

All soils were air-dried, pulverized, and sifted through a 2.0 mm sieve before exposed to cyanotoxins. To determine cyanotoxin sorption, a batch study was conducted consisting of 1 g of dry soil and 10 mL of deionized water, 0.25 μg mL^{-1} of BMAA, or 0.05 μg mL^{-1} microcystin-LR to accurately represent concentrations seen in HAB lakes. Each sample contained 0.05% sodium azide to inhibit microbial growth and potential toxin degradation. All soils were shaken on an orbital mixer at 110 rpm for 24 hours at room temperature (Wu et al. 2011). Calcined-clay acted as a positive control due to its

high cation exchange capacity (CEC) and sand a negative control due to its low CEC. Cyanotoxins remaining in the solution were measured using ELISA kits. As only one phase, water was analyzed for cyanotoxins any loss was assumed to be sorbed to the soil. Further, desorption was not measured in this study, but it will likely occur for cyanotoxins which impacts their final bioavailability. Though, any inhibition of cyanotoxin transport may increase the potential for biodegradation.

Cyanotoxin concentration in lettuce crops

To determine cyanotoxin accumulation in lettuce grown in toxin-laced water, three cyanotoxins (microcystin-LR, nodularin, and BMAA) at three environmentally relevant concentrations (5 μg L^{-1}, 100 μg L^{-1}, and 500 μg L^{-1}) (Goel 2009; Oudra et al. 2019), were provided to romaine lettuce (Fusion cultivar, Johnny's Selected Seeds ME, USA). Drip irrigation with only deionized water was used as a control. Nodularin did not receive the 500 μg L^{-1} treatment due to cost restraints and 500 μg L^{-1} is an order of magnitude higher than what has been measured in lakes with recurring nodularin blooms (e.g. the Great Salt Lake) (Mcculley 2014). A total of five replicates of each concentration were grown for 6 weeks in the Utah State University Research Greenhouses from August 11 to September 25, 2019 (Logan, Utah). Greenhouse temperatures averaged 23.6 ± 2.6 °C over the growth period. All plants grew from seed and in a randomized row design. Plants were exposed to 14 hours of light with an average daily photosynthetic photon flux density of 1,000 µmol s^{-1} m^{-2}. Each plant grew in 1-liter pots filled with 200 grams of Ottawa ASTM 20-30 Test Sand (Humboldt Mfg. Co., IL, USA) and 200 grams of potting soil (75% peat moss, 25% vermiculite, <1% gypsum, dolomite, and a wetting agent). The soil-sand mixture was used to reduce the amount of

cyanotoxins sorbing to soil and to increase pore space for plant roots to access the contaminated water. This growth media was not assessed in the soil sorption experiments, but the ability for soil to sorb significant amounts of microcystin-LR varies amongst studies (Järvenpää et al. 2007; Lee et al. 2017; Ai et al. 2020; Levizou et al. 2020). Both seeds and soil were sterilized with 3% H_2O_2 for 3 minutes and rinsed with deionized water (Contardo-Jara et al. 2018).

Plants were treated twice a day, every other day for six weeks (18 days of treatments) with 50 mL of Peters Professional 20-10-20 nutrient solution (ICL Fertilizers, OH, USA) containing a dose of a cyanotoxin at one of the three concentrations. The irrigated volume of cyanotoxins was kept constant to keep the amount of cyanotoxins in the soil the same throughout the experiment. Lettuce plants used as controls received 50 mL of the nutrient solution without any cyanotoxins. On days without cyanotoxin treatment, all plants were watered twice a day with at least 50 mL deionized water to prevent soil from drying out, with volume increasing as the plant size increased. Care was taken to not overwater that plants so that cyanotoxins were not washed out of the pot, regardless leachate was not collected. Total cyanotoxin mass added to each plant for each dosage, 5 μg L^{-1}, 100 μg L^{-1}, and 500 μg L^{-1}, was respectively 9 μg, 180 μg, and 900 μg. At the end of the growing season, the roots and shoots of each plant were harvested, and above- and below-ground plant biomass was measured. Approximately 20 g of fresh leaf and 3 g of root material was collected from each plant. A representative selection of leaf sizes was collected to account for potential variation in leaf cyanotoxin content. All plant parts were washed twice in Milli-Q water, patted dry, and weighed. Weighed samples were stored in aluminum foil bags and frozen until cyanotoxin extraction.

Cyanotoxin extraction

Extraction methods for microcystin-LR and nodularin were adapted from Romer-Olivia et al. 2014, and Abraxis extraction instructions (Abraxis, PA, USA). Methods for BMAA extraction were adapted from Vita et al. 2016. To remove the cyanotoxins sorbed into plant material, tissue samples were lyophilized (Harvest Right, UT, USA) and pulverized into a fine powder with a mortar and pestle. Toxins within the lyophilized powder were extracted with sonification for 25 minutes using either 4 mL of 6% glacial acetic acid in methanol and DI (75/25, v/v) for microcystin-LR and nodularin or 4 mL 0.1 N trichloroacetic acid (TCA) for BMAA. BMAA was sonicated in an ice bath to prevent protein degradation. After sonication, samples were centrifuged at 2500g at 4 °C for 15 minutes and the supernatant was collected. An additional 1 mL of extraction solvent was added to the samples and sonicated for an additional 25 minutes followed by the centrifugation step. The supernatants were added together and frozen until further processing. To release any protein-bound BMAA, 1 mL of 6 N HCl was added to the remaining precipitate and hydrolyzed in an inert atmosphere at 110 °C in a dry bath for 24 hours in the Utah State University Geology Laboratory. After protein hydrolysis, 4 mL of TCA was added, and samples were centrifuged again for 10 minutes. The supernatant containing the protein-bound BMAA was collected and frozen until sample clean-up.

Sample clean-up

Extracted samples were thawed and cleaned using solid-phase extraction (SPE) columns. The cleanup step attempted to remove any chemicals or particulates that could interfere with cyanotoxin quantification. A method blank was used to show that no

analytes were lost during SPE cleanup. For microcystin-LR and nodularin toxins, a Strata™-X 33 μm polymeric reversed-phase column (Phenomenex, CA, USA) was conditioned with 6 mL of 5% methanol followed by 6 mL of DI. The sample was filtered with a 0.45 μm hydrophilic PVDF syringe filter (Foxx Life Sciences, NH, USA) and added to the SPE column and rinsed with 2 mL of 5% methanol. 5 mL of 90% acetonitrile eluted the cyanotoxin from the column and was collected in a 20 mL glass scintillation vial.

For both free and protein-bound BMAA, a Strata™-X-CW 33 μm weak cation column was conditioned with 6 mL of 5% methanol followed by 6 mL of DI. The samples were filtered and added to the SPE column and rinsed with 5 mL of 0.1 N HCl. 5 mL of 5% ammonium hydroxide in methanol and DI (75/25, v/v) eluted the BMAA from the column and collected in a 20 mL HDPE or glass scintillation vial.

All cyanotoxin scintillation vials were placed in a 35 °C water bath and the solvents were evaporated to dryness with a nitrogen evaporator (24 Position N-EVAP, Organomation, MA, USA). Dried samples were rehydrated with 1 mL of DI for ELISA (Abraxis, PA, USA) analysis. All sample pH values were measured to ensure they fell within the working range of 5 to 11 of the ELISA kits. Half of the rehydrated sample was mixed with methanol and shipped overnight to Dr. Greg Boyer's laboratory at the State University of New York for cyanotoxin quantification by liquid chromatography and mass spectrometry.

LC-MS analysis

Microcystin-LR and nodularin were determined using an untargeted reverse phase chromatography coupled with single quadrupole mass spectroscopy (LC-MS) screening

method. Toxins were separated using an ACE C18 column (3.0 x 150 mm, MacMod Analytical, Chelmsford PA) and a 30–70% aqueous acetonitrile gradient containing 0.1% formic acid as the modifier (Boyer 2007). Individual toxins were identified on the basis of their retention time, their characteristic absorbance spectrum in the photodiode array detector, and their characteristic molecular ions. The LC-MS method described here used a Waters 2695 solvent delivery system coupled to a 2996 PDA detection and a ZQ4000 mass spectrometer (Waters Corporation, Milford MA). It specifically looks for the molecular ions (and/or their sodium adducts) of 22 common congeners (RR, dRR, mRR, H4YR, hYR, YR, LR, mLR, zLR. dLR, meLR, AR, FR, WR, LA, dLA, mLA, LL, LY, LW, LF, WR) and NOD. For many of these congeners, validated reference standards do not exist, therefore the individual congeners were quantified using a standard curve based on microcystin-LR (Abraxis Inc., Warminster PA). NOD was quantified using a standard curve based on nodularin (Abraxis Inc., Warminster PA). Detection limits were 3.0 μg mL^{-1} (0.3 μg g^{-1} fresh weight lettuce, FW) for both microcystin-LR and nodularin. Full method details and the standard operating protocols are available from Protocols.io (Boyer 2020).

BMAA was measured separately in the same extracts using tandem mass spectrometry (LC-MS/MS). BMAA was measured using a LC-MS/MS method that cleanly resolves BMAA from the isomeric compounds diamino butyrate (DAB) and amino ethyl glycine (AEG) modified from Lage et al. 2016. BMAA, DAB, and AEG were assayed by LC–MS/MS using one quantification ion and one or two confirmation ions for each compound. Separation was achieved with an TSKgel Amide-80 250 × 2.0 mm column (Tosoh Bioscience LLC, King of Prussia, PA) assembly with solvent flow of

0.3 mL min^{-1} from Alliance 2695 solvent delivery system coupled to a TQD tandem mass spectrometer (Waters, Milford, MA, USA). The solvent gradient was beginning with 70 % acetonitrile in water to 100% water over 10 min followed by a 5 min wash and re-equilibration step. All solvents contained 0.1% formic acid. Calibration was performed with single point calibrations of BMAA (method detection limit: MDL < μg g^{-1} dry weight). Detection limit for BMAA 2.3 μg mL^{-1} (0.23 μg g^{-1} fresh weight lettuce). Multiple reaction monitoring quantitation transitions were: BMAA (119.01 > 101.95, collision energy (CE) 11 eV), DAB (119.01 > 100.95, CE 11 eV), AEG (119.01 > 101.95, CE 11 eV). Confirmation transitions were: BMAA (119.01 > 75.94, CE 11 eV; 119.01 > 87.91, CE 13 eV), DAB (119.01 > 101.95, CE 11 eV), AEG (119.01 > 75.94, CE 11 eV; 119.01 > 100.95, CE 11 eV).

ELISA analysis

The kit producer instructions were followed for the quantification of cyanotoxins using ELISA. A spectrophotometer set to 450 nm was used to measure the absorbance of each sample. To calculate the amount of cyanotoxins within each sample, a four-parameter logistic function (4PLF) was used; where *a* is the minimum absorbance value obtained, *d* is the maximum, *c* is the point of inflection, *b* is the slope of the curve, and *x* and *y* are the independent and dependent variables.

$$y = d + \frac{a - d}{1 + \left(\frac{x}{c}\right)^b}$$

Cyanotoxin values were reported from the 4PLF as μg toxin per liter and corrected by multiplying the values by the mass of the sample (e.g. soil and lettuce). Soil samples were

reported as μg toxin per dry gram of sample and lettuce as μg toxin per kilogram of fresh weight.

Sample validation and recovery

To check for interference within the samples, three concentrations of cyanotoxin standards were spiked into a control for both the batch soil experiment and in lettuce shoots and roots from the greenhouse experiment. For microcystin-LR and nodularin, spikes of 0.25, 1.0, and 2.5 μg L^{-1} were made in triplicate. Spikes of 25, 100, and 250 μg L^{-1} were made for BMAA. Recovery of greater than ± 30% indicated interference with the quantification of the cyanotoxin in the respective sample matrix.

Estimated daily intake (EDI) calculations

To estimate the daily intake of cyanotoxins for an average size human we used the following equation:

$$\text{EDI} = \frac{T \times LC}{W}$$

The concentrations of toxins in the edible fractions of the lettuce (T, μg kg^{-1} fresh weight) and the daily consumption amounts of lettuce (LC, kilograms per day) were divided by the weight of an average-sized human (W, 60 kg adult). We assumed consumption of 85 fresh weight grams of lettuce based on the suggested serving size (U.S. FDA 2017). An EDI of > 0.04 exceeds the total daily intake limit set by the World Health Organization.

Meta-analysis

A meta-analysis of current publications on cyanotoxin uptake in crops was synthesized to discover linear relationships between cyanotoxin uptake, concentration, and different crop/plant parts. In total, 27 publications on cyanotoxin uptake in crops were assessed. Over 75% of these studies were on microcystin, specifically microcystin-LR. Cyanotoxins such as BMAA and nodularin had too few experiments to effectively create linear models. Due to insufficient data for other cyanotoxins, linear models were only created for microcystin-LR using data from 14 studies (Table A.3.2). Plant parts were defined to be either edible (fruit/grains, leaves, roots) or inedible (leaves and roots) to better represent the physical characteristics and uptake mechanism of each part.

RESULTS

Soil sorption of cyanotoxins

Microcystin-LR sorption for all soils ranged from 13.7 to 33.4% (K_d = 1.59 to 5.03 L kg^{-1}) of toxin added with the remainder (including the desorbed fraction) potentially being available for plant uptake. For BMAA, sorption was 11.9 to 51.7% (K_d = 1.35 to 10.73 L kg^{-1}). No difference in toxin sorption between BMAA and microcystin-LR was observed across the 8 soils ($p = 0.98$) (Figure 3.1). Both controls differed in the sorption of the two cyanotoxins ($p < 0.1$). Only 2 soils (Collinston and Quindoqua) sorbed BMAA and microcystin-LR in different quantities. The remaining 6 soils did not differ in the sorption of either cyanotoxin. Further, none of the 8 soils differed from each other in the sorption of either cyanotoxin ($p > 0.1$). Soils with high CEC (e.x. Mendon and Collinston) sorbed both BMAA and microcystin-LR the most. In contrast, higher pH (> 7.5) soils sorbed both toxins the least. Recovery validation was

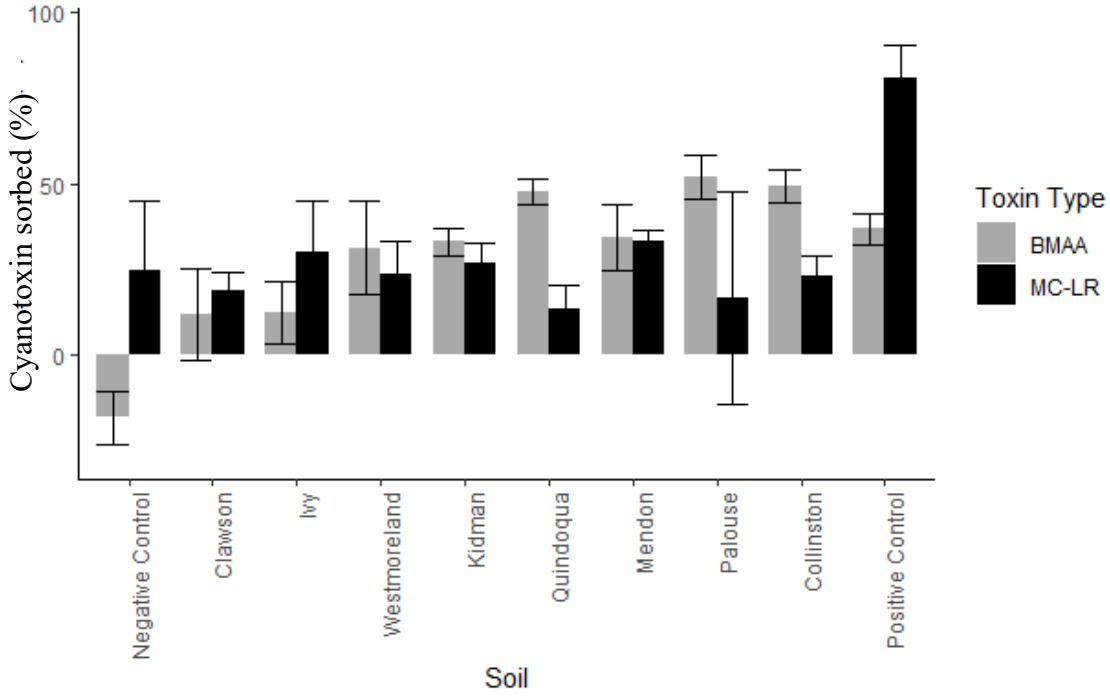

FIGURE 3.1: Cyanotoxin sorption for eight agricultural soils and controls.

TABLE 3.2: Cyanotoxin recovery validation in Mendon soil. A recovery percentage between 70 and 130% indicates negligible interference in cyanotoxin quantification

Spike (μg L^{-1})	Recovery (μg L^{-1})	SD (μg L^{-1})	Recovery %	CV %
BMAA				
25	41.1	7.2	164.2	17.5
100	85.7	16.5	85.7	19.3
250	256.8	16.0	102.7	6.2
Microcystin-LR				
0.25	0.34	0.03	134.1	7.5
1.00	0.88	0.10	87.9	11.4
2.50	2.01	0.34	80.2	16.8

satisfactory for all but the 25 μg L^{-1} BMAA spike (Table 3.2)

There were weak and slight relationships between the chemical soil characteristics, OM, CEC, and pH with cyanotoxin sorption (Figure 3.2). Microcystin-LR sorption saw the strongest relationship with CEC, followed closely by pH and OM. For BMAA, CEC explained the most variance in sorption to soil, followed by OM and pH. All relationships were statistically significant ($p = 0.049$ to $p < 0.001$).

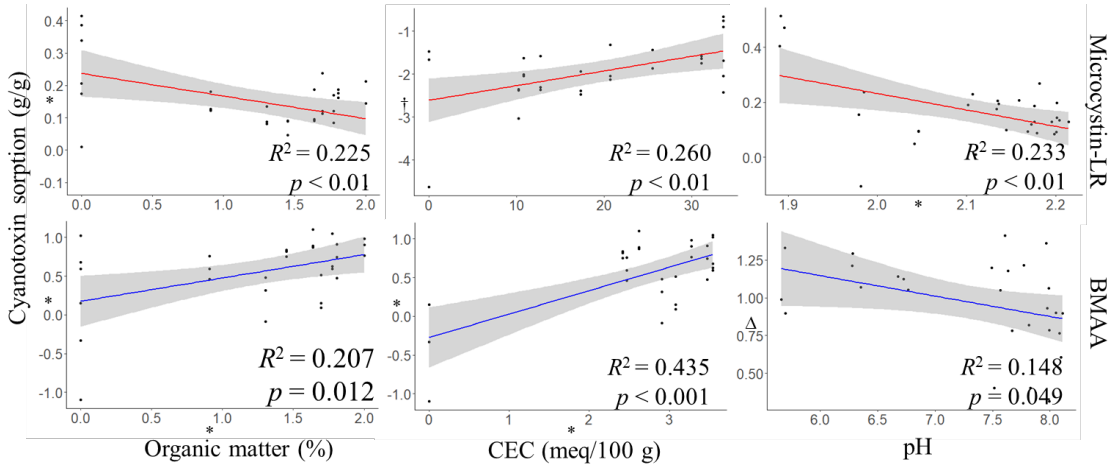

FIGURE 3.2: Relationship between chemical soil characteristics and cyanotoxin sorption. A (*) on an axis indicates a natural log transformation, (†) indicates a log transformation, and (Δ) is a square root transformation.

Significant relationships ($p = 0.026$ to $p < 0.001$) were found between the physical soil characteristics (proportion of clay, sand, and silt) and cyanotoxin sorption for both toxins (Figure 3.3). Microcystin-LR sorption had the strongest negative relationship with the silt content followed by a positive relationship with clay content. For BMAA, all physical characteristics significantly impacted sorption, with clay and silt increasing sorption and sand decreasing sorption.

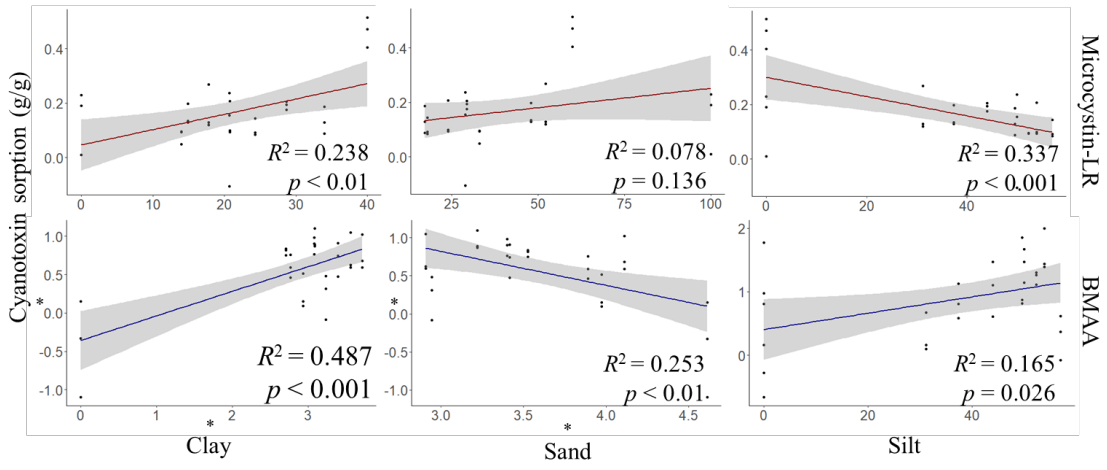

FIGURE 3.3: Relationship between physical soil characteristics and cyanotoxin sorption. A (*) on an axis indicates a natural log transformation, (†) indicates a log transformation, and (Δ) is a square root transformation

Lettuce uptake of cyanotoxins

Effects on growth. After 6 weeks of irrigation with cyanotoxin laden irrigation water, no differences in plant biomass were measured amongst any of the toxins or concentrations (Figure 3.4). No apparent visual indicators of plant toxicity via physical differences in plant color foliage quality were noted at any point during the experiment.

Cyanotoxin concentration in lettuce via ELISA. Results generated using ELISA encountered interference for all toxins and plant parts. To account for the interference, cyanotoxins found in controls were subtracted from treatment concentrations. Nearly half of the plants receiving BMAA did not differ in toxin concentration from the control and cannot be quantified. Cyanotoxin concentration in leaf and root tissue values were orders of magnitude higher than reported in similar studies (Table 3.3). Interestingly, toxin concentrations in both roots and shoots of lettuce increased with the treatment concentration of microcystin-LR. Recovery validation (Table 3.4) for BMAA was between 133% and 1158% and microcystin-LR (nodularin) between 131% and 7866%;

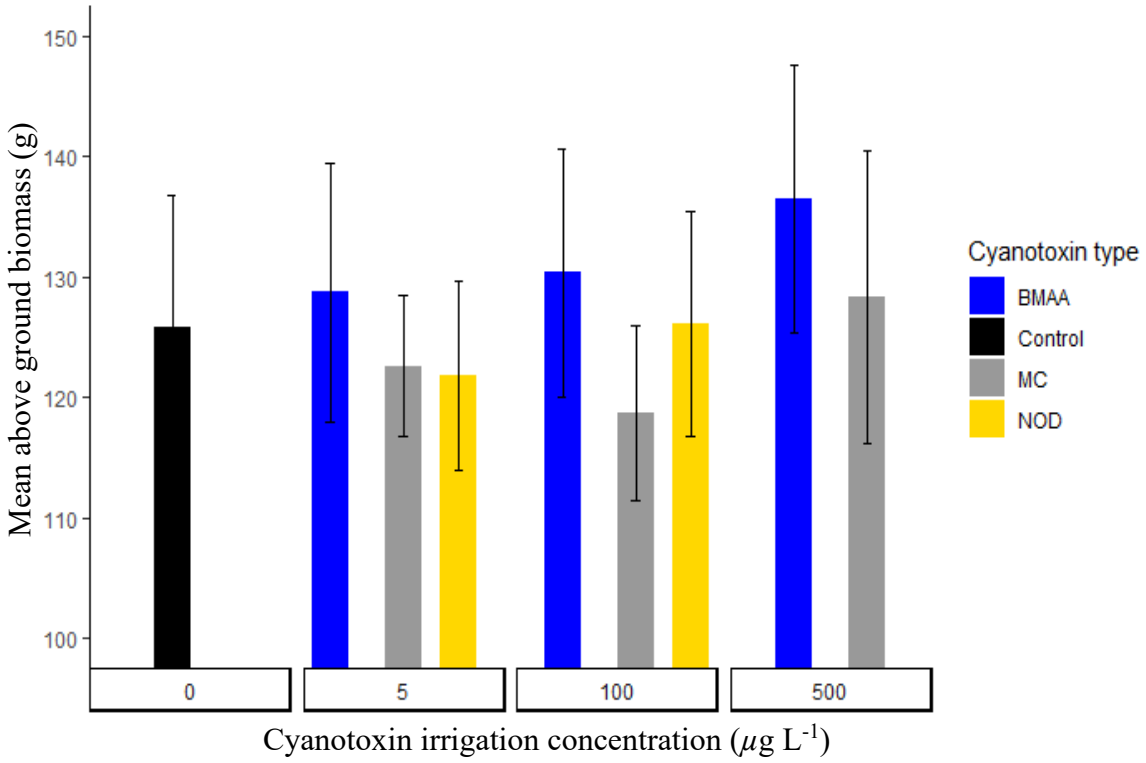

FIGURE 3.4: Lettuce plant mass after irrigation with cyanotoxins for the 6-week growth period.

indicating severe interference in the quantification. Root material appeared to have lower,but still unacceptable interference than leaf material for all toxins.

Cyanotoxin uptake via LC-MS. None of the three cyanotoxins were detected in any of the leaf or root samples. However, an isomer of BMAA, neurotoxic 2,4-Diaminobutanoic acid (DAB) was detected in both roots and leaves (Figure 3.5). A BMAA standard was spiked into a few DAB-positive samples to confirm that a shift in the chromatogram peak did not occur. Only the free form of DAB was found in leaves, while both protein-bound and free were detected in the roots. DAB concentrations were indifferent between treatments including the controls, indicating DAB production was not a product of cyanotoxin irrigation. Further, DAB is not known to be produced by lettuce

plants (Nunn and Codd 2017) and its presence in our samples is abnormal.

TABLE 3.3: Cyanotoxin concentration in lettuce plants via ELISA. Estimated daily intake (EDI) was calculated for a 60 kg adult consuming 85 grams of leaf material. ND indicates no difference in concentration between the control and treatment. PB stands for protein-bound BMAA

Treatment	Concentration (μg kg^{-1} FW)	EDI	Treatment	Concentration (μg kg^{-1} FW)	EDI
BMAA			Microcystin-LR		
Root (PB)			Root		
5	ND		5	3342	
100	ND		100	13574	
500	ND		500	34592	
Root (Free)			Leaf		
5	ND		5	9649.8	13.7
100	2501.6		100	11961.1	16.9
500	23540.0		500	18475.6	26.2
Leaf (PB)			Nodularin		
5	ND		Root		
100	9954.2	14.1	5	363960.0	
500	5718.8	8.1	100	208368.9	
Leaf (Free)			Leaf		
5	ND		5	374.4	0.53
100	ND		100	2110.5	3.0
500	38781.1	54.9			

TABLE 3.4: Cyanotoxin recovery validation for quantification using ELISA. Above Quantifiable Limit (AQL) indicates a value beyond the quantification limit of ELISA using the four-parameter logistic function (4PLF)

Spike (μg L^{-1})	Concentration (μg L^{-1})	SD (μg L^{-1})	Recovery %	CV %
BMAA				
Root (Protein-bound)				
25	155.0	4.1	620.2	16.1
100	197.4	22.8	197.4	50.7
250	677.2	162.1	270.9	36.9
Root (Free)				
25	94.6	13.2	378.4	26.4
100	161.9	2.1	161.9	61.8
250	331.5	120.6	132.6	75.4
Leaf (Protein-bound)				
25	194.4	7.2	777.6	12.9
100	557.0	169.7	557.0	18.0
250	AQL			
Leaf (Free)				
25	289.5	35.8	1158.1	8.6
100	AQL			
250	AQL			
Microcystin-LR				
Root				
0.25	1.0	0.4	388.9	25.7
1	1.7	0.6	173.2	57.7
2.5	3.3	0.5	131.4	76.1
Leaf				
0.25	19.7	0.0	7866.4	1.3
1	8.5	2.4	845.7	11.8
2.5	AQL			

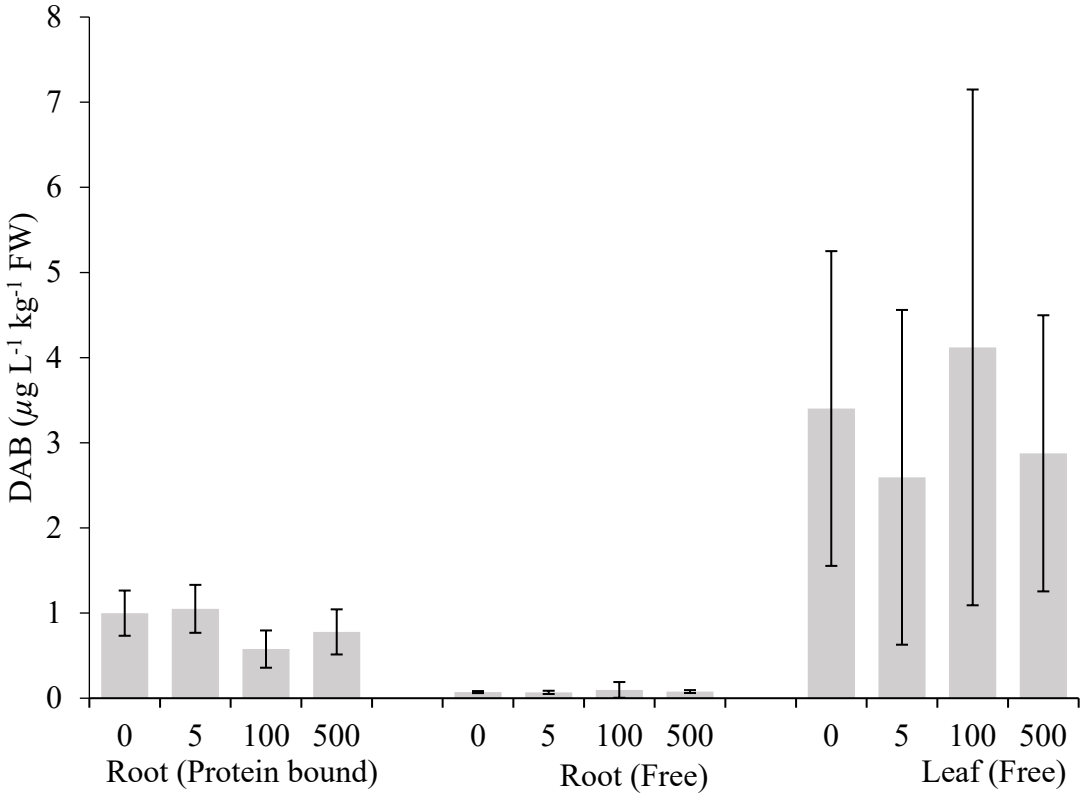

FIGURE 3.5: Protein-bound and free 2,4-Diaminobutanoic acid (DAB) concentrations in roots and leaves of lettuce plants.

Meta-analysis

Microcystin-LR uptake differed between edible and inedible plant parts in relation to the concentration of the toxin in irrigation water (Figure 3.6). The edible parts had stronger relationships between plant concentration and water concentration than their inedible counterparts. Fruit and grains had by far the strongest and most significant positive association between microcystin-LR concentration in their tissue and in irrigation water ($R^2 = 0.70$, $p < 0.001$). Edible leaves showed a weaker but significant relationship ($R^2 = 0.23$, $p < 0.05$). No relationships were observed for either inedible leaves ($R^2 = 0.027$, $p = 0.556$) or roots ($R^2 = 0.022$, $p = 0.328$). There were no significant differences in cyanotoxin concentration between analysis methods for any of

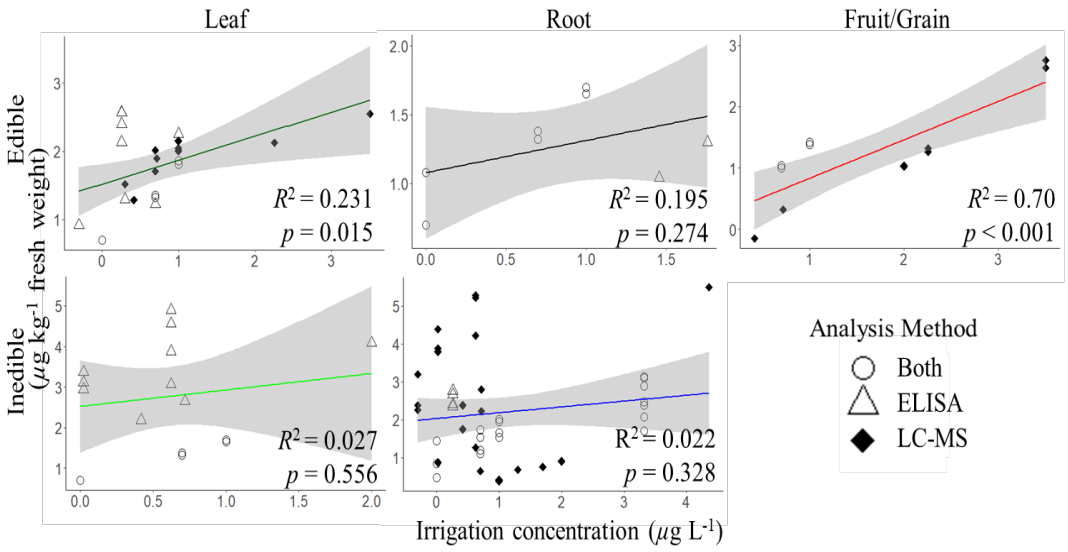

FIGURE 3.6: Relationship between microcystin-LR concentration and irrigation concentration amongst plant parts. Microcystin-LR plant (μg kg^{-1} fresh weight) and irrigation concentration (μg L^{-1}) both underwent a logarithmic base 10 transformation. Analysis method for each result is indicated by different symbols.

the plant parts ($p > 0.1$).

DISCUSSION

Cyanotoxins in agriculture pose an increasing threat to human health as HABs become more common in lakes due to climate change and human activity. In an attempt to quantify this threat, we assessed three aspects of cyanotoxins in food crops. First, we measured the degree to which microcystin-LR and BMAA partition between soil and water to determine the amount of toxins available for plant uptake. Second, lettuce plants were irrigated with microcystin-LR, nodularin, and BMAA to quantify the potential risk a food crop may have as an exposure route to humans. Finally, a review of current literature and a meta-analysis discovered relationships between the concentration of microcystin-LR in irrigation and the concentration in various plants.

Soil sorption

Soil can be an important factor in the partitioning of cyanotoxins between water, soil, and a plant. BMAA and microcystin-LR partitioned in water and soil depending on the soil's characteristics. Only one concentration of BMAA and microcystin-LR were studied, and soil uptake may change for different cyanotoxins concentrations. Dependent on the initial microcystin-LR concentration, soil can potentially bind the microcystin-LR in the water before it can be taken up by plant roots. Soils may act as a reservoir for binding cyanotoxins to make them temporarily unavailable for plant uptake, allowing time for biodegradation, and therefore reducing future exposure to humans via plant ingestion.

However, the chemical characteristics of the soil directly impact the fate of the cyanotoxins and how permanently they are bound. In this study, pH negatively influenced microcystin-LR sorption with a decrease in sorption by 0.60 g g^{-1} per one natural log pH increase. This result supports the established relationship that microcystin-LR hydrophobicity decreases with pH increase (De Maagd et al. 1999; Liang et al. 2011; McCord et al. 2018). The pKa values of microcystin-LR (pKa_1 = 2.09, pKa_2 = 2.19, pKa_3 = 12.48) determine that the dominant species is $(COO^{-})_2(NH_2^{+})$ in pH values between 2.19 and 12.48 giving the toxin an increasingly net negative charge. As the negative charge increases, the octanol-water partition coefficient decreases and subsequently the hydrophobicity of the compound. Most agricultural soils are generally above the 5.5 pH threshold where microcystin-LR becomes increasingly hydrophilic, reducing a soil's ability to remove microcystin-LR from irrigation water. Soil organic matter content also had a negative relationship with microcystin-LR sorption. Each of the soils used had organic matter concentrations between 0 and 6.4%, which falls in the range (1 to 6%) of

typical agricultural topsoil OM content (NRCS 2020). Wu et al. 2011 showed microcystin-LR sorption in soils decreases as OM increases to a threshold of ~8%.

Unfortunately, OM and pH are unlikely to be altered beyond the thresholds where cyanotoxins like microcystin-LR become largely unavailable to plant uptake. Increased CEC had a weak positive relationship with both microcystin-LR sorption and a moderate positive relationship with BMAA. High CEC values are associated with a greater abundance of negatively charged metal ions which can bind the positively charged microcystin-LR (above 5.5 pH). For physical soil characteristics, clay content also had a positive relationship with microcystin-LR since CEC and clay content are correlated in soils. However, for BMAA no studies to date have established relationships between any soil characteristics and BMAA sorption. BMAA is highly polar and has a neutral charge around a pH of 8, the isoelectric point for the two amine groups (Diaz-parga et al. 2018). Therefore, soils with high CEC (R^2 = 0.435) and clay content (R^2 = 0.487) may preferentially retain the toxin due to its polarity. Organic matter and pH are unlikely to be a soil characteristic that can be altered to reduce cyanotoxin mobility and bioavailability to plants. However, CEC and clay content may be a viable determinant of cyanotoxin fate in a soil-plant system. A soil with high CEC and clay content would be likely to increase the proportion of microcystin-LR or BMAA partitioning into the soil and potentially temporarily decrease the amount of cyanotoxins available for plant uptake. These soil conditions, which are conducive to cyanotoxin sorption, are most common in the agricultural portions of the U.S. (mainly the Midwest) where crops may be irrigated with cyanotoxin-laden water and their soils could lower the risk of uptake (Miller and White 1998; Bockheim and Hartemink 2013). However, only a few studies have quantified the

fate of cyanotoxins in a soil-plant system but only with a single soil type in each study (Lee et al. 2017; Levizou et al. 2017; Cao, Steinman, Wan, et al. 2018b; Li et al. 2019). Thus, further research is needed to determine the exact mechanisms affecting the retention and uptake of cyanotoxins in an agricultural setting.

Plant cyanotoxin concentration

This study was unable to quantify the ability and magnitude at which lettuce crops can concentrate cyanotoxins from irrigation water due to either unreliable analysis or that there simply was no root uptake. Numerous previous studies have shown various plants can translocate cyanotoxins such as microcystin-LR and BMAA from their roots into their shoots, therefore it is unlikely that the lettuce did not have the ability to accumulate cyanotoxins (Table A.3.2). There are several potential reasons that we could not quantify cyanotoxins in our lettuce plants. 1) Growth conditions specific to this study may have prevented the cyanotoxins from being bioavailable to the lettuce. 2) Extraction or analytical errors may have also contributed to the inability to accurately quantify toxin concentration in plant tissues, or 3) there was no uptake into the lettuce. No direct evidence exists that the cyanotoxins in the irrigation did not come into contact with lettuce roots. However, cyanotoxin concentrations in the growth media were not measured, and significant sorption may have occurred. Further, the half-life of microcystin-LR is as low as 1.7 days in soil with high microbial activity (Cao, Steinman, Yao, et al. 2018). Sufficient sorption and intensive microbial activity may have degraded cyanotoxin concentrations in the growth media below the threshold for plant root uptake. Regardless, our meta-analyses (see below) suggests that our cyanotoxin concentrations may have been below the analytical detection limits, which is a plausible explanation for

the non-detects.

The extraction methods for each cyanotoxin were nearly identical to other studies, which suggests our ELISA analyses were subject to contamination while our LC-MS results suffered from issues with analytical detection. The first quantification method, ELISA, exhibited extensive interference which resulted in unrealistically high concentrations of cyanotoxins. Curiously, interference was seen across each of the three toxins and all plant parts (leaves and roots). Multiple ELISA kits were used, and each had a suitable standard curve performance ($R^2 > 0.99$), indicating the interference was originating elsewhere. ELISA kits are known to generate false positives with saline samples, however, the deionized water used to resuspend the cyanotoxin extracts was not saline (Metcalf et al. 2000). Conflict exists on the ability for ELISA to accurately quantity cyanotoxins, particularly BMAA in surface waters and complex samples (e.g. soil and plants) due to the high probability of false positives, and high variability amongst replicates and variants (Vita et al. 2016; Guo et al. 2017). Even in water samples, BMAA and microcystin-LR recovery from ELISA can reach 400% (Faassen et al. 2013; Guo et al. 2017). Further, the manufacturer of the ELISA used in this study recently released a statement that certain filter types can lead to false low or high toxin concentrations. We measured potential filter influence by passing 5 mL, equal to the volume filtered in the extraction procedure, of deionized water through PVDF filters. The filters were falsely adding an average of 3.29 ± 0.27 μg L^{-1} to the BMAA samples. All cyanotoxin plant concentration and recovery samples were corrected for this inflation. No interference was found for microcystin-LR or nodularin.

False positives can also be generated by a loss of the enzyme coating on the

ELISA kit well plates. Because inflated concentrations were not detected for any of the standards or blanks, a compound unique to the lettuce and root extracts may be interacting with the enzyme coating and causing the positive interference. Future studies that quantify cyanotoxins from complex matrices that have limited prior research should be cautious of interference yet to be identified. Referencing experimental data to established relationships between cyanotoxin uptake and irrigation concentration can help to highlight potential interference. Based on current relationships between the concentration of microcystin-LR in leaf material and irrigation water we would expect to have measured between 57.5, 165.8, and 291.1 μg kg^{-1} FW for the 5 μg L^{-1}, 100 μg L^{-1}, and 500 μg L^{-1} microcystin-LR treatments. Instead, the concentrations we measured by ELISA was 168, 72, and 63 times higher than the expected value. When the predicted concentration values from the meta-analysis for our lettuce were converted to EDI, a human would consume 0.081, 0.235, and 0.412 μg microcystin-LR per kilogram of bodyweight respectively with each treatment. These estimated EDIs are between 2 to 10 times higher than the WHO 0.04 μg kg^{-1} limit.

All lettuce leaf and root samples analyzed by ELISA were also subjected to LC-MS quantification. LC-MS does not have the same potential issues with interference as ELISA does, and ELISA typically gives significantly higher toxin concentrations than LC-MS (Foss and Aubel 2015). Falsely inflated ELISA results could explain the lack of agreement with LC-MS. Therefore, the cyanotoxins might not be present in the plants in high enough quantities to reach detection limits or they are not being extracted successfully. Also, microcystin-LR has been shown to adsorb to polypropylene pipette tips even after one pipetting step (Hyenstrand et al. 2001; Altaner et al. 2017), which may

have been a source of cyanotoxin loss during our extraction procedure. During extraction, the chemical structure of a cyanotoxin may be altered enough such that LC-MS detection is limited due to over specificity and therefore unable to quantify the toxin (Foss and Aubel 2015). Interestingly, 2,4-Diaminobutanoic acid (DAB), an isomer of BMAA which is produced in tandem with BMAA was found in both leaf and root material and controls. Concentrations of DAB in plants that received BMAA were the same as the controls and BMAA was spiked into samples to confirm a shift in chromatograph peaks did not occur. DAB is typically produced in the environment by some diatoms, dinoflagellates, and cyanobacteria (Krüger et al. 2010; Lage et al. 2014; Violi et al. 2019). A few studies have quantified DAB in various plants, though mainly in the Fabaceae family (Fowden and Bryant 1958; Bell and Tirimanna 1965; Nigam and Ressler 1966). Regardless, the presence of DAB in lettuce in this study is unprecedented and requires further research to identify the source.

Meta-analysis

A review of 14 recent studies on the concentration of microcystin-LR in plants shows relationships of varying strength between uptake and irrigation cyanotoxin concentration amongst the different edible and inedible parts of the plant (Figure 3.6). Studies did differ in analytical methods (LC-MS and ELISA) and plant irrigation conditions (hydroponic, drip, or spray) or were not reported. Neither the analytical method used, nor the irrigation conditions affected the microcystin-LR concentration in plant material significantly ($p > 0.1$). However, these differences are still a limitation of the models created in the meta-analysis.

The microcystin-LR concentrations predicted for our lettuce plants by the models produced by the meta-analysis identified another potential reason that the lettuce samples failed to reach the 0.3 μg g^{-1} FW microcystin-LR detection limit of the LC-MS. For our three microcystin-LR treatments of 5 μg L^{-1}, 100 μg L^{-1}, and 500 μg L^{-1}, the model predicted microcystin-LR concentrations in lettuce leaves of 0.0575, 0.1658, and 0.2911 μg g^{-1} FW, all of which are at or below the LC-MS detection limit. While our experimental conditions did not exactly match those that generated the model, it is possible that our lettuce plants did not have had enough cyanotoxins in their tissue to be detected. More leaf tissue could have been collected from the lettuce to increase the cyanotoxin concentrations in the extractions to surpass the detection limit of the LC-MS. The models created for each of the plant parts, with continual updates, could be a useful tool for future studies to estimate plant cyanotoxin concentrations before the start of an experiment to optimize analytical methods.

Differences in toxin concentration in plant parts were due to the chemical and physical properties of individual toxins and the respective plant uptake mechanisms. Large toxins such as microcystin-LR and nodularin will concentrate first in the roots and second in leaf tissue where transpiration occurs. However, the large size of microcystin-LR and nodularin make it unlikely for these compounds to make it first into the xylem sap, phloem sap, and eventually the fruit tissue (Trapp and Legind 2011). Yet, microcystin-LR was measured in the fruit of tomatoes and green beans as well as in rice grains suggesting an uptake mechanism that allows a large cyanotoxin molecule to eventually reach the fruit and grains (Table A.3.2).

An uptake route for large cyanotoxins into plants has yet to be tested, however, studies on the plant uptake of nanoparticles (< 100 nm) suggest a potential root uptake mechanism that may also apply to cyanotoxins (Dietz and Herth 2011; Oliveri Conti et al. 2020). Microcystin-LR and nodularin are similar in size to some nano-particles, and when dissolved in water have a volume of 2.63 and 2.24 nm^3 respectively (Lanaras et al. 1991). Usually, plant roots are covered by an exodermis that prevents large molecules, such as microcystin-LR, from entering and translocating in the xylem (Steudle and Peterson 1998). However, newly formed lateral roots lack the exodermis and can allow for the transport of larger molecules into the xylem (Faiyue et al. 2010). Cracks in the exodermis of lateral roots also allow the passage of large molecules (Li et al. 2020). Large cyanotoxins such as microcystin-LR and nodularin may use the lateral roots to enter the xylem. Once in the xylem, translocation to the rest of the plant is restricted via pores in the filters of xylem nodes, the size of which is dependent on the species of plant (Zhu et al. 2008; Dietz and Herth 2011). The filter pores of maize (*Zea mays*) were found to be < 20 ± 1.4 nm, but allowed for the perfusion of particles < 4.9 nm in diameter (Shane et al. 2000). Pumpkins (*Cucurbita maxima*) also translocated magnetite particles (~20 nm) into leaf material (Zhu et al. 2008). Also, nanoplastics were translocated to *Lactuca sativa* leaves in quantities positively related to the transpiration rate of the plant (Li et al. 2020). Therefore, dependent on plant species, microcystin-LR and nodularin may be small enough to enter plants via new lateral roots and translocated throughout the plant through nodes with sufficiently large pores.

Cyanotoxin concentrations in fruit and grains may have the greatest relationship with the toxin concentration in irrigation water as plants preferentially protect

reproductive organs from contaminants by compartmentalizing toxins into cell vacuoles and walls (Kvesitadze et al. 2009). With magnitude increases in toxin concentration in the plant toxins may overwhelm the compartmentalization mechanisms and enter the fruit and grains proportionally to the concentration in the overall plant. Also, Oliveri Conti et al. 2020 hypothesized that fruits contain more microplastics than other plant parts because fruit is highly vascularized, and the plant has a more complex root system by the time it is capable of producing fruit leading to greater uptake. Toxin concentration in edible portions of plants may have stronger relationships with toxin concentration than the inedible part as edible material usually has the most energy, nutrients, and water allocated to its growth (e.g. lettuce leaves and carrots).

Using the same regression that predicted the potential for microcystin-LR concentration in lettuce, the concentration of the toxin in irrigation can be calculated for a given concentration value. For a human health context, the concentration of microcystin-LR in irrigation water that drives plant uptake above the 0.04 μg kg^{-1} daily intake limit was determined. Based on the daily consumption of 85 grams of lettuce, a concentration of 3.39 μg microcystin-LR per liter of irrigation water can cause uptake above the daily intake limit. Interestingly, this estimated microcystin-LR irrigation concentration only slightly exceeds the 2.44 ± 0.45 SE μg L^{-1} average microcystin-LR concentration measured in 851 HAB lakes from the 2007 and 2012 EPA National Lakes Assessment (Chapter 2). Therefore, if crops such as lettuce are irrigated with water from the average U.S. lake containing microcystin-LR, the concentration of cyanotoxin taken up in the lettuce may approach the recommended daily intake limit. Consuming 148 grams of tomato irrigated with 42.0 μg L^{-1} microcystin-LR will also exceed the limit (U.S. FDA

2017). As expected from plant toxin compartmentalization mechanisms, fruit and grains require a higher concentration of cyanotoxins in irrigation to match the concentration in edible leaves. As the depth of research expands on cyanotoxins in food, those who irrigate with cyanotoxin laden water can predict the concentration in food from known concentrations in irrigation water to project whether certain plants will exceed total daily intake limits.

The lack of a significant relationship in toxin concentration and edible root uptake may be due to roots reaching a maximum concentration of toxin before translocation to above-ground matter occurs (Miller et al. 2016). Existing root uptake models for organic contaminants predict that polar, neutral non-volatile chemicals are the most likely to be translocated from the root system into the xylem, a metric described by transpiration stream concentrations factors (TSCF) (Dettenmaier et al. 2009). TSCF values greater than one are taken up by plants more readily than water, and values lower than one are taken up less than water. A relationship exists between TSCF and the partitioning of organic containments between water and octanol (K_{ow}) with highly polar ($\log K_{ow} < 1$) and lipophilic ($\log Kow > 4$) having low TSCF values due to the contaminants partitioning to the lipid bilayer in plant roots rather than be transported via water into the xylem (Briggs et al. 1982; Hsu et al. 1990; Burken and Schnoor 1998; Dettenmaier et al. 2009; Miller et al. 2016). Experimental $\log K_{ow}$ values exist for microcystin-LR, ranging from -0.1 to -2.0 in the environmentally relevant pH range of 6 to 8 (De Maagd et al. 1999; Liang et al. 2011; McCord et al. 2018). Based on $\log K_{ow}$ values alone, microcystin-LR is not necessarily favored for root uptake, however, mechanisms such as the peptide transfer system may facilitate uptake (Oudra et al. 2019). As no experimental

log K_{ow} values exist for BMAA, the EPI SuiteTM estimate of log K_{ow} = -4.0 (KOWWIN v. 1.68) indicates a low potential for bioaccumulation in plants, however, low molecular weight and the ability for BMAA to be integrated into proteins vastly increases the potential for biomagnification in organisms (Murch et al. 2004). While still unclear, identifying the exact cyanotoxin concentration and partitioning mechanisms would allow for better predictions on the fate of cyanotoxins in plants.

CONCLUSION

The increase in frequency and severity of toxic HABs from climate and anthropogenic effects on the environment has placed an enhanced focus on the potential for human exposure to cyanotoxins. Impacts from cyanotoxins in irrigation water on soil, plant quality and uptake, and the risk of ingesting harmful quantities of the toxins are essential to understand to make informed decisions to protect human and economic health. Research must continue on a larger variety of cyanotoxins and food crops, as well as how soil and degradation mechanisms alter plant concentration. We also suggest caution in interpreting ELISA cyanotoxin results for complex matrices. Because cyanotoxin concentration in edible plants is positively related to the concentration in the irrigation, increased severity of HABs magnifies the risk of significant exposure above set thresholds on daily intake. To protect the health of both humans and the environment, HABs must be mitigated and appropriate irrigation regimes and contamination limits need to be instituted immediately.

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CHAPTER 4

CONCLUSION

This thesis includes a large-scale assessment of how HABs are changing in U.S. lakes and the risk for exposure from cyanotoxins in food crops irrigated with contaminated water. Chapter 2 identified that HABs are dominating in greater amounts of water bodies due to climate and anthropogenic drivers and that negative impacts on human and ecosystem health will increase with HAB abundance. Chapter 2 informs chapter 3 that nearly 45% of U.S. lakes experience a HAB that produced cyanotoxins which can end up as irrigation water for food crops. In chapter 3, soil was shown to remove nearly half of the cyanotoxins from water, however, the remainder is available for plant uptake. Many crop plants can accumulate a variety of cyanotoxins in quantities that exceed recommended health limits. Plants also accumulate toxins differently amongst tissue types, with fruit and grains accumulating microcystin-LR proportionally to the concentration in irrigation water. Edible plant tissue also saw a weak trend in toxin accumulation in proportion to irrigation water concentration. While cyanotoxin concentration in the lettuce was unable to be quantified in chapter 3, important method and analytical limitations to research on cyanotoxin accumulation in food crops were identified. When combined, both chapters present a framework of how cyanobacteria and their toxins are impacting the environment and those who occupy it. Hopefully, this framework will inform solutions to the health and economic problems associated with cyanobacteria. HABs are only expected to increase in frequency and severity in a future altered by climate changes and humans. Remediating the effects of climate change is no longer possible as controlling temperature and precipitation on a lake scale is impossible.

Completely removing cyanotoxins from water bodies is similarly impossible. However, implementing management and policies that control nutrient pollution can greatly reduce HABs and their toxins. Further, irrigation water quality standards can prevent harmful concentrations of cyanotoxins from being applied to agriculture and taken up into crops. Action should be taken to minimize the harm HABs may have on human lives, the environment, and the economy.

APPENDICES

Table A.2.1. NLA and PRISM variables. Natural logarithm transformations not shown.

Precipitation/Air Temperature	Water Chemistry	Lake Conditions	Physical Conditions
Average spring	Total nitrogen	Surface temperature	Maximum depth
Average summer	Total phosphorus	Epilimnion temperature	% agricultural basin
Average annual	Turbidity	Epilimnion depth	% developed basin
Average two weeks prior	Acid neutralizing capacity	Epilimnion volume	Secchi depth
Max. spring	Dissolved organic carbon	Epilimnion energy	Elevation
Max. summer	Conductivity	Season	Lake area
Max. annual	Dissolved oxygen	Lake origin	Urbanized shore
Max. two weeks prior	pH	Ecoregion	Latitude
Min spring	Nitrogen phosphorus ratio	Size class	Longitude
Min summer		EPA region	
Min annual			
Min. two weeks prior			
# days above 25 °C			

Table A.2.2. Change in mean (μ) and median ($\tilde{x}$) total phosphorus and nitrogen between 2007 and 2012.

Region	TP (μ)	TP ($\tilde{x}$)	TN (μ)	TN ($\tilde{x}$)
U.S.	9	15	-0.2	38.5
MAN	12.3	12	206.5	77
NAT	2.62	14	-284.6	14
CPL	45	11.5	195.3	41.5
NAP	6.2	5	-0.9	25.5
NPL	-15.7	-79	-589.2	-933
SAP	23.3	13	78.9	48
SPL	55.5	22	581.5	290
TPL	-3.7	-1.5	-176.8	-32
UMW	7.6	15	41.1	41.5
WMT	21.7	19	62.4	-1
XER	-78.2	21	-171.1	-113

Table A.2.3. Linear models for the remainder of ecoregions not shown in main text.

	Best model determined by AIC	R^2	*k*	*n*
ln(CHLA)				
CPL	0.55 ln(TN) + 0.37 ln(TP) - 0.32 DOC + 0.08 pH + 0.41	**0.58**	4	219
WMT	1.77 TN + 0.28 ln(TP) + 0.13 EPI.T + 0.15	**0.56**	3	253
SPL	0.55 ln(TN) + 0.17 ln(TP) + 0.12 ln(pH) + 0.1 ln(P_{annual}) + 0.17 Air.T_{25} + 0.03	**0.55**	4	217
TPL	0.73 ln(TN) - 0.53 DOC + 0.34 ln(TP) + 0.08 ln(P_{annual}) + 0.13 Air.T_{25} + 0.02	**0.55**	5	292
UMW	0.54 ln(TP) + 0.42 DOC + 0.29 ln(TN) – 0.03	**0.54**	3	297
NPL	0.95 ln(TN) - 0.51 ln(DOC) + 0.35 ln(TP) - 0.14 P_{prior} – 0.32	**0.47**	4	123
XER	-0.78 DOC + 0.63 ln(TN) + 0.26 ln(TP) + 0.21 ln(EPI.T) – 0.3	**0.46**	4	135
ln(BIOV)				
UMW	1.74 AREA - 0.41 * ELEVATION + 0.39 ln(TP) + 0.17 ln(TN) + 0.16	**0.24**	4	297
CPL	0.58 ln(TN) + 0.04 AREA + 0.01	**0.22**	2	219
WMT	0.41 ln(TN) + 0.29 ln(EPI.T) - 0.29 ln(DOC) + 0.16 ln(TP) + 0.33	**0.20**	4	253
SPL	0.47 ln(TN) - 0.14 ln(TP) + 0.14 Air.T_{25} - 0.13 P_{prior} – 0.01	**0.19**	4	217
XER	-0.34 ANC + 0.32 ln(TP) + 0.25 ln(TN) + 0.03	**0.19**	3	135
TPL	0.44 ln(TP) + 0.16	**0.18**	1	292
NPL	0.36 ln(TN) + 0.12	**0.12**	1	123
ln(MICL)				
UMW	0.38 ln (TN) + 0.23 pH - 0.14 Air.T_{spring}+ 0.1 P_{prior} - 0.11	**0.20**	4	297
NPL	-0.55 LakeOrigin + 0.51 ln(TN) + 0.22 Air.T_{prior} + 0.34	**0.18**	3	123
CPL	0.27 ln(TN) + 0.09 pH - 0.09	**0.11**	2	219
WMT	0.35 TP + 0.05 pH + 0.03 P_{spring} – 0.22	**0.09**	3	253
SAP	1.15 DOC + 0.17 ln(P_{annual}) + 0.15 pH + 0.09	**0.09**	3	198
XER	0.32 ln(TN) – 0.1	**0.05**	1	135

Table A.3.1. Cyanotoxin molecular weights, pKa values, and chemical structures. [1]De Maagd et al. 1999; [2]Klein et al. 2013; [3]Lide 2001; [4]Nunn and O'Brien 1989.

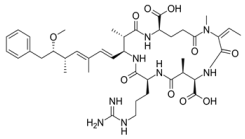
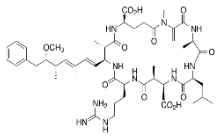
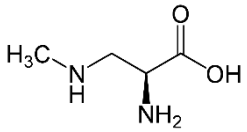
Microcystin-LR[1]	Nodularin-R[2,3]	BMAA[4]
995.2 g mol^{-1}	824.96 g mol^{-1}	118.13 g mol^{-1}
pKa_1 (Carboxyl) = 2.09 pKa_2 (Carboxyl) = 2.19 pKa_3 (Amino) = 12.48	pKa_1 (Carboxyl) = 2.17 pKa_2 (Carboxyl) = 3.96 pKa_3 (Amino) = 12.1	pKa_1 (Carboxyl) = 2.1 pKa_2 (Amine-α) = 6.63 pKa_3 (Amine-β) = 9.76
		

Table A.3.2. Data and publications used in the meta-analysis. Publications that did not specify their method of irrigation are labeled as NA. Analysis is either by ELISA, LC-MS, or both.

Plant	Irrigation	Publication	Dose μg L^{-1}	[MC-LR] μg kg^{-1}	Analysis
Edible Root					
Carrot	NA	Ai et al. 2020	57	20	ELISA
Carrot	NA	Ai et al. 2020	28.5	11	ELISA
Carrot	Drip	Lee et al. 2017	1	12	Both
Carrot	Spray	Lee et al. 2017	10	204	Both
Carrot	Drip	Lee et al. 2017	10	220	Both
Carrot	Spray	Lee et al. 2017	5	74	Both
Carrot	Drip	Lee et al. 2017	5	72	Both
Carrot	Spray	Lee et al. 2017	1	18	Both
Edible Leaves					
Lettuce	Spray	Bittencourt-Oliveria et al. 2016	10	143.35	LC-MS
Lettuce	Spray	Bittencourt-Oliveria et al. 2016	5	103.24	LC-MS
Lettuce	Spray	Bittencourt-Oliveria et al. 2016	2	32.99	LC-MS
Lettuce	Drip	Cao et al. 2018	5.22	78	LC-MS
Lettuce	Drip	Cao et al. 2018	2.61	19.3	LC-MS
Spinach	Drip	Cao et al. 2019	10	140	LC-MS
Lettuce	Drip	Cao et al. 2019	10	110	LC-MS

Table A.3.2. (cont.)

Lettuce	Spray	Cordeiro-Araújo et al. 2016	10	101	LC-MS
Lettuce	Spray	Cordeiro-Araújo et al. 2016	5	51.25	LC-MS
Lettuce	Spray	Hereman et al. 2012	10	177.8	ELISA
Lettuce	Spray	Hereman et al. 2012	2	19.8	ELISA
Lettuce	Spray	Hereman et al. 2012	5	16.8	ELISA
Lettuce	Spray	Hereman et al. 2012	0.5	8.31	ELISA
Lettuce	Spray	Lee et al. 2017	10	72	Both
Lettuce	Drip	Lee et al. 2017	10	64	Both
Lettuce	Spray	Lee et al. 2017	5	23	Both
Lettuce	Drip	Lee et al. 2017	5	21	Both
Lettuce	Drip	Lee et al. 2017	1	5	Both
Lettuce	Spray	Lee et al. 2017	1	5	Both
Lettuce	Drip	Levizhou et al. 2017	1.81	370	ELISA
Lettuce	Drip	Levizhou et al. 2017	1.81	365	ELISA
Lettuce	Drip	Levizhou et al. 2017	1.81	251	ELISA
Lettuce	Drip	Levizhou et al. 2017	1.81	135	ELISA
Water Spinach	Hydroponic	Wijewickrama & Manage 2019	3197.4	350.82	LC-MS
Water Spinach	Hydroponic	Wijewickrama & Manage 2019	180	132.86	LC-MS
Edible Fruit/Grains					
Rice	Hydroponic	Cao et al. 2018	5.22	2.1	LC-MS
Rice	Hydroponic	Cao et al. 2018	2.61	0.7	LC-MS
Tomato	NA	Gutierrez-Praena et al. 2014	100	10.52	LC-MS
Tomato	NA	Gutierrez-Praena et al. 2014	100	10.83	LC-MS
Green Bean	Spray	Lee et al. 2017	10	26	Both
Green Bean	Drip	Lee et al. 2017	10	24	Both
Green Bean	Spray	Lee et al. 2017	5	11	Both
Green Bean	Drip	Lee et al. 2017	5	10	Both
Rice	Hydroponic	Wijewickrama & Manage 2019	3197.4	567.52	LC-MS
Rice	Hydroponic	Wijewickrama & Manage 2019	180	20.97	LC-MS
Rice	Hydroponic	Wijewickrama & Manage 2019	3197.4	429.83	LC-MS
Rice	Hydroponic	Wijewickrama & Manage 2019	180	18.19	LC-MS

Table A.3.2. (cont.)

Inedible leaf					
Rice	Hydroponic	Cao et al. 2018	5.22	446	LC-MS
Rice	Hydroponic	Cao et al. 2018	2.61	153	LC-MS
Tomato	NA	Gutierrez-Praena et al. 2014	100	12298.18	LC-MS
Carrot	Spray	Lee et al. 2017	10	50	Both
Carrot	Drip	Lee et al. 2017	10	45	Both
Carrot	Spray	Lee et al. 2017	5	24	Both
Carrot	Drip	Lee et al. 2017	5	21	Both
Carrot	Spray	Lee et al. 2017	1	5	Both
Peas	NA	Saqrane et al. 2009	4.2	79190	LC-MS
Lentil	NA	Saqrane et al. 2009	4.2	36610	LC-MS
Lentil	NA	Saqrane et al. 2009	1.05	2330	LC-MS
Wheat	NA	Saqrane et al. 2009	4.2	1170	LC-MS
Peas	NA	Saqrane et al. 2009	1.05	880	LC-MS
Corn	NA	Saqrane et al. 2009	4.2	7650	LC-MS
Corn	NA	Saqrane et al. 2009	1.05	1290	LC-MS
Inedible root					
Rice	Hydroponic	Cao et al. 2018	5.22	634	LC-MS
Rice	Hydroponic	Cao et al. 2018	2.61	241	LC-MS
Lettuce	Drip	Cao et al. 2018	5.22	168	LC-MS
Lettuce	Drip	Cao et al. 2018	2.61	56.6	LC-MS
Tomato	Hydroponic	Corbel et al. 2016	23000	311800	LC-MS
Tomato	NA	Corbel et al. 2016	5	4.5	LC-MS
Tomato	NA	Corbel et al. 2016	100	8.1	LC-MS
Tomato	NA	Corbel et al. 2016	50	5.7	LC-MS
Tomato	NA	Corbel et al. 2016	20	4.8	LC-MS
Clover	Drip	Crush et al. 2008	2100	1370	Both
Lettuce	Drip	Crush et al. 2008	2100	780	Both
Rape	Drip	Crush et al. 2008	2100	120	Both
Rape	Spray	Crush et al. 2008	2100	50	Both
Ryegrass	Drip	Crush et al. 2008	2100	240	Both
Ryegrass	Spray	Crush et al. 2008	2100	120	Both
Lettuce	Spray	Crush et al. 2008	2100	300	Both
Clover	Spray	Crush et al. 2008	2100	1280	Both
Broccoli	NA	Jarvenpaa et al. 2007	10	2.4	LC-MS
Mustard	NA	Jarvenpaa et al. 2007	10	2.6	LC-MS
Lettuce	Spray	Lee et al. 2017	10	90	Both
Lettuce	Drip	Lee et al. 2017	10	102	Both
Lettuce	Spray	Lee et al. 2017	5	34	Both
Lettuce	Drip	Lee et al. 2017	5	54	Both

Table A.3.2. (cont.)

Lettuce	Drip	Lee et al. 2017	1	28	Both
Lettuce	Spray	Lee et al. 2017	1	7	Both
Green Bean	Spray	Lee et al. 2017	10	34	Both
Green Bean	Drip	Lee et al. 2017	10	45	Both
Green Bean	Spray	Lee et al. 2017	5	13	Both
Green Bean	Drip	Lee et al. 2017	5	16	Both
Green Bean	Drip	Lee et al. 2017	1	3	Both
Green Bean	Spray	Lee et al. 2017	1	3	Both
Lettuce	Drip	Levizhou et al. 2017	1.81	465	ELISA
Lettuce	Drip	Levizhou et al. 2017	1.81	580	ELISA
Lettuce	Drip	Levizhou et al. 2017	1.81	256	ELISA
Lettuce	Drip	Levizhou et al. 2017	1.81	227	ELISA
Lentil	NA	Saqrane et al. 2009	0.5	1600	LC-MS
Peas	NA	Saqrane et al. 2009	0.5	240	LC-MS
Wheat	NA	Saqrane et al. 2009	0.5	180	LC-MS
Wheat	NA	Saqrane et al. 2009	1.05	7600	LC-MS
Peas	NA	Saqrane et al. 2009	4.2	190850	LC-MS
Lentil	NA	Saqrane et al. 2009	4.2	162790	LC-MS
Lentil	NA	Saqrane et al. 2009	1.05	24520	LC-MS
Wheat	NA	Saqrane et al. 2009	4.2	16660	LC-MS
Peas	NA	Saqrane et al. 2009	1.05	6230	LC-MS
Corn	NA	Saqrane et al. 2009	4.2	18.71	LC-MS
Corn	NA	Saqrane et al. 2009	1.05	7.55	LC-MS