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**Accumulation of Cyanobacteria Toxins in Lettuce and Radishes Exposed to Water and
Aerosols from a Low-Toxin Lake**

BY

ANNE C EWERT

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THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
The Requirements for the Degree of

Master of Science
in
Integrative and Organismal Biology

May 2021

This thesis has been examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences: Integrative and Organismal Biology by:

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On Thursday, March 25th, 2021

Original approval signatures are on file with the University of New Hampshire Graduate School.

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ABSTRACT

ACCUMULATION OF CYANOTOXINS IN LETTUCE AND RADISHES EXPOSED TO LAKE WATER AND AEROSOLS FROM A LOW-TOXIN LAKE

By

Anne Ewert

University of New Hampshire, May 2021

Cyanobacteria are the oldest known photosynthetic organisms on Earth. They are found in a wide range of habitats worldwide but have recently become an increasing issue in both freshwater and marine systems due to anthropogenic eutrophication and climate change. Cyanobacteria produce an array of toxins harmful to wildlife and humans, some of which have been found to accumulate in plant and animal tissues. Microcystins (MCs) are the most common toxins produced, occurring in 40 – 75% of cyanobacteria blooms worldwide. They are highly stable, water and fat soluble, cyclic heptapeptides that cause acute toxicity in the liver by the inhibition of cellular protein phosphatase 1 and 2a, resulting in the breakdown of hepatic tissues. MCs are also tumor promoters and have been linked with non-alcoholic liver disease with long-term exposure. β -methylamino-L-alanine (BMAA) is a non-protein coding amino acid that acts as a neurotoxin, is produced by all groups of cyanobacteria, and has been linked to neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer's, and Parkinson's diseases. While many studies have reported the accumulation of MCs by various crop plants after exposure to high concentrations of toxin via irrigation water, relatively few studies have investigated such accumulation of BMAA, and none have investigated aerosolized cyanotoxins as a possible route of exposure.

In this study, lettuce and radish seedlings were placed in hydroponic systems under small low-tunnels on the shore of Lake Attitash in Amesbury, MA and exposed to low levels of natural, lake-derived cyanotoxins in four treatments via lake water in the hydroponic reservoirs and deposited aerosols emitted from the lake. After three weeks, plant tissues were collected, separated, and analyzed for MCs and BMAA via Enzyme-Linked Immunosorbent Assay (ELISA) analysis. In a second experiment, particulate and dissolved fraction lake aerosols were collected in one open and one sealed/HEPA filtered tunnel using modified Compact-Lake-Aerosol-Monitors (CLAMs) to quantify aerosols emitted from Lake Attitash and to determine if aerosols had penetrated the filtered tunnel treatments.

The average concentration of MCs and BMAA in water from Lake Attitash throughout the three-week experiment was $78.9 \text{ ng MC L}^{-1} \pm 7.06$, and $350.0 \text{ ng BMAA L}^{-1} \pm 75.0$. Lettuce plants had overall average dry weight MCs concentrations of $7.16 \pm 0.38 \text{ ng g}^{-1}$ in leaves, $5.65 \pm 0.21 \text{ ng g}^{-1}$ in stems, and $3.59 \pm 0.77 \text{ ng g}^{-1}$ in roots. Overall average MCs concentrations in radish tissues were $10.39 \pm 1.20 \text{ ng g}^{-1}$ in leaves, and $3.83 \pm 0.23 \text{ ng g}^{-1}$ in roots. BMAA concentrations displayed a similar trend, with dry weight concentrations of $7562.39 \pm 1148.31 \text{ ng g}^{-1}$ in lettuce leaves, $4018.54 \pm 269.43 \text{ ng g}^{-1}$ in lettuce stems, $551.88 \pm 96.39 \text{ ng g}^{-1}$ in lettuce roots, $4710.13 \pm 490.27 \text{ ng g}^{-1}$ in radish leaves, and $3223.60 \pm 557.46 \text{ ng g}^{-1}$ in radish taproots. This trend of higher toxin concentrations in the upper tissues of the plants is generally the opposite of what has been reported previously in the literature.

CLAM samples collected in the early fall of 2017 had average particulate and dissolved MCs aerosol concentrations of 2.60 pg m^{-3} and 10.16 pg m^{-3} , respectively, for an average total aerosol concentration of 12.76 pg m^{-3} . The total average BMAA aerosol concentration was $1176.91 \text{ pg m}^{-3}$, which was comprised of 630.86 pg m^{-3} dissolved and 546.05 pg m^{-3} particulate

aerosols. This represents the first report of concurrent BMAA and MCs aerosols from the same water body, and one of the first reports on the concentration of BMAA in lake aerosols.

Overall, there were few differences in toxin concentrations in each of the plants between treatments, and those that were present indicated that cyanotoxin aerosols were likely a significant source for accumulation in this system. Lettuce had significantly higher MCs and BMAA concentrations in the tissues exposed to aerosols (leaves and stems) compared to the roots, opposite the trend generally observed in the literature (Two-way ANOVA, $n = 36$, $p < 0.001$). In addition, there were no significant differences between the lake water and tap water treatments for lettuce leaf and stem tissues (two-way ANOVA, $n = 36$, $p = 0.068$), though lettuce roots from the lake water treatments had a higher average MCs concentration than roots from the tap water treatments (one-way ANOVA, $n = 12$, $p = 0.019$). For radishes, the leaves from the open tunnel/tap water treatment had a higher MCs concentration than the other three treatments (two-way ANOVA, $n = 12$, $p = 0.0037$), and interestingly, the taproot tissues from the lake water treatments had a lower average MCs concentration than those from the tap water treatments (two-way ANOVA, $n = 12$, $p = 0.036$). In conclusion, this study demonstrated that accumulation of lake-derived cyanotoxins by crop plants can occur from lakes with relatively low levels of toxins, and that aerosols, especially the dissolved fraction, are likely playing an important role in the contamination of crops. However, further research on lake-derived cyanotoxin accumulation in crops via exposure to aerosols and irrigation water is needed to evaluate this potential risk for low-level, long term exposure to these toxins.

INTRODUCTION

Cyanobacteria and Cyanotoxins

Cyanobacteria are a diverse group of photosynthetic prokaryotes that live in a wide range of aquatic, marine, and terrestrial environments. They are the oldest known photosynthetic organisms on Earth and were the primary driving force in shaping our atmosphere during the Precambrian Era approximately 3.5 billion years ago (Carr and Whitton 1982; Schopf 2000). Though many species of cyanobacteria live in terrestrial settings such as on bare rock, in soil, in desert crusts, and even in symbiotic relationships with terrestrial plants (Meeks 1998; WHO 1999; Richer et al. 2015), their primary habitats are aquatic and marine environments. The wide range of forms and traits found within the cyanobacteria allow them to inhabit waterbodies that range in salinity, temperature, and light availability from hot springs to arctic lakes, salt marshes, and the euphotic hypolimnetic zones of deep lakes. Within freshwater systems, nutrient-rich (eutrophic) lakes and reservoirs are often dominated by cyanobacteria, but low-nutrient (oligotrophic systems) can sustain them as well (Carey et al. 2012).

In recent decades, increases in cultural eutrophication have promoted cyanobacterial proliferation in waterbodies worldwide (WHO 1999; O'Neil et al. 2012; Paerl and Otten 2013). Cultural eutrophication of limnetic systems is a result of nutrient runoff and leaching from anthropogenic activities such as agricultural practices, industrial processes, impermeable surfaces, sewage treatment plants, and septic systems. Increased nitrogen and phosphorus levels often initially result in higher eukaryotic algal plankton biomass, which in turn causes reduced light penetration, and warmer surface water temperatures owing to increased light absorption.

Such conditions allow cyanobacteria to exploit their preferred niche habitats and eventually dominate the phytoplankton community. One such trait of cyanobacteria is higher growth efficiency in low-light conditions compared to eukaryotic algae. Because all cyanobacteria have both chlorophyll- α and phycocyanin photosynthetic pigments, they can utilize a wider spectrum of light and grow faster in low-light conditions (WHO 1999). In addition, some cyanobacteria use gas vesicles for buoyancy control, allowing for the positioning of the cyanobacteria at depths with ideal light intensity and/or nutrient availability. In warm waters, the growth rates of most eukaryotic phytoplankton decrease where those of many cyanobacteria increase (O'Neil et al. 2012). Waterbodies that experience high phytoplankton growth can become nitrogen limited, further encouraging cyanobacterial growth due to the ability of some genera to fix atmospheric nitrogen. In contrast, when conditions are not favorable because of light conditions, temperature, or nutrient availability, many cyanobacteria can store nutrients as well as form resting cells called akinetes that fall to the sediments and regrow in times with better conditions (WHO 1999). The combination of these traits and strategies allows cyanobacteria to dominate the phytoplankton community in eutrophic lakes, forming dense blooms, films, and scums.

Increased cyanobacteria densities, including more frequent and intense cyanobacterial blooms and scums, is of particular significance because of the potential for these organisms to produce toxins. Cyanobacteria produce diverse toxins including neurotoxins, genotoxins, cytotoxins, dermal irritants, carcinogens, and hepatotoxins (Table 1) (WHO 1999; Corbel et al. 2014). Because of this toxicity, as well as the negative ecological effects caused by large blooms of cyanobacteria, they are often termed "Harmful Cyanobacteria Blooms", or HCBs. Reports of poisoned wildlife and livestock due to HCBs date back to the late 19th century (Francis 1878), and HCBs continue to cause repeated cases of animal poisonings (Ressom et al. 1994), including

many confirmed and suspected cases of dog poisonings in the US in recent years (Backer et al. 2013).

Table 1. Examples of known cyanotoxins and their associated effects

Toxin	Effect
Aeruginosin	Genotoxin
Anatoxin	Neurotoxin
Aplysiatoxins	Irritant / Carcinogen
β -Methylamino-L-alanine (BMAA)	Neurotoxin
Cylindrospermopsin	Cytotoxin
Jamaicamides	Neurotoxin
Lyngbyatoxin	Irritant / Carcinogen
Microcystins (MCs)	Hepatotoxin
Nodularin	Hepatotoxin
Saxitoxin	Neurotoxin

The most common toxins produced by cyanobacteria blooms are microcystins (MCs), hepatotoxins that occur in 40-75% of blooms worldwide (Sivonen and Jones 1999). Microcystins are highly stable, water soluble, cyclic heptapeptides (Figure 1) that cause toxicity in the liver by the inhibition of cellular protein phosphatase 1 and 2a, which causes the breakdown of hepatic tissues (Kurki-Helasma and Meriluoto 1998). Acute exposure to microcystin at high doses causes death by liver failure or hemorrhage after a few hours (WHO 1999). Additionally, at lower exposure levels, MCs act as tumor promoters (Nishiwaki-Matsushima et al. 1991; Humpage and Falconer 1999) and induce oxidative stress in plant and animal cells (Pflugmacher et al. 2007; Wang et al. 2011; Freitas et al. 2015). Reported symptoms after an incidence of acute exposure to MCs through recreational activities included nausea, fever, and abdominal pain, followed by respiratory distress and liver damage (Giannuzzi et al. 2011). Tragically, the first documented case of human death directly attributed to MCs poisoning occurred in 1996 at a dialysis center in Caruaru, Brazil (Carmichael et al. 2001). As a result of using a water source dominated by cyanobacteria, 116 hemodialysis patients were

exposed to MCs intravenously, causing acute liver failure in 100 of the patients, and the eventual death of 76 (Carmichael et al. 2001).

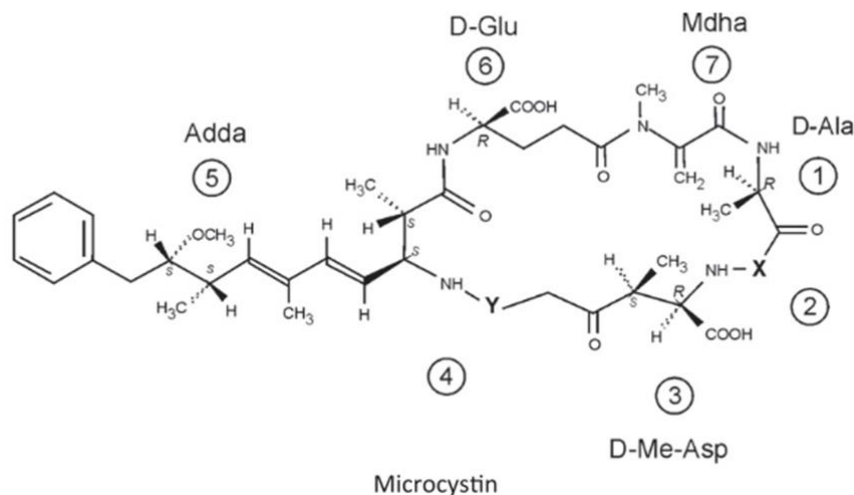


Figure 1. Molecular structure of Microcystin with major groups numbered. The two variable amino acids in microcystin are located at positions 2(x) and 4(y). Microcystin-LR is the most common variant, with L-Leu at 2 and L-Arg at 4. Image taken from (Pantelić et al. 2013).

There are over 100 reported congeners of microcystin (Niedermeyer 2014), each differentiated by the type of amino acids attached at two sites along the heptapeptide ring structure (Figure 1). Though MC-LR is the most commonly reported variant (Niedermeyer 2014), little information on the abundance, ecology, or toxicity of other MC congeners such as MC-RR and MC-YR exists. However, one study examining the toxicity of three MC variants in mice reported death 90 minutes after intraperitoneal injection of microcystin, with variant MC-LR exhibiting the highest toxicity level (Gupta et al. 2003). Fawell et al. (1999) also found that intraperitoneal injection of MC-LR was 30-100 times more toxic than by oral ingestion in mice and rats, and established a No Observed Adverse Effect Load (NOAEL) for tissue damage in the liver of $0.40 \mu\text{g kg}^{-1}$ of bodyweight per day for MC-LR. Based on these results, the World Health Organization (WHO) set guidelines for microcystin-LR exposure at $1 \mu\text{g L}^{-1}$ in drinking water and $0.04 \mu\text{g MC kg}^{-1}$ bodyweight for daily exposure (WHO 1999). More recently, the

U.S. Environmental Protection Agency set 10-day microcystin exposure limits in drinking water of $0.3 \mu\text{g L}^{-1}$ for infants and young children, and $1.6 \mu\text{g L}^{-1}$ for school-age children and adults (EPA 2015a).

Acute poisoning events of livestock and animals have been reported regularly in the literature, but a recent growing body of evidence is now raising awareness of the dangers of chronic human exposure to microcystins. In a study of 35 fishermen on Lake Chaohu, a lake that experiences intense summer cyanobacteria blooms located in Anhui Province in southeastern China, Chen et al. (2009) found microcystins in the blood serum of all study participants, as well as indications of hepatocellular damage. Li et al. (2011) also found signs of liver damage and elevated levels of MC in blood serum, this time in a population of children in the Chongqing area of Three Gorges Reservoir Region that had been chronically exposed to MCs through drinking water and aquatic food sources. In addition, Zhang et al. (2015) found a significant correlation between deaths due to non-alcoholic liver disease and areas of the United States that commonly experience cyanobacterial blooms. Considering this evidence, it is important to investigate the potential routes of chronic exposure to microcystin, not just acute episodic exposure.

The neurotoxin β -methylamino-L-alanine (BMAA) is another widespread cyanotoxin that has been linked to neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer's, and Parkinson's diseases (Bradley and Mash 2009; Pablo et al. 2009; Banack et al. 2010; Cox et al. 2018). Though less is known about BMAA than MCs, this non-protein encoding amino acid (Figure 2) has been found to cause motor neuron damage in several ways. Primarily, BMAA induces excitotoxicity in animal nerve cells through the overstimulation of NMDA glutamate receptors (Rao et al. 2006; Chiu et al. 2012), while targeting motor neurons specifically (Rao et al. 2006). Others have also found that it causes neuronal death by oxidative

stress (Lobner 2009) and degeneration of nerve axons (Tan et al. 2018). BMAA can also be misincorporated into proteins in place of L-serine, causing misfolding of the proteins which leads to the neurofibrillary tangles commonly associated with neurodegenerative disorders (Dunlop et al. 2013). In addition, Potjewyd et al. (2017) found that nitrosated BMAA (N-BMAA) caused breaks in the DNA strands of human neuroblastoma cells when BMAA was only minimally toxic on its own.

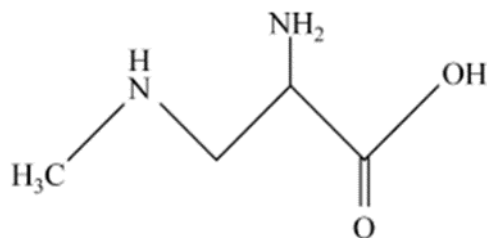


Figure 2. Molecular structure of β -N-methylamino-L-alanine (BMAA). BMAA is a hydrophilic, non-protein coding amino acid that has relatively high chemical stability. Taken from Jonasson et al. (2008).

The putative role of BMAA in human neurological disorders was initially discovered in connection with the unusually high rate of a neurodegenerative disease in the native Chamorro people of Guam, dubbed amyotrophic lateral sclerosis-parkinsonism dementia complex (ALS-PDC). In the 1950's, ALS-PDC was found in the Chamorro people at 50-100 times the incidence of ALS worldwide (Prasad and Kurland 1997), and it was thought that the BMAA found in the cycad seed flour there could be the culprit due to its structural similarity to the compound β -N-oxalyamino-L-alanine (BOAA) that had been linked to the paralytic disease lathyrism (Rao et al. 1964). When administered intraperitoneally at varying levels in chicks and rats, BMAA produced convulsions and decreased motor function (Vega et al. 1968; Polsky et al. 1972), confirming its potential as an acute neurotoxin. Shortly thereafter, in a study of the effects of BMAA fed to macaques over a period of 12 weeks, Spencer et al. (1987) reported behavioral signs of motor neuron dysfunction as well as structural damage to the central nervous

system in the animals. However, naturally-occurring levels of BMAA high enough to produce neurological damage weren't found in Guam until the discovery of a bioaccumulation route for BMAA from the roots of the cycad trees (where it is originally produced by a symbiotic cyanobacteria), into the cycad fruits, and then to multiple tissues in fruit bats which are considered a delicacy in Chamorro culture (Cox et al. 2003). Murch et al. (2004) also found protein-bound BMAA levels in cycad flour to be 50-100 times higher than the free BMAA levels measured previously.

Since it was determined that the cyanobacteria *Nostoc* living as a symbiont in Cycad trees was the original source of BMAA in the diet of the Chamorro people (Cox et al. 2003), connections between BMAA and neurodegenerative disorders on a global scale could be made as toxigenic cyanobacteria exist worldwide. For example, high concentrations of BMAA were found in the brain tissues of Chamorro patients diagnosed with ALS/PDC and in Canadian patients diagnosed with Alzheimer's, but none was found in patients who died from non-neurodegenerative causes (Cox et al. 2003). Despite the lack of cycad flour or flying foxes in the diet of the Canadian patients, they could still have been exposed to BMAA in some manner as cyanobacteria are found worldwide, and BMAA is produced by at least five major taxonomic groups (Cox et al. 2005). More recently, the development of neurofibrillary tangles and β -amyloid plaques in the brain tissues of vervet monkeys after chronic dietary exposure to BMAA is further evidence linking BMAA to ALS/PDC and Alzheimer's disease as these are the neurological hallmarks of both diseases (Cox et al. 2016). Additionally, mis-incorporated or protein-bound BMAA that has bioaccumulated in brain or other tissues could act as a slow-release source of the toxin, causing delayed damage to the nervous system over long periods of

time (Dunlop et al. 2013). Consequently, it is crucial to consider chronic, low-level exposure to this destructive neurotoxin.

Routes of Exposure

Oral ingestion via drinking water or recreational activities involving waters with high concentrations of toxigenic cyanobacteria is the most common route of exposure to cyanotoxins. Incidences of human illness after exposure to cyanobacteria through recreational activities have been reported globally since 1949 (Chorus et al. 2000; Stewart et al. 2006; Wood 2016), some of which were attributed directly to MCs in recent decades (Turner et al. 1990; Giannuzzi et al. 2011). Several studies that analyzed levels of MCs in ground-well and treated drinking water reported concentrations many times the World Health Organization (WHO) recommended level of $1\mu\text{g L}^{-1}$ (Chen et al. 2009; Mohamed and Al Shehri 2009), with some levels as high as $4.3\mu\text{g L}^{-1}$ (Li et al. 2011). After decades of recurring cyanobacteria blooms in Lake Erie (Kutovaya et al. 2012), the city of Toledo, Ohio issued a “do not drink” water advisory on August 2, 2014 after elevated levels of MCs in treated water were reported (Jetoo et al. 2015). This event triggered a state of emergency and left over 400,000 residents without drinking water for several days during a very hot summer. BMAA has also been reported in recreational and drinking waters across the globe including the U.S (Banack et al. 2015), the U.K (Metcalf et al. 2008) Australia (Main et al. 2018), the Netherlands (Faassen et al. 2009) and South Africa (Scott et al. 2014). In addition, multiple toxins including MCs, BMAA, anatoxin-a, and cylindrospermopsin were detected over a three-year sampling period in Lake Jordan, NC, which serves as a drinking water source (Wiltsie et al. 2018).

Consuming contaminated drinking water or engaging in common activities such as swimming, jet-skiing, or boating in waterbodies with high levels of cyanobacteria are perhaps

obvious routes of direct oral contact, but there are other ways that human exposure to cyanotoxins have been reported. For example, an analysis by Gilroy et al. (2000) found detectable levels of microcystin in 72% of health supplement samples produced by four companies that all used cyanobacteria collected from Upper Klamath Lake in Oregon. Cyanobacteria supplements are commonly used, as they are advertised for their high protein, vitamin, and mineral content and other beneficial health effects (Vaz et al. 2016). However, it is difficult, if not impossible, to guarantee that the species collected are not toxin-producers, especially when collected from a natural community and without any regulatory testing. In the case of Upper Klamath Lake, the cyanobacteria targeted for collection was *Aphanizomenon flos-aquae*, but the lake often experiences blooms of *Microcystin aeruginosa*, a known toxin-producer (Gilroy et al. 2000). In addition, other studies have confirmed the presence of MCs and/or BMAA in several different cyanobacteria-based health food supplements using multiple detection methods (Heussner et al. 2012; Vichi et al. 2012; Parker et al. 2015; Roy-Lachapelle et al. 2017; Bishop and Murch 2018), where some have failed to measure detectable levels of either toxin (McCarron et al. 2014; Rzymiski et al. 2015). Because health food supplements are not regulated by the FDA in the United States, quality assurance tests for cyanotoxins are not required, so it is impossible for a consumer to know whether the particular supplement they are taking contains cyanotoxins.

Another potential route of cyanotoxin exposure is through food. MCs accumulate in fish, shellfish, crayfish, shrimps, mussels, prawns, and aquatic plants harvested from natural or aquaculture systems (Ibelings and Chorus 2007; Smith et al. 2008; Mulvenna et al. 2012; Romero-Oliva et al. 2014). Over time these contaminated food sources may negatively affect the health of people consuming them. As previously mentioned, it has been recorded that fishermen

and children in regions of China were chronically exposed to microcystins through their drinking water and food collected from waterbodies with heavy cyanobacteria blooms (Chen et al. 2009; Li et al. 2011). BMAA was initially discovered in the diet of the Chamorro people of Guam but has also been found in the gelatinous *Nostoc sp.* colonies that are a seasonal dietary staple in the Peruvian highlands, though it is not known if there is a higher rate of neurodegenerative disease in this region (Johnson et al. 2008). It has been recorded that *Nostoc sp.* colonies are also part of the diet in Java (Zaneveld 1959) and the Philippines (Martinez 1988) and have been considered a delicacy in China for hundreds of years (Gao 1998), though toxin levels have not been reported from these regions. Most reports of BMAA in other foods have focused on foods from marine ecosystems. In the Southeastern U.S., BMAA has been found in a variety of seafoods including fish, bivalves, lobsters, crabs, and shrimp at relatively high concentrations (Brand et al. 2010), some of which were found to have associations with ALS patients (Field et al. 2013; Banack et al. 2014).

Studies involving cyanotoxins in food plants have also been conducted but are not as common. Studies of MCs in plants have primarily focused on the physiological effects of MCs uptake on plant growth in seeds and seedlings (Kurki-Helasmo and Meriluoto 1998; Chen et al. 2004; Järvenpää et al. 2007; Pereira et al. 2009; Wang et al. 2011) or on mature plants (Freitas et al. 2015), versus levels of MCs accumulated in foods meant for consumption at the time of harvest. In addition, the controlled-system studies that have measured uptake of MCs by more mature plants have either used pure extracted MCs diluted to known concentrations (Corbel et al. 2016) or lake water treated to release all intracellular toxins (Crush et al. 2008). A few studies have examined accumulation of MCs in field crops that have been irrigated by contaminated water containing intact cyanobacteria cells with measurable toxin levels (Codd et al. 1999;

Mohamed and Al Shehri 2009; Romero-Oliva et al. 2014). However, these studies only measured concentrations of MCs in the plants and water sources on one occasion, whereas the concentrations of MCs in irrigation water used throughout the development of the crops were unknown.

In comparison to the work that has been done with MCs, very little research on BMAA in food plants has been conducted. Uptake of BMAA by plants other than cycads has only been of interest in recent years, and work on this topic has primarily focused on the physiological effects of the molecule in submerged aquatic plants in laboratory settings (Esterhuizen et al. 2011; Esterhuizen-Londt et al. 2011; Contardo-Jara et al. 2013). Free and protein-bound BMAA levels in natural samples of aquatic plants collected from 12 reservoirs in Nebraska ranged from 1.86 to 13.4 $\mu\text{g g}^{-1}$ for free BMAA and 0.48 to 12.7 $\mu\text{g g}^{-1}$ for protein-bound BMAA (Al-Sammak et al. 2014), but no other concentrations of BMAA in natural plant samples have been reported. A few studies focused on the uptake and physiological effects of BMAA in crop plants in laboratory settings reported bioaccumulation of the toxin in root, stem, and leaf tissues of carrots and nasturtium (Niyonzima 2010), wheat (Contardo-Jara et al. 2014), and onions and lettuce (Esterhuizen-Londt and Pflugmacher 2019). For example, BMAA concentrations in mature wheat plants after 205 days of irrigation with water containing 10 $\mu\text{g L}^{-1}$ of pure BMAA extract had unexpectedly high levels, including 217 ng g^{-1} in the mature seeds (Contardo-Jara et al. 2018). This is an alarming result as grains constitute a large portion of diets worldwide. As of yet, only Esterhuizen-Londt and Pflugmacher (2019) have reportedly analyzed BMAA concentrations in field grown crops exposed to natural lake water with high cyanobacteria toxins via irrigation, though BMAA was not detected in any of these samples. Though these studies have demonstrated that cyanotoxins can be absorbed into food plants through irrigation water, no

controlled studies have examined microcystin or BMAA concentrations in plants after continual exposure to unprocessed lake water with a natural cyanobacteria community, so it is difficult to interpret how much of these toxins could be taken up from a natural system under normal growing conditions.

Previous studies have assumed that the only route of exposure for food crops to cyanotoxins is directly through irrigation water, but another potential route of exposure that has not been investigated thus far is the deposition of cyanobacteria aerosols released from surface waters such as rivers, lakes, ponds, and reservoirs onto exposed plant surfaces. Although it is widely assumed that cyanobacteria cells are released as aerosols from the surface of the water through turbulence such as wave action and bubble bursting (Cheng et al. 2007), recent results from lab experiments indicate that cyanobacteria aerosols may be emitted without turbulence (Murby and Haney 2016). This implies that cyanobacteria aerosols could be emitted in the absence of wave action or bubbling at the water surface.

Outside of a laboratory setting, cyanobacteria aerosols have been reported from several natural systems. In the summer of 2015, Lewandowska et al. (2017) detected toxigenic species of cyanobacteria in aerosol samples collected in the coastal zone and directly above the surface of the Baltic Sea, though the samples were not analyzed for toxins. Other studies have reported detectable levels of BMAA and MC in aerosols. BMAA has been detected in lake aerosols in New Hampshire, USA (Banack et al. 2015), and MCs were found in aerosols from lakes in New Zealand (Wood and Dietrich 2011) California, USA (Backer et al. 2010), and New Hampshire, USA (Murby and Haney 2016). Spatial studies have identified clusters of patients with ALS in New England (Caller et al. 2009, 2013; Torbick et al. 2018) and non-alcoholic fatty liver disease across the US (Zhang et al. 2015) around lakes that experience frequent cyanobacteria blooms,

identifying aerosolized toxins as a likely cause. As agricultural fields are often placed adjacent to surface waters and irrigation sources, it is possible that the crops are exposed not only to toxins in the irrigation water during watering times, but constantly to aerosolized toxins emitted from irrigation ponds, lakes, and rivers.

Research Objectives

The goal of this research was to investigate the uptake of cyanotoxins by food plants exposed both directly to natural lake water at the roots and indirectly via deposition of aerosolized toxins released from the surface of a lake. To accomplish this, a controlled field experiment was conducted where lettuce and radishes were grown on a lake with a history of intense late-summer cyanobacteria blooms. We chose to run the experiment on the lake to enable exposure to fresh lake water as well as to the aerosolized toxins released from the lake. The plants were exposed to lake water taken directly from the lake as well as lake aerosols in four separate treatments: exposure to both aerosols and lake water, exposure to lake water only, exposure to aerosols only, and no exposure. Hydroponic reservoirs were used instead of a soil-based system because cyanobacteria live freely within soils and could have interfered with the amount of toxins absorbed through the roots from the irrigation water alone. Lettuce was chosen because it is known to grow well hydroponically, and it is a very commonly consumed product. Radishes were chosen as a second crop type because they have a very different leaf texture to lettuce and because previous studies have reported dramatically higher toxin concentrations in radishes than other crops grown in the same system (Mohamed and Al Shehri 2009). Including radishes in this study helped provide further information on differences between toxin concentrations in different plant types and whether this particular vegetable stores cyanotoxins at high rates. In addition, because radishes have hairy leaves, including them as a second plant

type provided information on the effect leaf texture has on the level of cyanobacteria aerosols retained on the leaf surfaces. The overall goal was to determine if lettuce and radishes could absorb MCs and BMAA from natural lake water and aerosols from a lake with low to moderate toxin concentrations, and if so, to what extent these two sources contributed to the overall toxin concentration in the final harvested plants. As the issue of chronic, low-level exposure to cyanobacteria toxins becomes more apparent, it is important to better understand the entire range of exposure routes that people and animals have to these toxins.

METHODS

Study Site

Lake Attitash is a shallow, 1.46 km² natural lake that lies within the towns of Amesbury and Merrimac in the northeast corner of Massachusetts (N42.8487704, W-70.9929935) (Figure 3). It consists of one large central basin with a maximum depth of 9.75 m and an average depth of 3.5 m. Most of the lake is relatively shallow, with a single deep site in the southeast corner of the lake. Back River, located in the northwest corner, is the main inlet tributary of the lake. The only surface water outflow is also located at the north end of the lake, just east of the Back River inlet. Because the inlet and outlet are located so close to each other, water in the main basin of the lake does not flush fully and likely contributes to nutrient accumulation in the lake. The shoreline is mostly developed, with much of the area immediately surrounding the lake comprised of medium and high-density housing. Beginning in the mid 1980's, residential septic systems surrounding the lake were switched over to the city sewers, but impermeable surfaces and turf lawns still contribute to the nutrient load of the lake. Very little of the shoreline remains forested or as natural wetland.

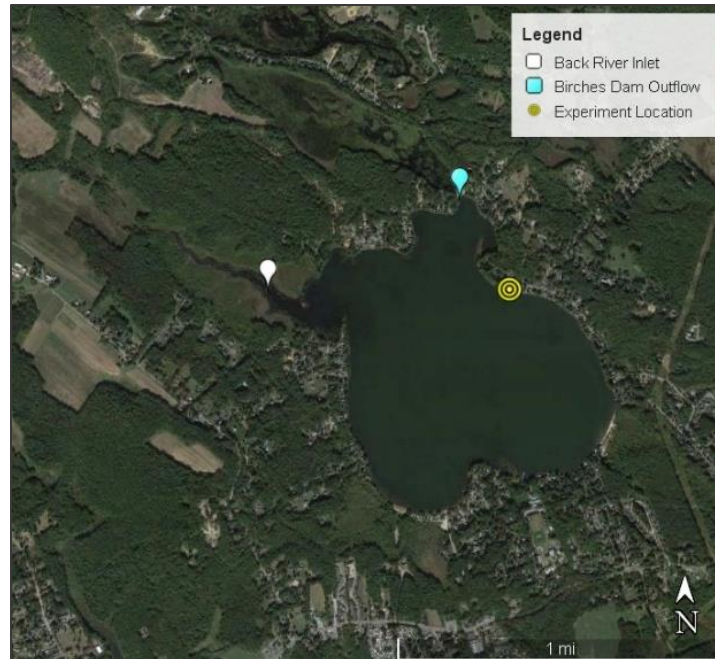


Figure 3. Aerial photography of Lake Attitash, Amesbury, MA taken in October of 2016. The primary inflow, outflow, and experiment location are labeled. The green coloration of the water indicates a heavy phytoplankton bloom at the time the image was taken, a common occurrence in the late Summer and Fall at this site.

Though the surrounding 10.1 km² watershed remains mostly undeveloped, there are several areas of agricultural and industrial development as well as residential areas in the immediate watershed that contribute nutrient runoff to the lake. Along the flow of the Back River, there are multiple slow moving, seasonally stagnant wetlands that accumulate mobilized nutrients during times of low flow and then release them downstream toward the lake during rain events. Just before the Back River enters Lake Attitash, there is also a large agricultural area that includes cropland as well as a composting operation that contributes runoff to the inflow of the lake. Since the 1970's, the trophic status of Lake Attitash has degraded from mesotrophic to highly eutrophic based on the Carlson (1977) trophic state index. This index uses secchi depth, total phosphorus, and chlorophyll- α measurements to calculate a unitless number that falls within the range of 0 – 100 that represents the general productivity of a lake. Within this range, each division of 10 units represents a doubling in phytoplankton biomass. Carlson and Simpson

(1996) define a eutrophic system as having secchi readings between 0.5 to 2 m, chlorophyll- α levels between 7.3 – 56 $\mu\text{g L}^{-1}$, and total phosphorus levels between 24 – 96 $\mu\text{g L}^{-1}$. Despite efforts to mitigate nutrient inflow into the lake, phosphorus levels remain high, stimulating cyanobacteria growth. The Massachusetts Department of Public Health has posted public health advisories every summer since it began monitoring the lake in 2009. In recent years, water quality parameters continue to indicate that the lake remains eutrophic, and the phytoplankton community of the lake has been dominated by cyanobacteria in the late summer (Table 2) (UNH CFB data records).

Table 2. Historical water quality parameters of Lake Attitash indicating eutrophic conditions.

Year	Date	Extracted Chl- α ($\mu\text{g L}^{-1}$)	Total Phosphorus ($\mu\text{g L}^{-1}$)	Secchi (m)	Percent Cyanobacteria	Dominant Cyanobacteria Genera
2015	24-Sep	44.0	25.7	0.767	69.5	<i>Aphanizomenon</i> & <i>Dolichospermum</i>
2014	9-Sep	16.6	-	1.34	76.0	<i>Dolichospermum</i> & <i>Microcystis</i>
2012	20-Sep	13.1	27.0	1.45	56.3	<i>Aphanizomenon</i> & <i>Microcystis</i>

Lake Cyanotoxin Plant Exposure Field Experiment: Design & Construction

Seedling Preparation and Sowing Stage Sample Collection

Lettuce and radishes were chosen as the two plant types to be included in the study because they are both popular consumer vegetables, previous literature has reported cyanotoxin levels in these types of plants, and they have a relatively short time to harvest. The lettuce variety used was Johnny’s Selected Seeds ‘Summer Crop Lettuce’ (MUIR OG MT0, Product #3881.G11, Lot 53462). This variety of lettuce was chosen because it was described by the seller as suitable for hydroponics with a high heat tolerance, which was a significant factor

because lettuce are normally cool-weather crops, but this experiment was conducted in the summer. This lettuce type also has an open leaf arrangement which allowed for exposure of all leaves to lake aerosols. In contrast, lettuce varieties such as iceberg and romaine have very tightly packed leaves, which would only allow the outer leaves exposed to lake aerosols. Cherry Belle radishes from NESeed Company lot# 88112 (Hartford, CT, USA) were used as this variety grew well in test runs.

Seedlings for the experiment were started under greenhouse conditions in the UNH research greenhouses. Standard growing trays with 3.18 cm (1.25 in) Oasis Rootcubes[®] (OASIS[®] Grower Solutions, Kent, OH, USA) were used to grow the lettuce and radish seedlings. Samples of seeds, growing media cubes, and greenhouse water were collected for toxin analysis the day the seeds were planted. Seeds were stored in the original seed packets placed inside plastic storage bags at room temperature in a dry, dark cabinet. Growing media and water samples were stored frozen at -20 °C until processed for toxin analysis.

Due to high temperatures in the greenhouses at the time of sowing, lettuce seeds were kept in a germination chamber at 16.5 °C for four days, then at 21 °C for another two days before moving them out into the propagation greenhouse. Once in this greenhouse, two drain holes were drilled in the bottom of the lettuce trays to allow water to slowly drain throughout the day after watering and prevent rotting due to over-saturation. Lettuce seedlings were grown for 18 days post-sowing before being moved into the experiment. For the radishes, the seed holes in the growth cubes were enlarged using the butt-end of an 8 mm (5/16 in) drill bit to allow for the large cotyledon leaves to sprout and grow upward properly. In trials, the radishes proved to be particularly sensitive to over-saturation, so four drain holes were drilled to allow for faster and

more thorough drainage after watering. Radishes were sown and raised in the propagation greenhouse only for 10 days before being moved into the field experiment.

All radish and lettuce trays were watered twice daily with unaltered tap water (provided by the town of Durham, NH) until first true leaves had emerged and were then watered with 17N-4P-17K fertilizer dosed at 100 mg L⁻¹ nitrogen. For the last 5 days prior to the experiment, lettuce seedlings were moved to a standard grow-out greenhouse so they could be watered with a higher concentration fertilizer mix of 17N-4P-17K dosed at 150 mg L⁻¹ nitrogen. This was done because they had started to show signs of nutrient stress including slight yellowing of the leaves. Radishes were grown in the propagation house for the entire 10 days they before being moved into the field experiment. Excess start seedlings that were not used in the field experiment were separated into roots, stems, and leaves, then frozen and stored at -20 °C for MCs and BMAA analysis.

Overall Experimental Design

In August of 2016, the radish and lettuce seedlings were grown to maturity for 3 weeks on a dock on the northeast shore of Lake Attitash in Amesbury, Massachusetts and exposed to varying levels of cyanotoxins through the lake water itself as well as airborne aerosolized toxins (Figure 4, Figure 6). To accommodate the experiment, a 3.35 by 4.88 m (11 by 16 ft) platform was built and attached to the existing dock structure on the property. The top surface of the platform was painted white to prevent heat build-up. This location directly over the lake, approximately 6 m from the shoreline, made it possible to consistently expose the plants to cyanobacteria aerosols emitted from the lake in the air and to cyanotoxins in the lake water applied directly to the roots of the plants. Cyanobacteria are present naturally in soils, and cyanotoxins can become bound to soil particles, so a hydroponic system with discrete reservoirs

was utilized to isolate the lake water and aerosols as the two main sources of cyanotoxins present in the study.

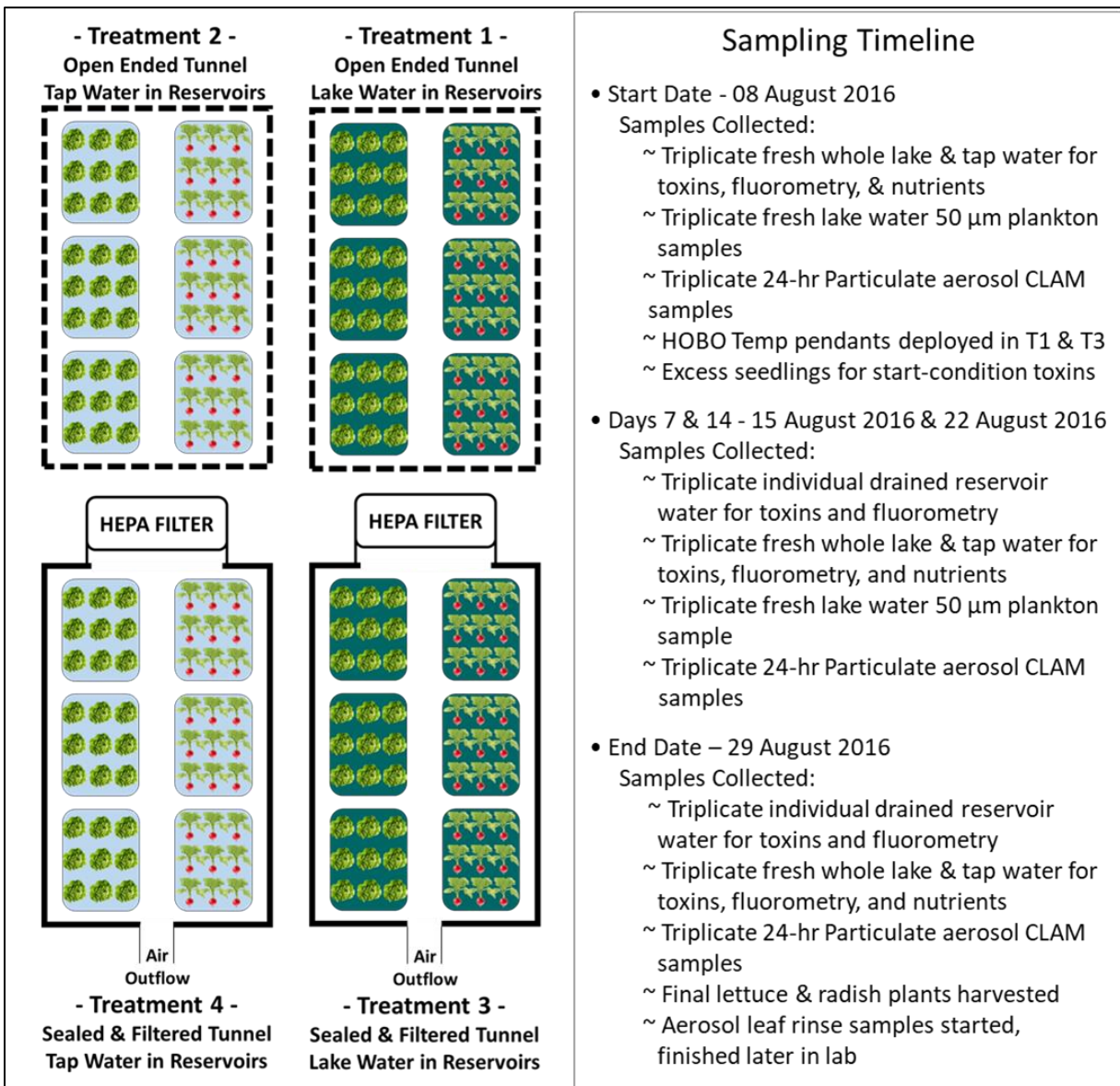


Figure 4. Overall lake cyanotoxin plant exposure field experiment design and sampling plan. Three reservoirs of each plant type were placed in each treatment tunnel, each with nine individual plants. Hydroponic reservoirs were filled with fertilized tap water or lake water. Open ended tunnels allowed for exposure to aerosolized cyanotoxins released from the lake, whereas filtered tunnels removed particulate aerosols.

Hydroponic Reservoir Design and Construction

Hydroponic reservoirs were built to accommodate nine individual plants by modifying 18.9 L (5 gal) clear polypropylene storage bins (HDX Model # PC151306-001) (Figure 5). Nine holes were drilled in the lid of each reservoir to support the 51 mm (2 in) diameter net pots that hold the individual plants. The useable space of the lids was 340 by 267 mm (13.38 by 10.5 in) so the nine holes were drilled in rows of three with 102 mm (4 in) of space between each row from center to center of each hole. Holes in the same row were 146 mm (5.75 in) apart from center to center. A small plumbing system was built using 13 mm (0.5 in) PVC pipe and fittings to drain and refill each reservoir without removing the lid and disrupting the plants. A 6.4 mm (0.25 in) diameter hole was drilled at one end of each reservoir to accommodate an aeration line. Reservoirs were completely wrapped with heavy duty aluminum foil to stop light penetration. Reduction of light in the reservoirs was essential to reduce solar heating of the water and to prevent growth of phytoplankton within the reservoirs during the experiment. A layer of black plastic was used to cover the foil of the lids to avoid damage from reflection of light back toward the seedlings.



Figure 5. Individual hydroponic reservoir design constructed of a modified clear plastic storage bin. For each reservoir, nine holes were drilled in the lid to support nine plants, a small hole was drilled in the upper corner to allow for the aeration line, a plumbing system was constructed to drain and refill the reservoir, and foil was used to block out sunlight. Duct tape was used on the bottom edges to prevent the foil from tearing. Reservoirs were placed on individually leveled platforms to ensure water levels inside the reservoirs were uniform. Not pictured: Black plastic was later used to cover the lids to prevent seedlings from being burned by excess sunlight reflected off the foil

Experimental Treatment Tunnel Design and Construction

Four small 1 x 1.6 m (3.25 x 5.25 ft) low-tunnels were constructed on the dock to protect the plants in the reservoirs from excessive wind that could damage the leaf tissues as well as from rain, that could contaminate the experimental reservoirs (Figure 6). Each low tunnel was built using three arches made of 2.1 m (7 ft) long pieces of 13 mm (0.5 in) PVC tubing that were evenly spaced and attached to a base made of wooden boards 0.6 by 1.2 m (2 by 4 ft) using 13 mm (0.5 in) electrical conduit pipe clips. A fourth piece of 13 mm (0.5 in) PVC tubing was attached lengthwise along the tunnel to the peak of the arches to provide better structural stability against high winds that could blow across the lake during common summer storms. The base of each tunnel was secured to the dock using six L-brackets. The tunnels were covered using 6 mm clear greenhouse film that was secured to the PVC arches using PVC snap clamps, and to the wood base frame using a metal wire lock base system commonly called ‘wobble-wire’ (Atlas

Manufacturing INC, Alapaha, GA, USA). Because lettuce and radishes are both cool-weather crops, the tunnels were additionally covered with 30% black shade cloth to help prevent overheating.

Three reservoirs of both lettuce and radishes were placed inside each tunnel. Reservoirs were spaced 152 mm (6 in) away from the sides of the tunnel to allow for vertical growth of the plants without contact with the plastic of the low tunnel and 203 mm (8 in) away from either end of the tunnels, with 32 mm (1.25 in) of space between the central reservoirs and the ones at either end. The plumbing system of each reservoir was constructed to emerge through the greenhouse film on the side of the tunnel so that each could be drained and refilled without removing the reservoir from the tunnel. Because the level of the additional dock space built to accommodate the experiment was not consistent, individual platforms were built for each reservoir and each was leveled individually depending on its location on the dock. This ensured that the same amount of water would be contained in all reservoirs without spilling out, and that each plant within each reservoir would sit at the same water depth. To maintain healthy growing conditions, each tunnel had a small aeration system to prevent stagnation in the reservoirs. Each aeration system was built with a Tetra 77854 Whisper Air Pump (Tetra, Melle, Germany) clear flexible PVC airline tubing (4.76 mm ID, 6.35 mm OD; 0.19 in ID, 0.25 in OD), airline T's, small aquarium air stones, and pinch clamps to regulate air flow to each reservoir. The air pumps were secured just outside each tunnel, due to lack of space inside, and were placed in adapted small storage containers to prevent weather damage.

Treatment Tunnel Experimental Conditions

Plants in the experiment were grown under one of four treatment conditions, where each of the four tunnels provided a different exposure combination to the cyanotoxins present in the lake water and/or aerosols (Figure 4, Figure 6). Two of the treatments allowed for exposure to aerosolized toxins by having open-ended tunnels for free movement of air through the tunnels. Particulate aerosolized toxins were excluded from the other two treatment tunnels by completely sealing these tunnels on all sides with greenhouse film (Figure 7). Idylis AC-2126 model HEPA filters were fitted to these two sealed tunnels to provide airflow through the tunnels at an average rate of 79.5 L s^{-1} while removing particles larger than or equal to $0.3 \mu\text{m}$. To expose plants to cyanotoxins present in the lake water, the reservoirs in one of the open tunnels and one of the closed tunnels were filled with water collected directly from the lake. The reservoirs in the remaining open and closed tunnels were filled with tap water. Both the tap water and lake water treatments were supplemented with equal amounts of OASIS[®] Hydroponic Fertilizer (OASIS[®] Grower Solutions, Kent, OH USA) dosed at the manufacturer-recommended level of 150 mg L^{-1} nitrogen for optimal growing conditions.



Figure 6. Four experimental treatment tunnels constructed on the dock expansion. Treatments 1 and 2 are open-ended to allow for free flow of air and exposure to cyanotoxin aerosols emitted from the lake. Treatments 3 and 4 are fully sealed and fitted with HEPA filters to remove particulate aerosols from the air entering these tunnels. In this image, the outflow ports for treatments 3 and 4 are visible; the HEPA filters are on the other side of the tunnels. Treatments 1 and 3 have lake water in the hydroponic reservoirs to expose plants to cyanotoxins directly via root uptake, where treatments 2 and 4 have tap water in the reservoirs to prevent this type of exposure. Shade-cloth was used to reduce temperatures inside the tunnels.

Overall, Treatment 1 (lake water and aerosols) was the full exposure treatment with lake water in the reservoirs and an open-ended tunnel for aerosol exposure to investigate the possible interaction between these two routes. Treatment 2 (aerosols only) had tap water in the reservoirs and an open-ended tunnel to isolate the effect of exposure to aerosolized toxins only. Treatment 3 (lake water only) had a sealed, filtered tunnel with lake water in the reservoirs to isolate the effect of direct cyanotoxin uptake through the roots. Finally, Treatment 4 was the control treatment with tap water in the reservoirs and a sealed, filtered tunnel to prevent exposure to cyanotoxins in both the air and water as much as possible for the duration of the experiment.



Figure 7. Image of Treatments 3 and 4 fitted with HEPA filters to exclude particulate aerosolized cyanotoxins. To protect the HEPA filters from rain, smaller enclosures were constructed out of PVC tubing, plywood, 2 x 4 ft boards, greenhouse film, and shade cloth. Home attic soffit vents were placed on one side to allow for inflow of air into the air filter, while preventing rainwater from entering. HEPA filters were placed inside the enclosures, which were connected to the treatment tunnels with greenhouse film. Also visible are the drain/fill pipes of three reservoirs as well as the boxes that were constructed to store the aeration pumps for each treatment tunnel.

Lake Cyanotoxin Plant Exposure Field Experiment: Implementation

Lettuce and radish seedlings were placed in the reservoirs inside the four treatment tunnels on August 8th, 2016 and then harvested on August 29th, 2016. Originally the experiment was planned to run for four weeks but was ended a week early due to the unexpected rapid growth of the plants observed at the end of week 2. It was decided that if the plants continued to grow for another week, they could become stressed from overcrowding, which would affect normal growth and uptake mechanisms.

Weekly Lake and Tap Water Collection and Sampling

To fill hydroponic reservoirs in the experiment, lake water and tap water was pumped into two separate 140 L (37 Gal) Rubbermaid Roughneck® tote bins and mixed thoroughly to ensure uniformity of the water in the reservoirs (Figure 8). Amesbury City tap water was aerated in the large bin for at least 1 h to off-gas residual chlorine. Lake water was pumped into the second large bin directly from lake just off the side of the dock, approximately 0.75 m below the surface of the water. Once filled, both large bins were covered to prevent photodegradation and heating from the sun.



Figure 8. Water used to fill hydroponic reservoirs. Left: tap water. Right: lake water. Coloration and cloudiness in the lake water indicates the presence of phytoplankton, including cyanobacteria

Samples of unfiltered tap water and lake water were collected from the large bins for phycocyanin fluorometry analysis using a dual-channel AquaFluor™ fluorometer (Turner Designs, Sunnyvale, CA USA) with a minimum detectable limit of $2 \mu\text{g L}^{-1}$ as determined in the lab. All fluorometry samples were collected in triplicate using 20 mL HDPE vials and held on ice in a dark cooler until they could be frozen and stored at $-20 \text{ }^{\circ}\text{C}$. Triplicate fluorometry samples of filtered lake water using a $50 \mu\text{m}$ Nitex mesh filter were also collected to determine the approximate proportion of small phytoplankton forms. Triplicate samples of unfiltered tap

water and lake water were also collected from the large bins for toxin analysis in 30 mL Histo containers and held on ice in a cooler until frozen at -20 °C for storage. Samples for total phosphorus and total nitrogen analysis were collected in triplicate from the large bins, acidified, and held in 500 mL Nalgene® bottles (Nalgene® Nunc™ International, Rochester, NY USA) on ice in a cooler until frozen and stored at -20 °C. In-Situ water chemistry parameters of the tap water and lake water in the large bins were collected using a YSI EXO2 multiparameter sonde (Yellow Springs Instruments, Yellow Springs OH). The parameters recorded by the sonde included temperature (°C), dissolved oxygen concentration (mg L⁻¹), dissolved oxygen percent saturation (%DO), specific conductivity (µS cm⁻¹), pH, oxygen-reduction potential mV (ORP), chlorophyll- α (µg L⁻¹), phycocyanin (µg L⁻¹), fluorescent dissolved organic matter RFU (fDOM), and turbidity (FNU). To correct for pH effects, ORP values were adjusted to a pH of 7 and recorded as E7 (Hutchinson 1957). The YSI EXO2 was also used to collect lake vertical profile data off the side of the dock on August 15th and August 22nd, 2016. To characterize the percent composition of the phytoplankton community, four-liter net plankton samples were collected in triplicate from the lake water bin using a 50 µm mesh plankton net, stored in 225 mL opaque white HDPE cups, and preserved at a concentration of 4% formalin-sucrose (Haney and Hall 1973). Phytoplankton were later identified to genus with the University of New Hampshire Phycokey (Baker 2012) and imaged using an Olympus BH-2 microscope with an Olympus DP25 camera and CellSens Entry 1.16 imaging software (Olympus Corporation, Shinjuku, Tokyo, Japan). Once water chemistry parameters and phytoplankton sampling was completed in the large tote bins, this water was then transferred to individual hydroponic reservoirs using a 18.9 L (5 Gal) bucket that was modified with plumbing to attach to the drain/fill plumbing on each of the reservoirs Figure 9.

Weekly Reservoir Water Changes and Sampling

Following the initial filling of the treatment reservoirs on Day 1 of the field experiment, all treatment reservoirs were drained and refilled at the end of each week to maintain consistent nutrient levels throughout the experiment and to refresh the plankton communities in the lake water treatments. During these water change events, each reservoir was drained and then refilled using the water that was collected into large bins as described previously. To refill the reservoirs, either tap or lake water was pumped from one of the large bins into a modified 18.9 L (5 Gal) bucket (Figure 9), then fertilized and transferred into the appropriate hydroponic reservoir. During the water changes at the end of weeks 1 and 2, triplicate samples of fresh fertilized water were collected, acidified, and stored on ice until stored frozen for TN & TP analysis to check fertilizer dosages against recommended levels. These high concentration nutrient analyses were conducted using the HACH Method 10127 kit for total phosphorus and HACH Method 10072 kit for Total Nitrogen using a HACH DR 3900 spectrophotometer (HACH, Loveland, CO). Drained water from each reservoir was sampled individually for whole water toxin analysis well as for whole water phycocyanin and chlorophyll- α fluorometry. Toxin and fluorometry samples were held on ice in a cooler until frozen and stored at -20 °C until analysis. As reservoirs were drained, this water was collected in one of two large bins (lake water vs tap water). Bins were then sampled for overall average high range nutrient analysis and in-situ water chemistry parameters using the EXO2 multiparameter sonde. To refill the reservoirs, the same procedure and sampling methods were followed as previously described. At the end of the third week on the last day of the experiment, August 29th, 2016, reservoirs were drained, and the above described sampling procedure was conducted on the drained water only.



Figure 9. Refilling a reservoir with fertilized tap water using the modified 18.9 L (5 Gal) bucket.

Monitoring Conditions Inside Treatment Tunnels

To check on the conditions inside the treatment tunnels during the experiment, an Extech Mini Thermo-Anemometer 45118 (Extech Instruments, Nashua, NH USA) was used to measure the airflow rate exiting the sealed tunnels and the inside/outside temperature for each tunnel on the four sampling dates. Four HOBO temperature/light pendants (Part# UA-002-64, Onset Computer Corporation, Bourne, MA USA) were used to monitor the tunnel and reservoir conditions inside Treatment 1 and Treatment 3 at 30-min intervals to determine if there were differences in air and reservoir temperatures between the open and sealed tunnels and to verify that light was not penetrating the hydroponic reservoirs. Light data measured by the HOBO pendants were converted from Lux to Photosynthetically Active Radiation (PAR) by linear regression of data collected side-by-side by the HOBO pendants used in the field experiment and a Li-Cor Quantum Sensor with a Li-1000 Datalogger (LI-COR, Lincoln, NE).

Lake Aerosol Sampling

Lake aerosols were collected on 25 mm circular Whatman™ GFF glass fiber filters using a modified Compact Lake Aerosol Monitor (CLAM) (Murby and Haney 2016; Langley 2019) (Figure 10). The CLAM was run for 24-hours four times throughout the experiment on 8/8/2016, 8/15/16, 8/22/16, and 8/29/16. Before use, glass fiber filters were washed with MilliQ water, then combusted at 500 °C for 1 h to reduce the effective pore size of the filters to 0.3 µm (Nayar and Chou 2003). The CLAM was constructed using three Gilian® BDXII air pumps (Sensidyne, St. Petersburg, FL USA) housed inside a clear plastic case and attached with clear vinyl PVC tubing to 25 mm Swinnex (Millipore Sigma, Burlington, MA USA) that each held a single filter. Air was pumped at 2.5 LPM from a collection funnel placed approximately 30 cm above the lake surface and through the GFF filter as described by Murby and Haney (2016). Filters were stored frozen at -20 °C until processed for toxin analysis.

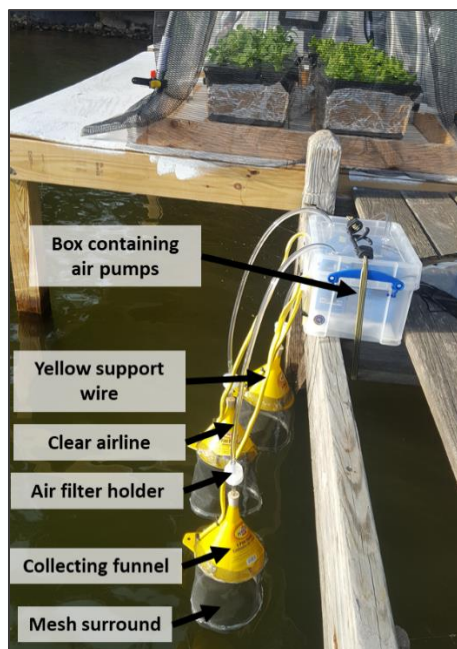


Figure 10. Compact Lake Aerosol Monitor (CLAM) used to collect aerosols emitted from the surface of Lake Attitash during the 2016 experiment.

Lake Cyanotoxin Plant Exposure Experiment: Sample Collection and Processing

Initial Field Sample Collection and Storage

Plants were harvested from the treatment tunnels in the field on day 21 of the experiment. For each reservoir, lettuce plants were collected and then separated into two parts and radish plants were collected and then separated into three parts. These parts were then stored in plastic bags on ice until they could be further processed in the lab. In the lab, plants were stored refrigerated at 5 °C or stored on ice due while waiting for processing. Initially in the field, the upper portion of the lettuce plants including the leaves and stems were separated from the roots by cutting the stem of the plant at the level of the top of the growth medium cube. For each of the 24 reservoirs, leaves and stems of all nine lettuce plants were stored in a single large bag to prevent leaves from becoming damaged or separated from the main plant stem. Additionally, all nine net pots with growth medium cubes and all root tissue from each reservoir was stored in a single storage bag. Radish plants were separated into three parts (leaves, taproot, and adventitious roots) by cutting the plant just below the taproot ‘bulb’ where the lower uptake root began and just above the root ‘bulb’ of the radish where the leaves emerged (Figure 11). Radish leaves from each reservoir were stored in large plastic bags, and taproots from each reservoir were combined and stored in smaller quart-sized bags. All other root material cut from the ‘bulbs’ of the taproots was placed with the net pots associated with the appropriate reservoir. Adventitious roots from lettuce and radish were later separated from the net pots and growth medium in the lab.

Sample Collection and Imaging for Cyanotoxin Aerosols Deposited on Leaf Surfaces

To determine the concentration of cyanotoxins on the surface of the leaves at the end of the experiment due to aerosol deposition, all plant leaves from each of the 24 reservoirs were

rinsed and this rinse water was later analyzed for BMAA and MCs concentrations. Leaf aerosol rinsing occurred in the field on the experiment end date for Treatment 1 only (open tunnel with lake water); the plants from the other three treatments were rinsed in the lab over the following two weeks.

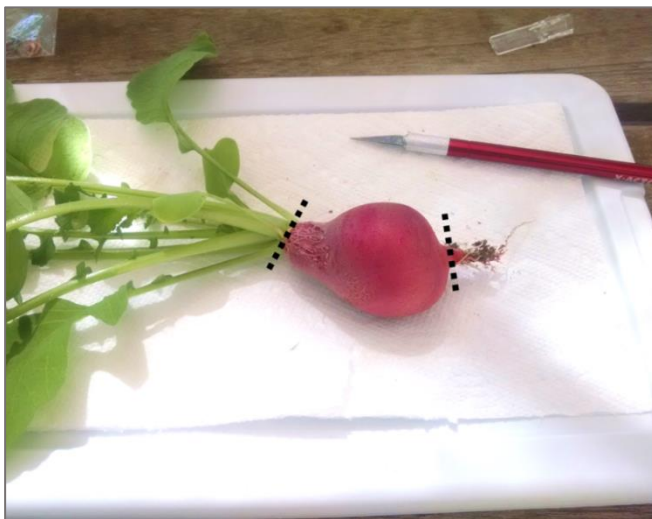


Figure 11. Visual representation of where radishes were cut to separate the leaves, taproot 'bulb', and lower adventitious roots. Leaves from the nine plants associated with each reservoir were stored in a single bag until further processing, as were taproot 'bulbs'. Adventitious roots were removed from the taproot 'bulb' and stored with the net pots and growth medium cubes for each reservoir until brought back to the lab for further separation and cleaning.

It was anticipated that the total amount of deposited aerosolized cyanotoxins on each plant would be very low. Therefore, in order to collect enough aerosolized toxins from the leaf surfaces of each reservoir to be detectable, all nine plants from one reservoir were rinsed as a batch in the same water, and the final rinse water was then tested for MCs and BMAA. This was done by placing the leaves of each plant, one plant at a time, into a small container with MilliQ water, then covering and gently inverting for 60 s. These rinsed leaves were then spin-dried in an OXO Good Grips® salad spinner (OXO, New York, NY, USA) for another 60 s to recover as much rinse water as possible, which was returned to the initial rinse container. Once dry, the rinsed leaves were transferred to a new storage bag for further processing, and the next plant was

rinsed. This was repeated for all nine plants of each reservoir, after which the amount of remaining rise water was measured and then stored frozen in 500 mL clear Nalgene[®] containers at -20°C until processed for toxin analysis. Because the lettuce leaves were larger than the radishes, 500 mL of MilliQ water was used to rinse lettuce and 250 mL was used to rinse radishes. To account for losses in water and transfer of toxins during the rinse cycles, cyanotoxin concentrations in the aerosol rinse water were based on an average of the start rinse and final rinse water volumes.

To report the amount of deposited aerosol cyanotoxins present on the leaves in units of weight and surface area, all parts of the plants were weighed to the nearest 0.01 g using an OHAUS Adventurer[™] AR2140 digital scale (OHAUS, Parsippany, NJ). All leaves were photographed, and leaf surface area measured using ImageJ ver. 1.51j8 (Schneider et al. 2012). Lettuce leaves were removed from their stems, then weighed and imaged separately. Initially, each leaf area was measured in triplicate, but once it was determined that the coefficient of variation was less than 1% between measurements, the remaining leaves were only measured once. These tissue weights and surface area measurements were used to calculate the amount of deposited cyanotoxins present on the leaf surfaces in units of leaf surface area.

For cyanotoxin analysis of deposited aerosol rinse samples, 1.8 mL of each sample was first pipetted into 2.0 mL polypropylene microcentrifuge tubes. Cyanotoxins were extracted by triplicate cycles of freezing at -80 °C for 15 min, thawing, vortexing (Vari-Whirl Mixer 58810-006, VWR Scientific, Radnor, PA) for 30 s, and sonication (Fisherbrand CPXH, ThermoFisher Scientific, Waltham, MA) for 3 min (Silva-Stenico et al. 2009). Once toxins were extracted, samples were and concentrated 10x to approximately 0.18 mL (minimum volume needed for toxin analyses) using either a Jouan RC1010 or a Savant SpeedVac Concentrator with external

cold vapor trap. To calculate concentration factors, sample weights were measured throughout the extraction and SpeedVac process using the OHAUS scale to the nearest 0.1 mg.

Toxin analyses followed the procedures recommended for the Enzyme-Linked Immunosorbent Assay (ELISA) kits for BMAA (Abraxis, Warminster PA) and MC (Envirologix, Portland ME). ELISA analysis measures only free molecules, and therefore does not include MCs or BMAA molecules that may be protein bound. All ELISA standards including negative controls were run in duplicate. Standard curves were developed for each toxin kit using a 4-parameter logistic model and the standards provided with each kit; 0, 5, 25, 100, 250, and 500 $\mu\text{g L}^{-1}$ for the Abraxis BMAA kit and 0.1, 0.3, 0.6, and 1.2 $\mu\text{g L}^{-1}$ for the Envirologix MCs kit. MilliQ water was used as a negative control for the MC analyses.

Preparation and Analysis of Lettuce and Radish Tissue Samples

Once imaged, plant leaves from each reservoir were combined and homogenized using a food processor (NutriBullet model NB-101b, Capital Brands, Los Angeles, CA) for 30 s, then frozen and stored at $-20\text{ }^{\circ}\text{C}$ in resealable plastic storage bags. Lettuce stems and radish taproots were homogenized using a smaller food processor (MagicBullet[®], Capital Brands, Los Angeles, CA) blender for 30 s, then frozen and stored at $-20\text{ }^{\circ}\text{C}$ in resealable plastic storage bags.

To collect as much adventitious root tissue as possible, each individual growth medium cube was removed from its net pot and cut in half. All visible root material was then removed from the foam, and any roots still attached to the net pot were removed as well. All adventitious roots from a reservoir were combined and rinsed using 500 mL DI water to remove growth medium particulate and any residual water from treatment reservoirs. Adventitious roots from Treatment 1 (open tunnel with lake water) were homogenized using the MagicBullet[®] after adding 10-15 mL of MilliQ water to help blend the tissues. Because this method introduced

excess liquid to the samples, roots from the three other treatments were frozen and stored at -20 °C, then later homogenized using a marble mortar and pestle.

Subsamples of all three tissue types from all reservoirs were taken in triplicate and dehydrated overnight in pre-weighed aluminum foil dishes at 60 °C for dry weight calculation. All start seedling samples used for MC extraction and analysis were homogenized using a manual glass 40 mL Pyrex tissue homogenizer. However, for BMAA extraction samples, seedling tissues and seeds were homogenized using a ceramic mortar and pestle to increase sample processing efficiency.

For cyanotoxin analysis of plant tissue samples, approximately 0.3 g of each sample was first placed in 1.5 mL polypropylene microcentrifuge tubes with 1.0 mL MilliQ water. Cyanotoxins were extracted using the triplicate freeze, thaw, vortex, and sonicate method described previously. Once toxins were extracted, plant tissues were removed by centrifugation at 10,000 RPM using a Heathrow Scientific®, LLC Gusto Mini Centrifuge (Vernon Hills, IL) until at least 0.5 mL of supernatant was visually separated from the plant tissue. Centrifugation for each tissue type varied from 15 min for leaf and radish bulb tissues to 30 min for lettuce stems. Supernatant was removed and concentrated to 0.18 mL (minimum volume needed for toxin analyses) using either a Jouan RC1010 or a Savant SpeedVac Concentrator with external cold vapor trap. Because pre-SpeedVac volumes varied from sample to sample, final concentration values ranged from an average of 3.56x for lettuce leaves to 5.93x for lettuce roots, with an overall average concentration of 4.74x for all tissue types. To calculate concentration factors, sample weights were measured throughout the extraction and SpeedVac process using the OHAUS scale to the nearest 0.1 mg. ELISA analyses followed the recommended procedures for each kit as previously described.

Preparation and Analyses of Weekly Fresh and Drained Water Samples

Weekly fresh tap and lake water samples as well as drained reservoir water samples were processed in triplicate for analysis of MCs using 1.8 mL of each sample in a 2.0 mL centrifuge tube. Samples were extracted using the triplicate freeze, thaw, vortex, and sonication method and a 10x concentration as previously described. For BMAA analysis these samples were extracted in the same manner but were concentrated approximately 20x to help ensure detectable levels of BMAA in the samples. For the 20x concentration, two separate 1.8 mL subsamples were extracted and concentrated approximately 10x, then combined and concentrated further. Samples were analyzed for BMAA and MCs concentrations using the previously described ELSIA kits and standard curves.

Greenhouse Sowing and Start Seedling Tissue Sample Analyses

Greenhouse water samples were extracted, concentrated, and analyzed for MCs and BMAA as previously described for the other water samples. For BMAA, water samples were analyzed in triplicate, but only single samples were analyzed for greenhouse water MCs. For lettuce seeds, radish seeds, and greenhouse oasis growth cube samples, approximately 0.3 g of material was extracted, concentrated, and analyzed in the same manner as described previously for the other lettuce and radish tissue samples.

Tissue and oasis samples representing the experiment 'start condition' were collected from excess start seedlings not used in the field experiment and were processed and analyzed as previously described for other plant tissue samples. For BMAA analysis these samples were done in triplicate where possible, but due to limited amounts of tissue this was not possible for the root samples. For MC analysis, single samples were processed and analyzed for all tissues

and oasis cube material. All sowing samples and start seedling samples were analyzed using the Abraxis BMAA and Envirologix MC ELISA kits along with the 4-parameter logistic curves based off the standards provided in each kit as previously described.

Preparation and Analyses of CLAM Aerosol Collection Samples

Aerosol GFF filters from the 24 h CLAM collections were first cut into 12 pieces, then extracted in 1.8 mL of MilliQ water in 2.0 mL centrifuge tubes. Following the triple freeze-thaw-vortex-sonication process, extracted filter samples were centrifuged for 3 min at 10,000 RPM and supernatant was pipetted off into a second centrifuge tube. The supernatant was centrifuged again, and the final supernatant was removed and speedvac concentrated to 0.2 mL (average 6.77x). Samples were analyzed as previously described for MC or BMAA using Abraxis BMAA ELISA kits and Envirologix MC ELISA kits with standard curves derived from provided standards.

Plant Tissue Cyanotoxin Spiking Experiment

To estimate the BMAA and MC extraction recovery rates from the plant tissues, spiking experiments were conducted on lettuce leaves, radish leaves, and radish taproots as these tissues constituted the majority of the total plant mass for each plant type. To create spiking solutions for BMAA and MCs, the 500 and 250 $\mu\text{g L}^{-1}$ standards from two recently used Abraxis BMAA ELISA kits and the 1.2 $\mu\text{g L}^{-1}$ standards from several recently used Envirologix MCs ELISA kits were combined. Toxin concentrations of the spiking solutions were confirmed via ELISA for percent recovery calculations of the plant tissues. For each tissue type, triplicate samples were made for each toxin by first placing approximately 0.3 g of tissue in a 2.0 mL centrifuge tube, then 0.5 mL of either the BMAA or MC spiking solution was then added, followed by 0.5 mL of MilliQ water. Samples were extracted using the freeze-thaw-vortex-sonication method

previously described. For control samples, 1.0 mL of MilliQ water was added to the 0.3 g of plant tissues in lieu of the 0.5 mL of spiking solution and 0.5 mL of MilliQ. Control samples were then extracted as previously described, though they were not Speedvac concentrated as this could have brought them above the readable ranges of the two ELISA kits.

Subsamples of each plant tissue sample were taken in triplicate, weighed, and dehydrated overnight in pre-weighed aluminum foil dishes at 60°C for dry weight toxin concentration calculations. For both toxins, extraction efficiencies were very similar for the three tissue types. The overall average for all plant tissues for MCs was 51.2 % ± 1.5 % and 66.4 % ± 2.7% for BMAA (Table 3. BMAA and MCs extraction efficiencies with standard error for lettuce leaves, radish leaves, and radish taproots Table 3).

Table 3. BMAA and MCs extraction efficiencies with standard error for lettuce leaves, radish leaves, and radish taproots

Plant Tissue Type	MCs Extraction Efficiency	BMAA Extraction Efficiency
Lettuce Leaves	52.7 ± 2.6 %	73.8 ± 1.3 %
Radish Leaves	50.3 ± 3.5 %	61.0 ± 2.6 %
Radish Taproots	50.7 ± 2.3 %	64.5 ± 6.0 %

In addition to the plant tissue spiking experiment, triplicate samples of both of the spiking standards as well as plain MilliQ water were analyzed for toxin concentrations to investigate the effect of the extraction process on purified toxins standards as well as on pure MilliQ water. To do this, triplicate samples for the BMAA and MCs standards were made using of 0.5 mL spiking solution added to 0.5 mL of MilliQ water. The same triplicate freeze/thaw/sonicate extraction process used for all other samples was conducted, and then samples were analyzed using the appropriate ELISA kit as previously described. To calculate extraction efficiency, the concentrations of these extracted spiking solution samples were compared to the concentrations

of the unmodified spiking solutions, which were confirmed by ELISA as well. As a control, triplicate samples of 1 mL of MilliQ water were also extracted and analyzed for each toxin kit. The BMAA spiking solution had a 121.8% extraction efficiency with no BMAA detected in the MilliQ water control. Interestingly, the MC spiking solution had an 89.7 ± 1.8 % extraction efficiency, but the MilliQ water had a positive reading of 106.4 ± 3.6 ng MCs L⁻¹. This indicates that there could have been unknown contamination of the MilliQ or the extraction vial that caused a false positive reading for MCs in the ELISA analysis.

Lake Aerosol Tunnel Exclusion Experiment

In late September and early October of 2017, a second experiment was run in the same location on Lake Attitash to investigate the possibility that lake aerosols may have entered the sealed HEPA filtered tunnel treatments during the 2016 experiment. The available space on the dock was limited at that time so only one open and one filtered tunnel could be placed on the dock at once (Figure 12). The tunnels were set up in the same manner as during the summer of 2016, except that the sealed tunnel was placed at the end of the dock and the open tunnel was placed closer to shore. Lake aerosols in the tunnels were collected using three adapted CLAM units that included a liquid trap after the GFF filter to collect toxins smaller than 0.3 μm (Figure 13). It was assumed that toxins that passed through the filters were not bound to cells, and therefore were considered dissolved or free toxins. These liquid traps were included because recent unpublished data from other studies in the lab had indicated that dissolved toxins were passing through the pre-combusted GFF filters.



Figure 12. Two experimental tunnels with three CLAM units to measure lake aerosols inside the open-ended and HEPA filtered treatments during late September and early October of 2017.

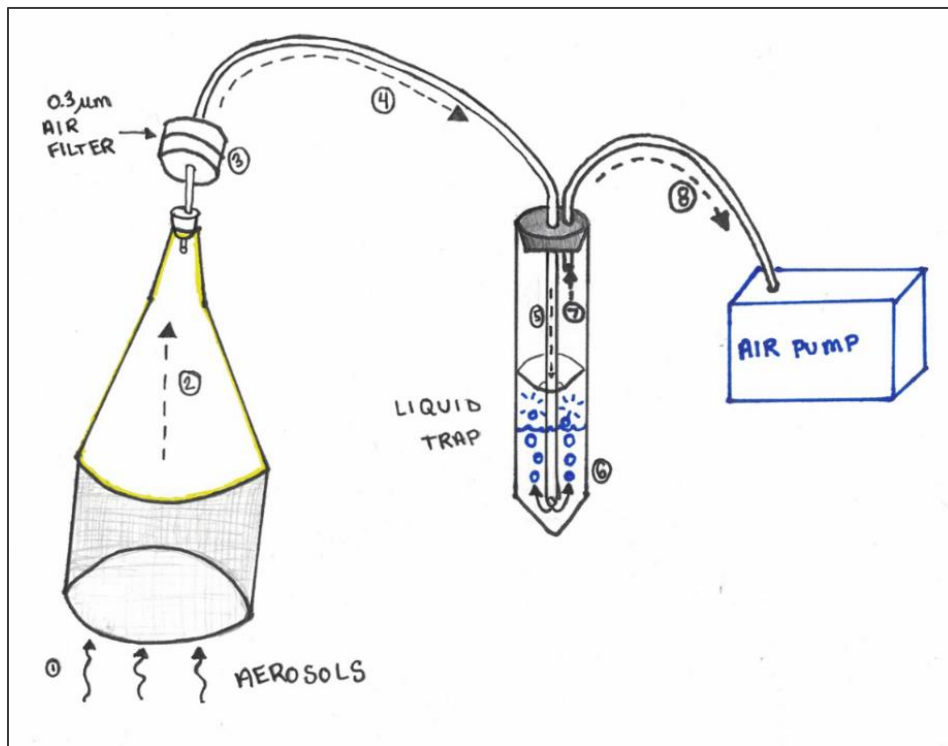


Figure 13. Diagrammatic representation of a single modified aerosol collection pump unit with the liquid trap. The vacuum air pump pulls air up through the mesh surround and funnel (1 & 2), then through the 0.3 μm filter (3) where particulate aerosols are removed. Filtered air is then drawn down into the liquid trap (4 & 5), where it bubbles up through the liquid (6), which traps toxins that are $<0.3 \mu\text{m}$ in size. Air is then finally pulled up out of the trap and to the air pump (7 & 8). Three of these units were combined into a single CLAM unit for triplicate sampling.

At the time of this experiment only three CLAM units with traps were available. Two CLAM units were used for particulate and dissolved MC aerosol collection with GFF filters attached to the collection funnels and 35 mL of 80% MeOH in the traps. One of these methanol-trap units was used for the HEPA filtered tunnel and the other was placed in the open-ended tunnel (Figure 14). As BMAA is not soluble in MeOH, but is water soluble (Jonasson et al. 2008), the third CLAM unit was placed in the open tunnel primarily for particulate and dissolved BMAA aerosol collection with GFF filters attached to the collection funnels and 35 mL of MilliQ water in the traps (Figure 14). In addition, the GFF filters and milliQ-traps from this third CLAM unit were analyzed for MCs to compare the 80% MeOH trap method and the water trap method for MC aerosol collection where possible and to further compare the amount of particulate aerosols on the GFF filters in the open and sealed tunnel.

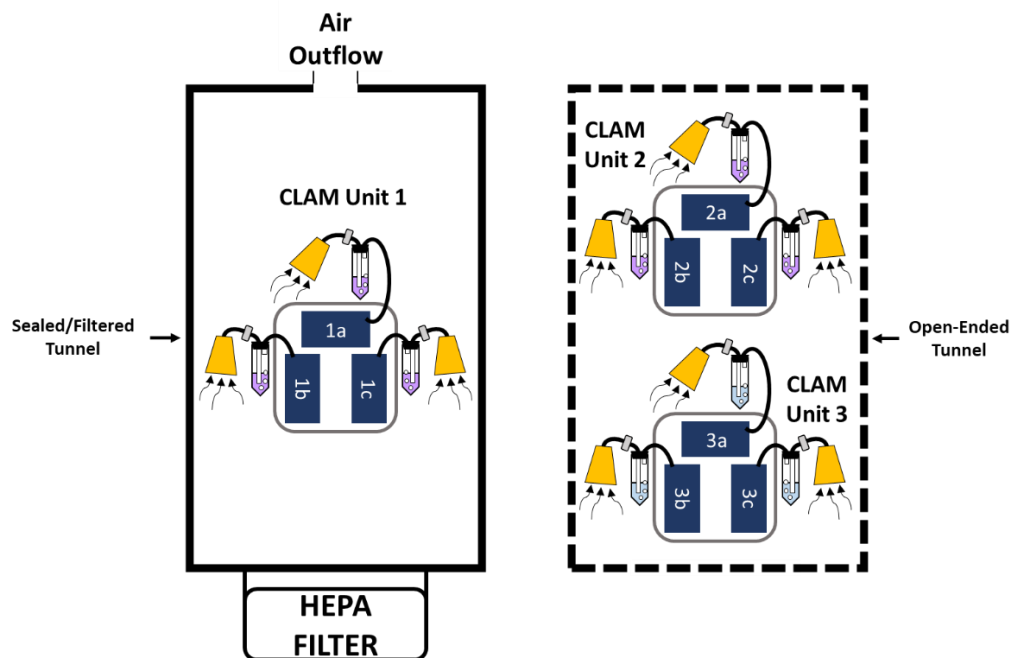


Figure 14. Diagrammatic representation of the 2017 lake aerosol tunnel experiment. CLAM Units 1 and 2 had 80% MeOH in the traps (purple traps); filters and traps from these were used for MCs analysis. CLAM Unit 3 was had MilliQ water in the traps (blue traps); filters were used for BMAA analysis and trap liquid was used for both BMAA & MC analyses.

The CLAM units with traps were run on three separate dates: September 24th, October 2nd, and October 8th, 2017. On the first sampling date the three CLAMS were placed inside the two tunnels and run for 12 h from 8:00 am to 8:00 pm with the air pumps set to 2.5 LPM. During this first sampling, field conditions and the long run time resulted in the evaporation of the methanol trap samples for this date, though all GFF filters were viable. For the subsequent two shorter duration runs, the CLAMS were run for 8 h beginning at 8:00 am, the tunnels were covered with 30% shade cloth, and the traps were wrapped in foil to reflect light and prevent heat buildup. In addition, the CLAM unit used for the sealed tunnel was adapted so that only the funnels were inside the tunnel (Figure 15). This made it possible to seal the tunnel fully and run the HEPA filter to flush out the air initially inside the tunnel before turning the CLAM pumps on. All samples collected from the 8 h aerosol collections were run without issue.

At the end of each aerosol sampling run, GFF filters were collected and stored in individual snap-caps on ice in a cooler until stored at -20°C for further processing. Methanol trap samples were transferred to individual 20 mL HDPE scintillation vials, and water trap samples were split and stored in two 20 mL HDPE scintillation vials per sample, which were later reduced in volume and recombined during sample processing. Trap samples were also stored on ice in a cooler until frozen and stored at -20°C for further processing. All GFF filters were processed in the lab for particulate toxin analysis of BMAA and MCs as previously described. All liquid trap samples were concentrated to 0.18 mL via SpeedVac, extracted, then tested for BMAA and MCs.



Figure 15. Placement of the CLAM unit to collect air from inside the HEPA filtered tunnel. The side of the tunnel was cut and re-sealed to allow the funnels to collect air from inside the tunnel, while the air pumps and traps remained outside on the dock.

During each of the three air samplings, whole lake water samples were also collected in triplicate in 30 mL Histo containers and placed on ice in a cooler until they could be frozen and stored at -20 °C for BMAA and MCs analysis. Triplicate whole lake water and filtered <50 µm lake water samples were collected and stored in 20 mL HDPE vials at -20 °C for fluorometric analysis using an AmiScience FluorQuik dual channel fluorometer (Fremont, CA). A single 4 L >50 µm plankton sample was also collected on and preserved with 4% formalin-sucrose 7 October 2017 to determine phytoplankton community composition.

Data Analysis

Raw data were organized and stored in MS Excel, and all graphs were generated using MS Excel as well. To calculate toxin concentrations from the raw ELISA results, SigmaPlot 12.5 (SYSTAT Software Inc., Chicago, IL) was used to generate variables for the 4-parameter-

logistic-curve (4PLC), and final calculations were conducted in an MS Excel spreadsheet based on the standard formula for the 4PLC modified to solve for x (Equation 1).

Equation 1. *Modified 4-parameter-logistic-curve to solve for x, where:*

x = toxin concentration

y = optical density result from ELISA

a = minimum

d = maximum

c = EC50

b = Hill slope

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

Statistical analyses including one-way and two-way analysis of variance (ANOVA) and Tukey's honest significant difference test were performed to identify statistically significant differences in data sets ($p < 0.05$) using the 'agricolae' and 'car' packages in RStudio software (R Core Team 2017). To determine treatment effects of the 2016 experiment, analyses for differences in MCs concentrations were run on lettuce and radish data separately as the loss of radish root data, combined with the methodological separation of plants into different parts made it impossible to compare them across the board. In addition, an analysis of the lettuce and radish leaves across treatments, as the only physiologically comparable portions of both plants, revealed an interaction between the plant type and the treatments, indicating that leaves would need to be analyzed separately by plant type as well (two-way ANOVA, $n = 24$, $p = 0.0063$). To determine treatment effects of the 2016 experiment on final dry weight BMAA concentrations, an analysis of combined lettuce and radish leaf data was conducted, but otherwise lettuce and radish data were analyzed separately by plant type due to the loss of radish adventitious root data and the lack of physiological similarity between the remaining tissue types.

RESULTS

Lake Cyanotoxin Plant Exposure Field Experiment

Experimental Tunnel Temperature and Light Conditions

Daily average air temperatures and reservoir water temperatures were calculated using data from the HOBO temperature/light pendants, and though the overall average air temperature over the course of the experiment in the filtered tunnel was 1.2 degrees higher at 29.0 °C than the average temperature of the open tunnel at 27.9 °C, this was not a significant difference (One-way ANOVA, $p = 0.19$) (Table 4. Overall average temperature and light levels (\pm standard error) in an open and filtered treatment tunnel throughout the three-week 2016 field experiment. Table 4). A similar pattern occurred for the reservoir water temperatures, where the reservoir in the filtered tunnel had an overall average temperature 0.8 °C higher than the average temperature of the reservoir in the open tunnel, though this difference was not statistically significant (One-way ANOVA, $p = 0.26$). This indicates that the HEPA filters were pushing enough air through the filtered tunnels to prevent excessive heat buildup, which could have negatively affected the growth of the plants in those tunnels. In addition, the light levels in the reservoirs of both the filtered and open tunnels remained at or just above 0 $\mu\text{Mol m}^{-2} \text{s}^{-1}$ the duration of the experiment, proving that wrapping the reservoirs in foil to prevent light penetration was successful.

Light levels experienced by plants did not differ significantly between the open and filtered tunnels throughout the duration of the experiment as neither the daily average 24-hour, daytime (7:00 am – 6:00 pm), or maximum light levels were different between tunnel types (One-Way ANOVA, $p_{24\text{-Hour}} = 0.51$, $p_{\text{daytime}} = 0.97$, $p_{\text{maximum}} = 0.69$) The filtered tunnel had a 24-hour average light level of $331.8 \pm 22.4 \mu\text{Mol m}^{-2} \text{s}^{-1}$, a daytime average light level of $621.2 \pm$

46.6 $\mu\text{Mol m}^{-2} \text{s}^{-1}$, and an average daily maximum light level of $1388.9 \pm 46.6 \mu\text{Mol m}^{-2} \text{s}^{-1}$ where the open tunnel had a 24-hour average light level of $352.1 \pm 21.0 \mu\text{Mol m}^{-2} \text{s}^{-1}$, a daytime average light level of $619.0 \pm 40.3 \mu\text{Mol m}^{-2} \text{s}^{-1}$, and an average daily maximum light level of 1348.8 ± 66.8 (Table 4).

Table 4. Overall average temperature and light levels (\pm standard error) in an open and filtered treatment tunnel throughout the three-week 2016 field experiment.

Tunnel Type - Sensor Location	Temperature \pm SE ($^{\circ}\text{C}$)			PAR Light \pm SE ($\mu\text{Mol m}^{-2} \text{s}^{-1}$)		
	Overall	Maximum	Minimum	24-Hour	Daytime	Maximum
Filtered - Air	29.0 ± 0.7	45.2 ± 1.2	20.2 ± 0.45	331.8 ± 22.4	621.2 ± 46.6	1388.9 ± 73.4
Open - Air	27.9 ± 0.6	41.7 ± 1.0	19.1 ± 0.5	352.1 ± 21.0	619.0 ± 40.3	1348.8 ± 66.8
Filtered - Reservoir	26.7 ± 0.5	30.1 ± 0.8	23.8 ± 0.5	n/a	n/a	n/a
Open - Reservoir	25.9 ± 0.4	28.2 ± 0.6	23.6 ± 0.4	n/a	n/a	n/a

In-Situ Lake Attitash Water Chemistry Parameters

Vertical profiles of water chemistry parameters were collected off the dock on August 15th and August 22nd, 2016 to monitor water quality in the lake and compare it to water used in the hydroponic reservoirs. Due to the relatively shallow depth and location close to shore, the water column was well-mixed and overall average parameters were calculated to characterize the lake water (Table 5). Overall water quality conditions were very similar on the two dates, with slightly higher turbidity, phycocyanin and chlorophyll- α concentrations on August 22nd indicating an increased concentration of cyanobacteria in the lake on this date.

Table 5. Average in-situ limnetic parameters of Lake Attitash collected using the YSI EXO2 multiprobe on August 15th and 22nd, 2016 with standard error

PARAMETER	August 15th, 2016	August 22nd, 2016
Temperature (°C)	27.4 ± 0.02	25.9 ± 0.01
Dissolved Oxygen (mg L ⁻¹)	7.8 ± 0.01	7.5 ± 0.02
Percent Saturation DO	98.0 ± 0.17	92.1 ± 0.26
pH	7.8 ± 0.00	7.5 ± 0.01
E ₇ (mV)	223.5 ± 0.22	171.9 ± 0.78
SpCond (µS cm ⁻¹)	204.5 ± 0.03	203.1 ± 0.12
Chlorophyll-α (µg L ⁻¹)	9.1 ± 0.04	9.6 ± 0.16
Phycocyanin (µg L ⁻¹)	22.6 ± 0.07	25.2 ± 0.27
Turbidity (FNU)	2.0 ± 0.02	3.5 ± 0.10

Hydroponic Reservoir Water Quality Conditions

Once lake and tap water were collected in the large storage bins for use in the hydroponic reservoirs each week, this water was sampled for nutrient analysis, water chemistry parameters, fluorometry, phytoplankton identification, and toxin analysis. Water chemistry parameters of the lake water in the large bin were unsurprisingly similar to those seen in the profiles collected off the dock (Table 6) and to most of the parameters from the tap water. The tap water had higher E₇, specific conductivity, and total dissolved solids levels compared to the lake water, likely due to the water treatment process of the tap water. Phytoplankton chlorophyll-α and phycocyanin pigment levels in the fresh tap water measured in-situ were below detectable limit. Tap water total nitrogen concentrations were lower than in the lake water, but the tap water total phosphorus values were relatively high compared to what was found in the lake, again likely due to the water treatment process (Table 6). However, after the hydroponic fertilizer was added to the water as it was moved into the hydroponic reservoirs, these differences were inconsequential as the average total nitrogen concentration of the fertilized water was 151.3 ± 4.1 mg L⁻¹ and the

average total phosphorus concentration was $50.9 \pm 0.8 \text{ mg L}^{-1}$; several orders of magnitude above the original nutrient concentrations.

Natural total phosphorus levels in the lake water fluctuated each week but increased overall between the first and last sampling dates (Table 6). In the lake water, in-situ phycocyanin levels increased steadily over the three-week period, a pattern that was confirmed by the samples collected and frozen for fluorometric analysis in the lab (Table 6). Most of the lake cyanobacteria were not typical large bloom-forming species, since the $< 50 \text{ }\mu\text{m}$ phytoplankton indicated these smaller forms accounted for an average of 92.2% of the phycocyanin in the whole lake water samples. Chlorophyll- α levels fluctuated each week, showing a decrease during the second week and then an increase in the third week sampling (Table 6). Overall, the three-week fluorometry and nutrient results indicate that Lake Attitash was once again experiencing eutrophic conditions with a moderate cyanobacteria population that increased steadily throughout the duration of the experiment in 2016.

Table 6. Weekly water chemistry sampling of tap and lake water collected in large (140 L) tote bins for subsequent use in hydroponic reservoirs. Sampling data include in-situ water chemistry parameters collected using the YSI EXO2 multiparameter probe, frozen fluorometry phycocyanin results from the Turner Aquafleur handheld fluorometer, and lab results of nutrient levels. 'BDL' means 'below detectable limit'.

Water Type	Lake Water				Tap Water				
	Date	Aug 8 th	Aug 15 th	Aug 22 nd	Average ± SE	Aug 8 th	Aug 15 th	Aug 22 nd	Average ± SE
Temperature °C		27.2	27.7	25.7	26.9 ± 0.6	27.0	21.3	19.9	22.7 ± 2.2
Dissolved Oxygen mg L ⁻¹		8.2	7.8	7.5	7.9 ± 0.2	8.2	8.9	6.9	8.0 ± 0.6
Percent Saturation DO		103.8	100.4	92.6	98.9 ± 3.3	103.0	105.0	75.3	94.4 ± 9.6
pH		8.0	7.9	7.7	7.8 ± 0.1	7.8	7.9	7.7	7.8 ± 0.1
E ₇ mV		111.4	202.5	154.9	156.3 ± 26.3	100.6	604.3	405.7	370.2 ± 146.5
SpCond µS cm ⁻¹		208.3	204.5	203.0	205.3 ± 1.6	301.8	299.9	326.0	309.2 ± 8.4
Turbidity FNU		1.9	2.0	2.3	2.1 ± 0.2	0.1	0.1	0.3	0.2 ± 0.1
TDS mg L ⁻¹		135	133	132	133.3 ± 0.9	196	195	212	201.0 ± 5.5
In-Situ Chl-α µg L ⁻¹		13.4	8.2	13.3	11.6 ± 1.7	BDL	BDL	BDL	BDL
In-Situ PC µg L ⁻¹		14.0	19.4	27.4	20.3 ± 3.9	BDL	BDL	BDL	BDL
Frozen PC µg L ⁻¹		18.9	27.4	45.9	30.7 ± 8.0	BDL	BDL	BDL	BDL
Total Nitrogen µg L ⁻¹		580.7	-	634.7	607.7	228.0	-	248.8	238.4
Total Phosphorus µg L ⁻¹		22.9	16.0	31.2	23.4 ± 4.4	232.6	177.7	256.8	222.4 ± 23.4

Lake Attitash 2016 Net Phytoplankton Community Composition

From August 8th to August 29th of 2016, the net phytoplankton community in the lake water used in the hydroponic reservoirs was diverse and dominated by the Cyanophyceae with an overall average percent composition of 72.3%, followed by the Bacillariophyceae (Diatoms) at 24.5%, then the Chlorophyceae at 2.2% Figure 16. Organisms from the other major groups constituted the remaining 1% combined (Table 7). Over the three-week experimental period, the Cyanophyceae increased from 56.0% on August 8th to 87.6% on August 29th, 2016, which is consistent with the phycocyanin trend observed in the fluorometry samples (Table 6). Of the Cyanophyceae present, a very thin form of *Lyngby sp.* dominated the overall average phytoplankton assemblage at 63.5%, with *Dolichispermum sp.* (17.1%) and *Microcystis sp.* (7.2%) being the next numerous genera. Other cyanobacteria genera present at low concentrations were *Snowella sp.*, *Gleotheca sp.*, *Coelosphaerium sp.*, *Aphanocapsa sp.*, and *Planktothrix sp.* (Table 8).

With the increase in Cyanophyceae over the three-weeks, Bacillariophyceae accordingly decreased from 39.2% of the overall large-form phytoplankton assemblage on August 8th to 10.8% on August 29th, 2016. The vast majority of diatoms observed each week were *Melosira sp.*, with an overall average composition of 98.9%, and appearances by a few individuals from other genera including *Cyclotella*, *Synedra*, *Rhabdonema*, and *Navicula* (Table 9).

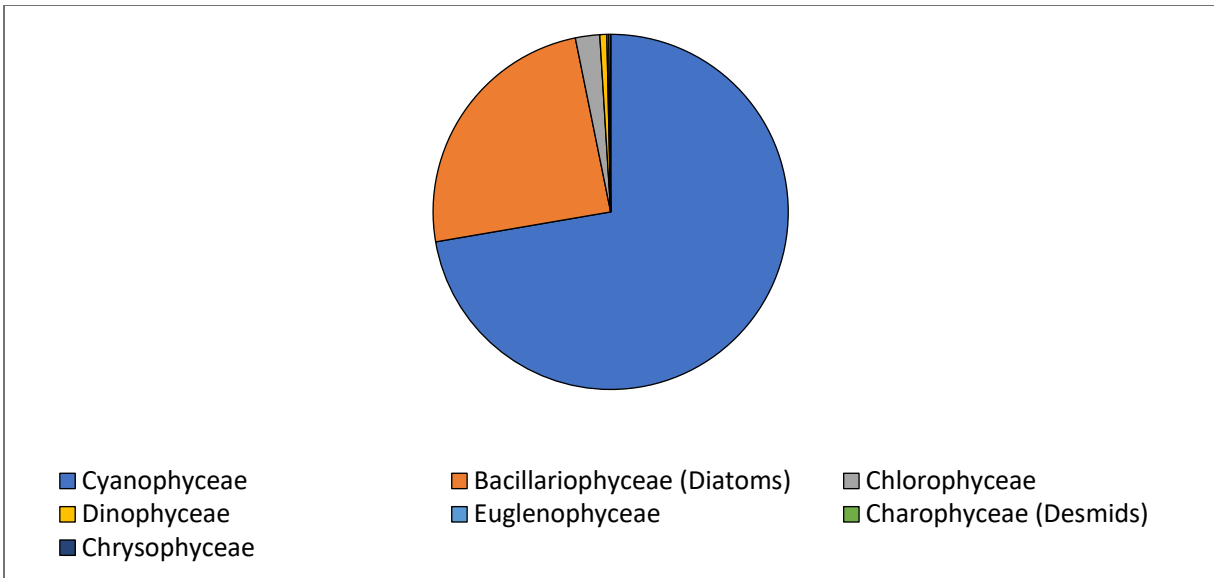


Figure 16. Average phytoplankton community composition from August 8th, 2016 to August 29th, 2016. The phytoplankton assemblage was dominated by the Cyanophyceae at 72.3%, followed by Bacillariophyceae (diatoms) at 24.5%, and then the Chlorophyceae (greens) at 2.2%.

Table 7. Lake Attitash weekly and overall average net phytoplankton community percent composition

Group	8/8/2016	8/15/2016	8/23/2016	8/29/2016	Average
Cyanophyceae	56.0	77.6	83.3	87.6	72.3
Bacillariophyceae (Diatoms)	39.2	18.6	15.7	10.8	24.5
Chlorophyceae	2.8	3.2	0.6	0.9	2.2
Dinophyceae	1.3	0	0.3	0.3	0.6
Euglenophyceae	0.5	0	0	0	0.2
Charophyceae (Desmids)	0	0	0.1	0.1	0.2
Chrysophyceae	0	0	0	0	0.0

Table 8. Lake Attitash weekly and overall average net Cyanophyceae community percent composition

Cyanobacteria Genera	8/8/2016	8/15/2016	8/23/2016	8/29/2016	Average
<i>Lyngbya</i>	36.0	65.1	84.8	67.9	63.5
<i>Dolichospermum</i>	17.4	26.6	12.2	12.4	17.1
<i>Microcystis</i>	2.6	5.5	2.6	18.0	7.2
<i>Snowella</i>	0	1.6	0.2	1.3	0.8
<i>Gleothoece</i>	0	0.9	0	0	0.2
<i>Coelosphaerium</i>	0	0.2	0	0.3	0.1
<i>Aphanocapsa</i>	0	0	0.2	0.1	0.1
<i>Planktothrix</i>	0	0	0	0.1	0.02

Cyanotoxins in Fresh Hydroponic Reservoir Water

Along with water chemistry parameters, nutrients, and phytoplankton analysis, the tap and lake water collected each week to be used in the hydroponic reservoirs was also tested for MCs and BMAA. The concentration of MCs in the lake water was $67.6 \pm 3.0 \text{ ng L}^{-1}$ at the beginning of Week 1, $62.7 \pm 3.6 \text{ ng L}^{-1}$ at the beginning of Week 2, and significantly higher in Week 3 at $106.4 \pm 1.6 \text{ ng L}^{-1}$ (One-way ANOVA, $n = 9$, $p < 0.0001$) (Figure 17). Microcystins were not detected in any tap water samples. BMAA concentrations in the lake water fluctuated in a similar way to the MC concentrations. For Week 1, the BMAA concentration in the lake water was $254.6 \pm 90.3 \text{ ng L}^{-1}$, which decreased slightly to $210.9 \pm 102.7 \text{ ng L}^{-1}$ for Week 2, and then increased to $584.5 \pm 84.1 \text{ ng L}^{-1}$ for Week 3. Interestingly, BMAA was also detected in the tap water in all three weekly samples with a concentration of $56.1 \pm 19.3 \text{ ng L}^{-1}$, $301.6 \pm 80.0 \text{ ng L}^{-1}$, and $399.8 \pm 72.1 \text{ ng L}^{-1}$ for weeks 1, 2, and 3, respectively. BMAA concentrations did not differ significantly between the lake water and tap water on any of the three sampling dates (Two-Way ANOVA, $n = 18$, $p = 0.18$) (Figure 18).

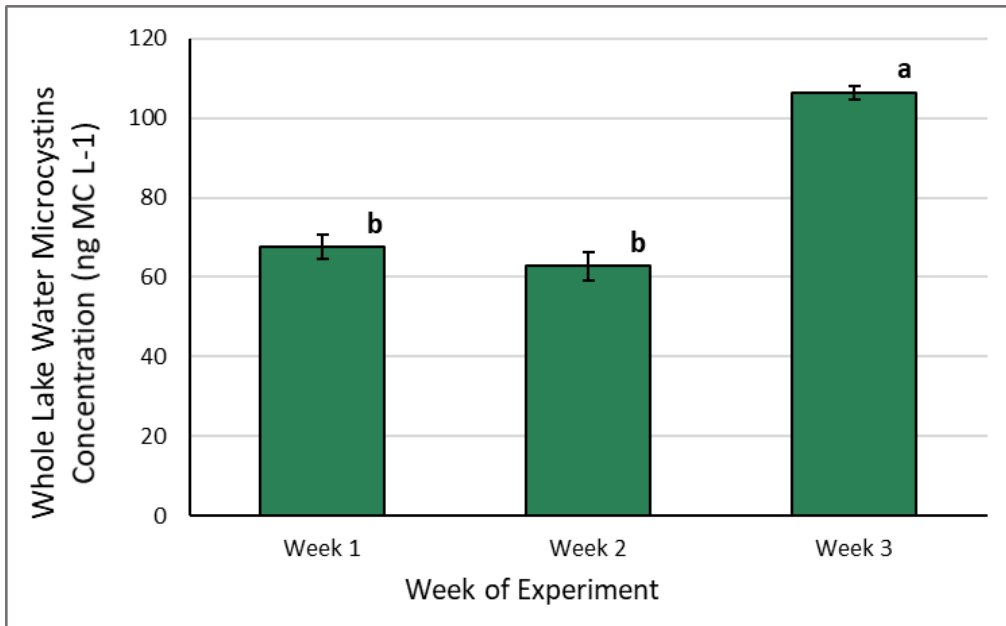


Figure 17. Concentration of free microcystins in whole lake water used in the hydroponic reservoirs each week with standard error. The microcystins concentration in Lake Attitash was significantly higher in Week 3 than in Weeks 1 and 2 (One-way ANOVA, $n = 9$, $p < 0.0001$).

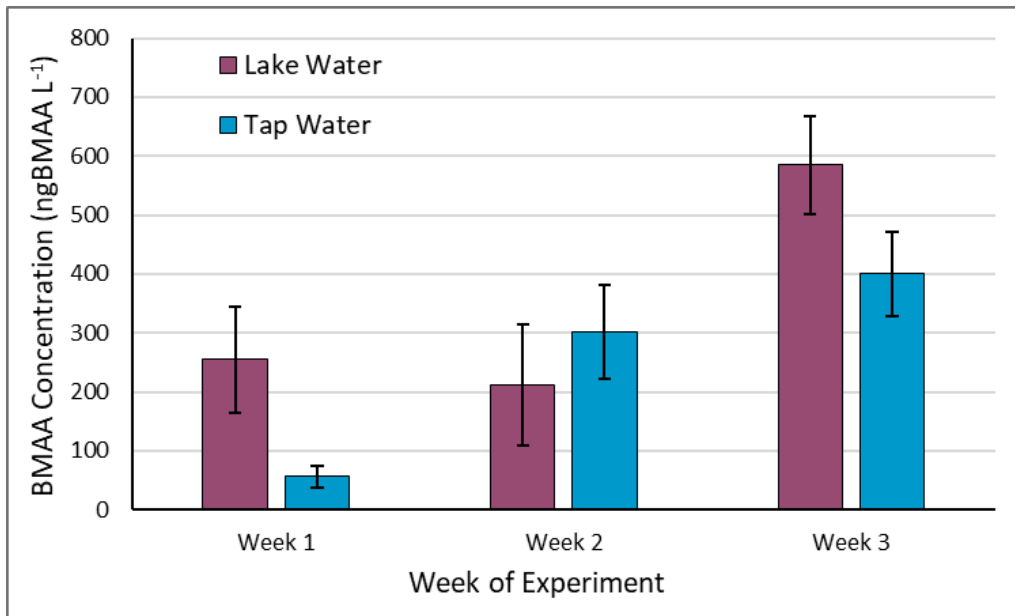


Figure 18. BMAA concentrations of fresh lake and tap water used in the hydroponic reservoirs each week with standard error. Toxin concentrations did not differ between water types for any week of the experiment (Two-Way ANOVA, $n = 18$, $p = 0.18$).

Drained Hydroponic Reservoir Water Conditions

At the end of each week samples were collected individually from all hydroponic reservoirs as they were drained for MCs analysis, frozen phycocyanin fluorometry, and water chemistry parameters to track changes inside the reservoirs during the experiment. Water from the lake water treatment reservoirs had increasing overall average MCs concentrations each week with 9.2 ± 1.0 ng MCs L⁻¹ at the end of Week 1, 20.4 ± 1.3 ng MCs L⁻¹ at the end of Week 2, and a significantly higher concentration of 51.0 ± 6.4 ng MCs L⁻¹ at the end of Week 3 (One-Way ANOVA, n = 36, p < 0.0001) (Figure 19). Drained tap water reservoirs had significantly higher microcystin concentrations each week with an average concentration of 4.9 ± 0.6 ng MCs L⁻¹ at the end of Week 1, 10.0 ± 1.2 ng MCs L⁻¹ at the end of Week 2, and a concentration of 17.4 ± 0.6 ng MCs L⁻¹ at the end of Week 3 (One-Way ANOVA, n = 36, p < 0.0001) (Figure 19). Overall, microcystins levels of lake water reservoirs were significantly higher than that of the tap water reservoirs each week (One-Way ANOVA, n = 72, p < 0.0001). Drained reservoir water was not sampled for BMAA concentrations. Interestingly, almost all phycocyanin and chlorophyll- α frozen fluorometry results for the drained reservoir water samples were below detectable limit except for the lake water samples collected on the last day of the experiment. The lake water reservoir samples from this date had an average phycocyanin concentration of 2.1 ± 0.3 μ g L⁻¹, a value just above the detectable limit of the instrument of 2.0 μ g L⁻¹. These very low fluorometry results indicate that there were few, if any, live cyanobacteria cells present in the water at that time, and therefore the toxin detected in the drained reservoir water was probably not cell-bound.

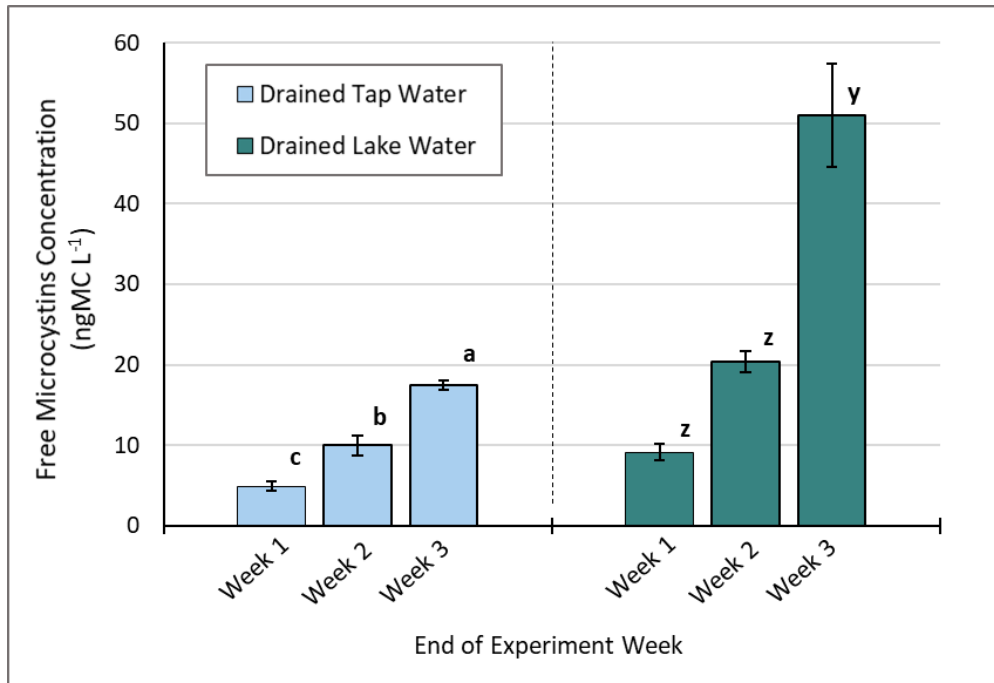


Figure 19. Weekly average free microcystin concentrations of drained reservoir water with standard error. Each bar represents the overall average MCs concentration of drained water from all reservoirs of each water type. The MCs concentration of drained tap water increased significantly each week (One-Way ANOVA, $n = 36$, $p = 2.61e-11$), where the average MCs concentration of drained lake water was significantly higher for the third week, but the first and second weeks were not statistically different (One-Way ANOVA, $n = 36$, $p < 0.0001$).

As the individual hydroponic reservoirs were drained, this water was combined in a large storage bin by water type and tested for water chemistry parameters as well as nutrient concentrations. Dissolved oxygen levels were high for both water types each week, ranging from 101.4% to 96.4%, indicating the aeration systems in the tunnels were effective (Table 9). Total dissolved solids and specific conductivity levels were higher than natural water levels due to the added hydroponic fertilizer, though they decreased each week, likely due to higher nutrient uptake rates with growth of the plants. Overall average nutrient concentrations of the drained tap and lake water were similar between water types. The reservoir lake water had a total nitrogen concentration of $124.7 \pm 9.9 \text{ mg L}^{-1}$ and a total phosphorus concentration of $35.3 \pm 3.1 \text{ mg L}^{-1}$ where the average total nitrogen and phosphorus concentrations of the reservoir tap water were very comparable with $121.7 \pm 14.1 \text{ mg L}^{-1}$ and $37.2 \pm 4.0 \text{ mg L}^{-1}$, respectively. These

concentrations indicate that nutrient levels did not change appreciably throughout the experiment, which prevented nutrient deficiency in the plants (Uchida 2000).

Table 9. Weekly in-situ water chemistry parameters of combined drained reservoir water collected with the YSI EXO2 multiparameter probe.

Date	Lake Water Reservoirs			Tap Water Reservoirs		
	8/15/2016	8/22/2016	8/29/2016	8/15/2016	8/22/2016	8/29/2016
Temperature (°C)	29.6	25.1	27.9	31.6	24.8	29.1
Dissolved Oxygen (mg L⁻¹)	7.6	8.3	7.7	7.3	8.0	7.4
Percent Saturation						
DO	100.6	101.4	98.0	99.5	96.9	96.7
pH	7.2	6.8	5.9	7.3	6.7	6.6
E₇ (mV)	127.2	164.0	166.8	132.4	125.4	163.9
SpCond (µS cm⁻¹)	1307.0	1162.8	1007.6	1411.3	1217.2	1050.1
Turbidity (FNU)	0.2	1.8	2.0	0.3	0.3	0.4
TDS (mg L⁻¹)	850	757	655	917	791	682

Lake Attitash 2016 MCs Aerosol Monitoring

To quantify toxins emitted from the lake as aerosols throughout the duration of the experiment a Compact Lake Aerosol Monitor (CLAM) was deployed for a 24 h collection once a week and on the last day of the experiment. Unfortunately, due to equipment issues, a particulate aerosol MCs concentration of 8.13 ± 1.50 pg MC m⁻³ collected on August 8th, 2016 is the only available result from these runs.

Plant Tissue Microcystins Concentrations

Low levels of microcystins were detected in many of the samples collected during the sowing and seedling stages of the lettuce and radish plants before they were placed in the treatment tunnels for the duration of the experiment. The lettuce seeds collected at the sowing stage had a dry weight MCs concentration of 1.06 ± 0.26 ng g⁻¹ (n = 3), and the radish seeds had a dry weight MCs concentration of 4.64 ± 1.02 ng g⁻¹ (n = 3). MCs were not detected in the greenhouse tap water used to grow the seedlings, but interestingly, MCs were detected in the

Oasis[®] growth cubes (2.16 ng MCs g⁻¹ DW, n = 2) (Table 10). Start condition lettuce seedling samples had a MCs concentration of 4.34 ng g⁻¹ DW in the leaves and stems, 5.15 ng g⁻¹ DW in the roots, and 1.64 ng g⁻¹ DW in the Oasis[®] growth cubes (Table 10). The radish seedlings had higher MCs levels with 27.50 ng g⁻¹ DW in the leaves, 30.47 ng g⁻¹ DW in the stems, and 6.69 ng g⁻¹ DW in the roots, with 3.31 ng g⁻¹ DW in the Oasis[®] growth cubes (Table 10).

For the final plant tissue samples collected at the end of the three-week field experiment in 2016, microcystins were detected in all parts of both plant types in all treatments except for the adventitious roots from the radishes. These were invalid because of a chemical interference with the ELISA that resulted in non-readable samples for all adventitious radish root samples. Radish leaves had the highest concentration of MCs, averaged across all four treatments, MCs concentrations ranged from a high of 10.39 ± 1.20 ng g⁻¹ DW in radish leaves to 3.59 ± 0.77 ng g⁻¹ DW in lettuce roots (Table 10, Figure 20). Lettuce leaves had the second highest MCs concentration with 7.16 ± 0.38 ng g⁻¹ DW, and radish taproots had an overall average MCs concentration of 3.83 ± 0.23 ng g⁻¹ DW. For lettuce, the leaves and stems had higher MCs concentration than the roots (Two-way ANOVA, n = 36, p < 0.0001), and for radishes the leaves also had a significantly higher MCs concentration than the radish taproots (One-way ANOVA, n = 24, p < 0.0001). In addition, radish leaves overall had significantly more MCs per gram dry weight than lettuce leaves (one-way ANOVA, n=24, p = 0.018).

Table 10. Overall average dry weight free MCs concentrations of lettuce and radish samples collected at different stages throughout the growth and experiment process. Samples collected during the sowing stage include lettuce seeds ($n = 1$), radish seeds ($n = 1$), and Oasis Rootcube® ($n = 2$). Initial start-condition seedling tissue samples include lettuce leaves/stems ($n = 1$), lettuce roots ($n = 1$), lettuce rootcube ($n = 1$), radish leaves/stems ($n = 1$), radish roots ($n = 1$), and radish rootcube ($n = 1$). Final experimental plant tissues represent results averaged across all four treatments ($n = 12$ for all sample types). Concentrations include standard error where possible.

Sample Type	Sowing Samples ng MCs g ⁻¹ DW	Start Seedlings ng MCs g ⁻¹ DW	Final Experimental Plant Tissues ng MCs g ⁻¹ DW
Lettuce Leaves	-	4.34	7.16 ± 0.38
Lettuce Stems	-	-	5.65 ± 0.21
Lettuce Roots	-	5.15	3.59 ± 0.77
Lettuce Seeds	1.06 ± 0.26	-	-
Lettuce Oasis Rootcube®	2.16	1.64	-
Radish Leaves	-	27.50	10.39 ± 1.20
Radish Taproots	-	-	3.83 ± 0.23
Radish Seedling Stems	-	30.47	-
Radish Seedling Roots	-	6.69	-
Radish Seeds	4.64 ± 1.02	-	-
Radish Oasis Rootcube®	2.16	3.31	-

Concentrations of MCs in the lettuce leaves, stems, and roots were not different between treatments overall (two-way ANOVA, $n = 36$, $p = 0.068$) (Figure 21). However, lettuce roots from lake water treatments had significantly higher MCs than lettuce roots from the tap water treatments (one-way ANOVA, $n = 12$, $p = 0.019$) despite tunnel type (Figure 22). In lettuce leaves, MCs ranged from a high of 7.81 ± 0.73 ng g⁻¹ DW in the open tunnel/tap water treatment to 5.90 ± 0.78 ng g⁻¹ in the filtered tunnel/tap water treatment (Figure 21, Table 11). MCs concentrations in the lettuce stems showed a similar pattern to the leaves with a high of 6.09 ± 0.57 ng g⁻¹ from the open tunnel/tap water treatment and a low of 5.30 ± 0.57 ng g⁻¹ from the filtered tunnel/tap water treatment. Lettuce roots from the filtered tunnel/tap water treatment had the lowest MCs concentration at 1.87 ± 0.37 ng g⁻¹, and the filtered tunnel/lake water treatment produced roots with the highest MCs at 6.03 ± 2.54 ng g⁻¹ DW (Figure 21, Table 11).

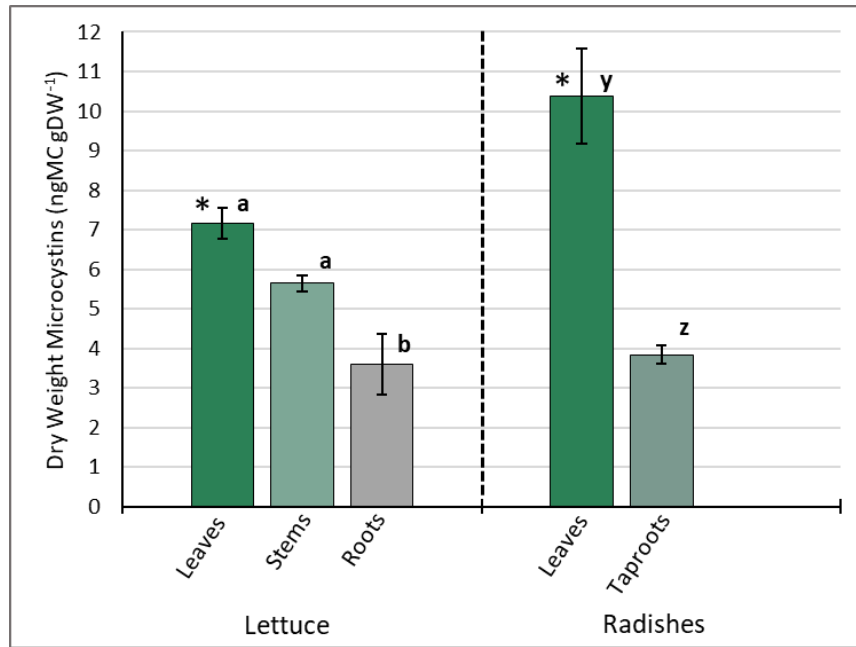


Figure 20. Overall MCs concentrations in different plant parts from across all four treatments with standard error. Lettuce leaves and stems (a) had a significantly higher MCs concentration than lettuce roots (b) (Two-way ANOVA, $n = 36$, $p = 3.55e-05$). Radish leaves (y) also had a significantly higher MC concentration than radish taproots (z) (One-way ANOVA, $n = 24$, $p = 2.14e-05$). The asterisks denote that the radish leaves had a significantly higher MCs concentration than the lettuce leaves (One-Way ANOVA, $n = 24$, $p = 0.018$).

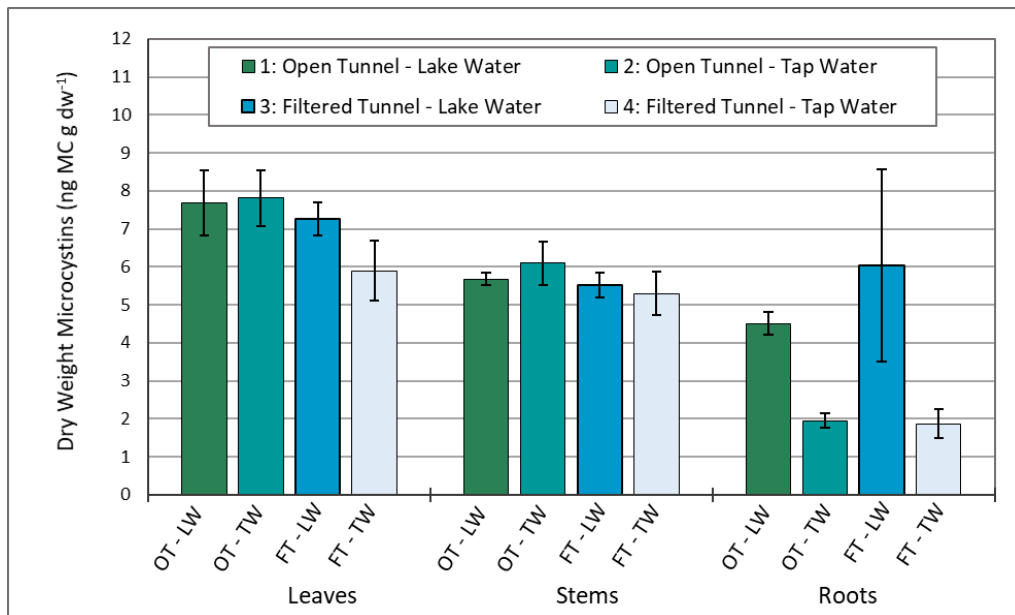


Figure 21. Free microcystins concentrations with standard error from lettuce leaves, stems, and roots across all four exposure treatments. MC concentrations were not significantly different between treatments overall for any tissue type (two-way ANOVA, $n = 36$, $p = 0.068$).

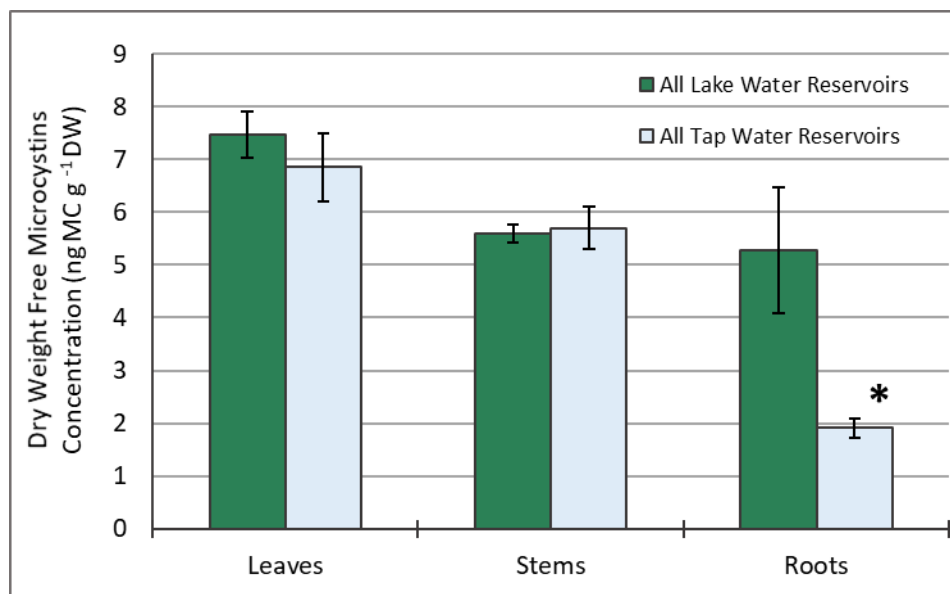


Figure 22. Free microcystins concentrations in lettuce leaves, stems, and roots with standard error by reservoir water type. As indicated by the asterisk, lettuce roots from tap water reservoirs had significantly less MC than lettuce roots from lake water reservoirs (one-way ANOVA, $n = 12$, $p = 0.019$). There were no significant differences in MC concentration in the leaves (one-way ANOVA, $n = 12$, $p = 0.45$) or stems (one-way ANOVA, $n = 12$, $p = 0.83$) between reservoir water types.

Radish leaves from the four treatments showed a MCs concentration pattern similar to the lettuce leaves. Radish leaves from the open tunnel/tap water treatment had a higher MCs concentration than the three other treatments overall (one-way ANOVA, $n = 12$, $p = 0.0037$) at $16.11 \pm 2.35 \text{ ng g}^{-1} \text{ DW}$, where leaves from the filtered tunnel/tap water treatment had the lowest amount of MCs overall with $6.65 \pm 0.75 \text{ ng g}^{-1} \text{ DW}$ (Table 11, Figure 23). For radish taproots, MCs concentrations were not significantly different across all four treatments overall (one-way ANOVA, $n = 12$, $p = 0.074$). However, radish taproots from the tap water treatments had a significantly higher average MCs concentration than taproots from the lake water treatments (two-way ANOVA, $n=12$, $p = 0.036$), though there were no differences between the two tunnel types for radish taproots (two-way ANOVA, $n = 12$, $p = 0.97$). In contrast to the other sample types, radish taproots from the open tunnel/lake water treatment had the lowest dry weight MCs concentration at $3.03 \pm 0.15 \text{ ng g}^{-1}$, where radish taproots from the open tunnel/tap water

treatment had the highest dry weight MCs concentration at $4.64 \pm 0.44 \text{ ng g}^{-1} \text{ DW}$ as seen in the lettuce leaves, lettuce stems, and radish leaves (Table 11, Figure 23).

Table 11. Free Microcystins concentrations with standard error from final experimental lettuce and radish tissue samples exposed to lake water and aerosols at four treatment levels for three weeks.

Plant	Tissue Type	Treatment #	Treatment Description	Free Microcystins Concentration ($\text{ng g}^{-1} \text{ DW}$)
Lettuce	Leaves	1	Open Tunnel - Lake Water	7.68 ± 0.85
Lettuce	Leaves	2	Open Tunnel - Tap Water	7.81 ± 0.73
Lettuce	Leaves	3	Filtered Tunnel - Lake Water	7.26 ± 0.45
Lettuce	Leaves	4	Filtered Tunnel - Tap Water	5.90 ± 0.78
Lettuce	Stems	1	Open Tunnel - Lake Water	5.68 ± 0.16
Lettuce	Stems	2	Open Tunnel - Tap Water	6.09 ± 0.57
Lettuce	Stems	3	Filtered Tunnel - Lake Water	5.52 ± 0.32
Lettuce	Stems	4	Filtered Tunnel - Tap Water	5.30 ± 0.57
Lettuce	Roots	1	Open Tunnel - Lake Water	4.51 ± 0.30
Lettuce	Roots	2	Open Tunnel - Tap Water	1.95 ± 0.19
Lettuce	Roots	3	Filtered Tunnel - Lake Water	6.03 ± 2.54
Lettuce	Roots	4	Filtered Tunnel - Tap Water	1.87 ± 0.37
Radish	Leaves	1	Open Tunnel - Lake Water	8.50 ± 0.35
Radish	Leaves	2	Open Tunnel - Tap Water	16.11 ± 2.35
Radish	Leaves	3	Filtered Tunnel - Lake Water	10.29 ± 0.42
Radish	Leaves	4	Filtered Tunnel - Tap Water	6.65 ± 0.75
Radish	Taproots	1	Open Tunnel - Lake Water	3.03 ± 0.15
Radish	Taproots	2	Open Tunnel - Tap Water	4.64 ± 0.44
Radish	Taproots	3	Filtered Tunnel - Lake Water	3.73 ± 0.42
Radish	Taproots	4	Filtered Tunnel - Tap Water	3.92 ± 0.35

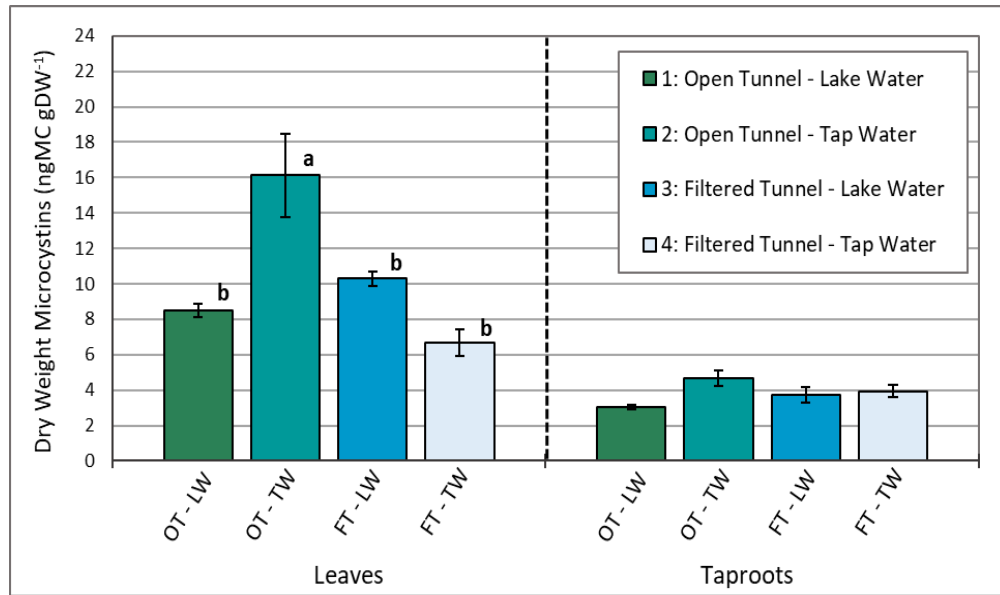


Figure 23. Dry weight free microcystin concentrations in radish leaves and taproots across the four treatments with standard error. Radish leaves from Treatment 2 (a) had a significantly higher MC concentration than leaves from the other three treatments (b), all of which were not significantly different from each other (two-way ANOVA, $n = 12$, $p = 0.0037$). Radish taproot MCs concentrations were not significantly different between treatments (one-way ANOVA, $n = 12$, $p = 0.074$)

Plant Tissue BMAA Concentrations

Samples of lettuce and radish seeds, greenhouse water, growth medium, and seedlings were collected during different preparation stages to determine if BMAA was present in the plants before they were placed in the treatment tunnels of the experiment. At the sowing stage in the greenhouse, BMAA was detected in the lettuce seeds, radish seeds, and Oasis[®] growth cube samples collected at concentrations of $1100.75 \pm 26.14 \text{ ng g}^{-1} \text{ DW}$, $7749.78 \pm 5920.95 \text{ ng g}^{-1} \text{ DW}$, and $323.04 \pm 89.50 \text{ ng g}^{-1} \text{ DW}$, respectively (Table 12). BMAA was not detected in the tap water in the greenhouse used to water the seeds and seedlings as they grew.

Start condition lettuce seedlings had an average BMAA concentration of $3244.06 \pm 48.80 \text{ ng g}^{-1} \text{ DW}$ in the leaves, $1422.42 \text{ ng g}^{-1} \text{ DW}$ in the roots, and $162.38 \pm 52.81 \text{ ng g}^{-1} \text{ DW}$ in the oasis growth cubes (Table 12). Start condition radish seedlings had an average BMAA concentration of $3223.08 \pm 250.10 \text{ ng g}^{-1} \text{ DW}$ in the leaves, $3042.65 \pm 143.98 \text{ ng g}^{-1} \text{ DW}$ in the

stems, $965.64 \text{ ng g}^{-1} \text{ DW}$ in the roots, and $152.45 \pm 22.89 \text{ } \mu\text{g g}^{-1} \text{ DW}$ in the Oasis[®] growth cubes (Table 12). Due to their small size, there was not enough material available for multiple extractions of lettuce or radish seedling roots.

Table 12. Overall average dry weight free BMAA concentrations of lettuce and radish samples collected at different stages throughout the growth and experiment process. Samples collected during the sowing process include lettuce seeds (n = 3), radish seeds (n = 3), and Oasis Rootcube[®] (n = 6). Initial start-condition seedling tissue samples include lettuce leaves/stems (n = 3), lettuce roots (n = 1), lettuce rootcubes (n = 3), radish leaves/stems (n = 3), radish roots (n = 2), and radish rootcubes (n = 3). Final experimental plant tissues represent results averaged across all four treatments (n = 12 for all sample types). Concentrations include standard error where possible.

Sample Type	Sowing Samples ng BMAA g ⁻¹ DW	Start Seedlings ng BMAA g ⁻¹ DW	Final Experimental Plant Tissues ng BMAA g ⁻¹ DW
Lettuce Leaves	-	3244.06 ± 48.80	7562.39 ± 1148.31
Lettuce Stems	-	-	4018.54 ± 269.43
Lettuce Roots	-	1422.42	551.88 ± 96.39
Lettuce Seeds	1100.75 ± 26.14	-	-
Lettuce Oasis Rootcube [®]	323.04 ± 89.50	162.38 ± 52.81	-
Radish Leaves	-	3223.08 ± 250.10	4710.13 ± 490.27
Radish Taproots	-	-	3223.60 ± 557.46
Radish Seedling Stems	-	3042.65 ± 143.98	-
Radish Seedling Roots	-	965.64	-
Radish Seeds	7749.78 ± 5920.95	-	-
Radish Oasis Rootcube [®]	323.04 ± 89.50	152.45 ± 22.89	-

As with microcystins, at the end of the three-week field experiment, BMAA was detected in all final plant tissue samples from both lettuce and radishes except for the radish adventitious root samples that interfered with the ELISA, resulting in non-readable samples for this tissue type. The overall average dry weight BMAA concentrations across all treatments ranged from a high of $7562.39 \pm 1148.31 \text{ ng g}^{-1}$ in lettuce leaves to $551.88 \pm 96.39 \text{ ng g}^{-1}$ in lettuce roots, with radish taproots at a mid-range concentration of $3223.60 \pm 557.46 \text{ ng g}^{-1}$ (Table 12). Within the lettuce, overall average BMAA concentrations were significantly different in each part of the plant, with the highest concentration in the leaves, followed by the stems, and then the lowest concentration in the roots (two-way ANOVA, n = 36. p < 0.0001) (Figure 24). Across plant

types, lettuce leaves also had a significantly higher overall BMAA concentration than radish leaves (three-way ANOVA, $n = 24$, $p = 0.027$). For radishes, the difference between the overall average BMAA concentrations of the radish leaves and taproots closely approached significance (one-way ANOVA, $n = 24$, $p = 0.058$) (Figure 24).

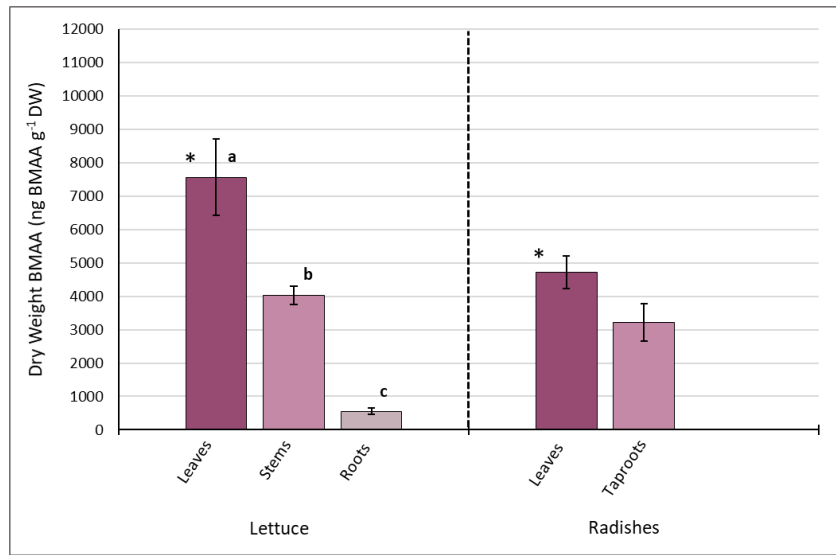


Figure 24. Overall average dry weight BMAA concentrations across all four treatments in lettuce and radish plant parts with standard error. The BMAA concentration in lettuce leaves (a) was significantly higher than that in the lettuce stems (b), and lettuce roots (b) had a significantly lower BMAA concentration than stems (two-way ANOVA, $n = 36$, $p = 3.23e-07$). The asterix denote significance between overall average BMAA concentrations in lettuce and radish leaves (three-way ANOVA, $n = 24$, $p = 0.027$). In radishes, BMAA concentrations approached significance between radish leaves and taproots (one-way ANOVA, $n = 24$, $p = 0.058$).

With the combined lettuce and radish leaf data, there was no significant effect of tunnel type for the treatments (three-way ANOVA, $n = 24$, $p = 0.30$), but lettuce and radish leaves from the lake water treatments had a higher BMAA concentration than those from the tap water treatments (three-way ANOVA, $n = 24$, $p = 0.042$) (Figure 25).

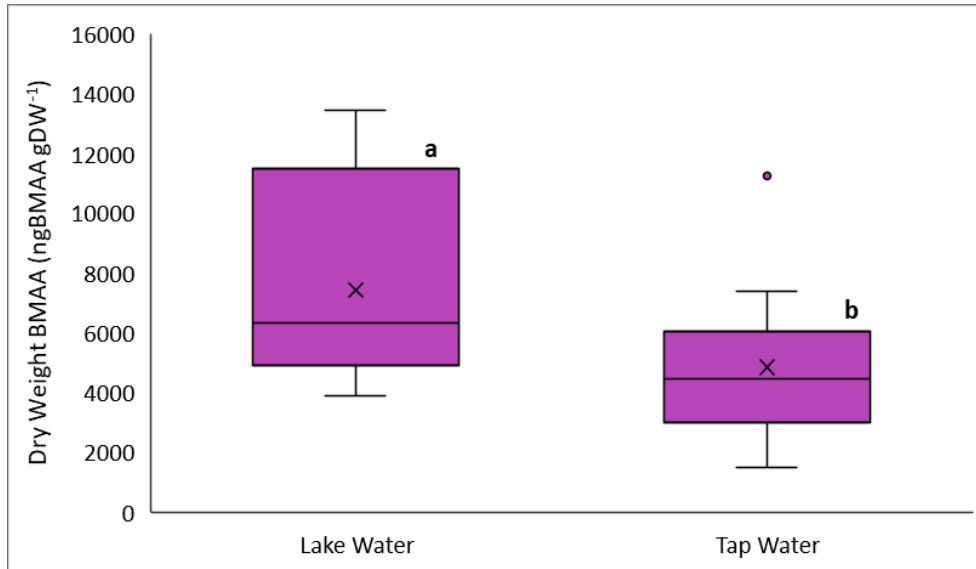


Figure 25. Boxplot of average lettuce and radish leaf dry weight BMAA concentrations from lake water and tap water treatments. Leaves from lake water treatments (a) had a significantly higher dry weight BMAA concentration than leaves from tap water treatments (b) (three-way ANOVA, $n = 24$, $p = 0.042$).

Within the lettuce, dry weight BMAA concentrations in the leaves, stems, and roots were not significantly different between overall treatments (two-way ANOVA, $n = 36$, $p = 0.50$) (Figure 26). For lettuce leaves, the open tunnel/lake water treatment (Treatment 1) produced the highest BMAA concentration of $10970.31 \pm 1960.80 \text{ ng g}^{-1} \text{ DW}$, and the filtered tunnel/tap water treatment (Treatment 4) was lowest with $4673.97 \pm 979.07 \text{ ng g}^{-1} \text{ DW}$ (Table 13). In comparison, BMAA in the lettuce stems from the open tunnel/tap water treatment (Treatment 2) was highest with $4515.01 \pm 328.76 \text{ ng g}^{-1} \text{ DW}$, and lowest in stems from the open tunnel/lake water treatment (Treatment 1) at $3505.21 \pm 314.91 \text{ ng g}^{-1}$. Lettuce roots had much lower BMAA concentrations than leaves and stems overall, and roots from the filtered tunnel/tap water treatment (Treatment 4) had the highest BMAA concentration with $886.65 \pm 317.17 \text{ ng g}^{-1} \text{ DW}$ and the open tunnel/lake water treatment (Treatment 1) had the lowest with $405.06 \pm 77.42 \text{ ng g}^{-1} \text{ DW}$; opposite the trend seen in the lettuce leaves.

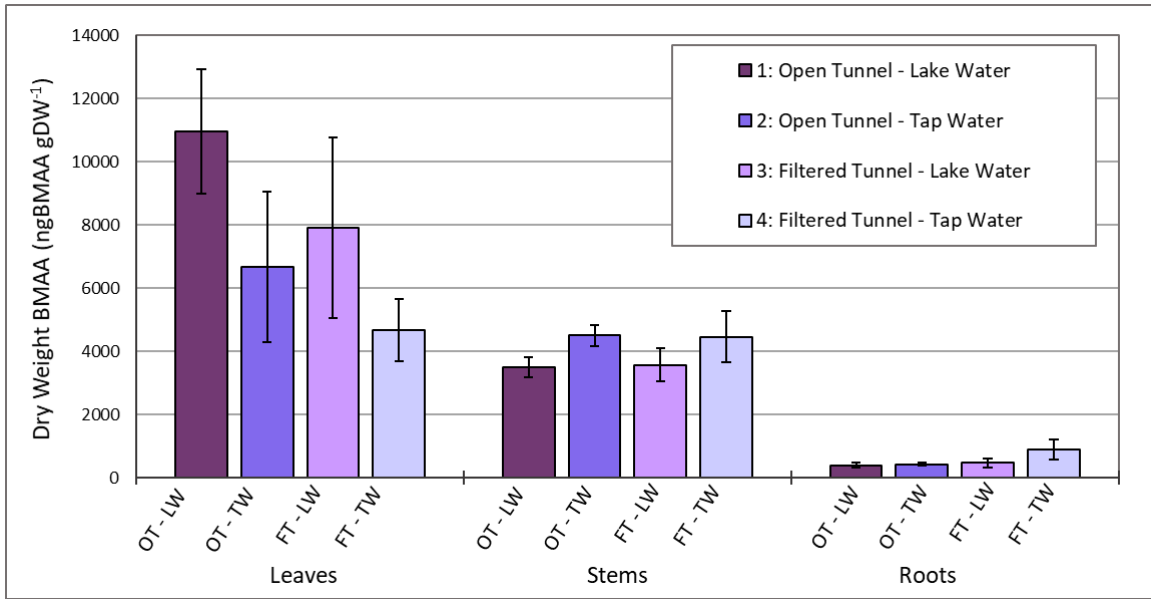


Figure 26. Free BMAA dry weight concentrations in lettuce tissues across four levels of exposure to lake cyanotoxins with standard error. BMAA concentrations were not significantly different between overall treatments for leaves, stems, or roots (two-way ANOVA, $n = 36$, $p = 0.50$).

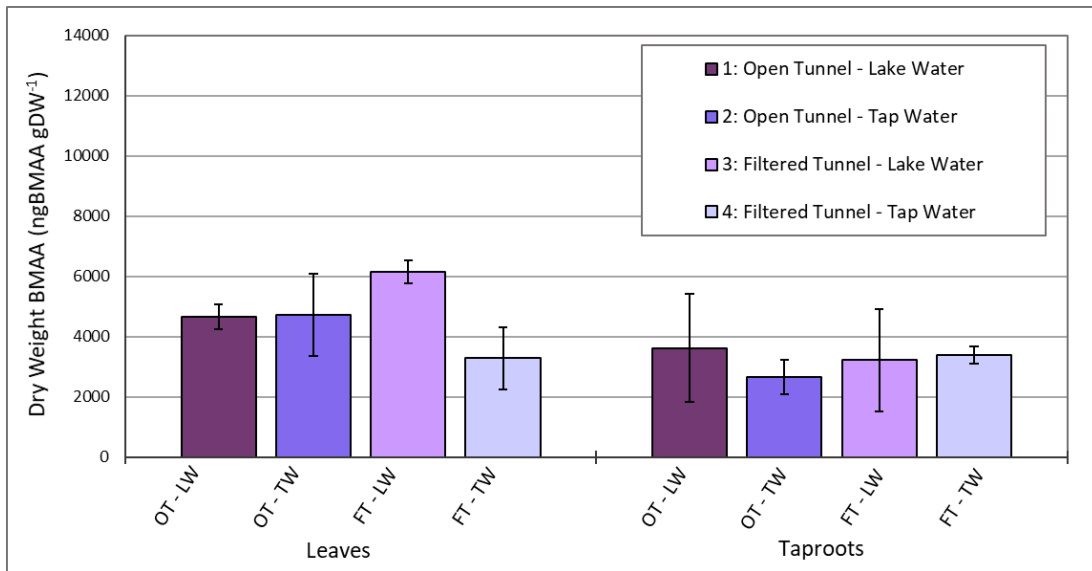


Figure 27. Free BMAA dry weight concentrations in radish tissues across four levels of exposure to lake cyanotoxins with standard error. BMAA concentrations were not significantly different between overall treatments for leaves or taproots (two-way ANOVA, $n = 36$, $p = 0.65$).

Similar to the lettuce, BMAA concentrations did not differ between the four treatments overall for radish leaves or taproots (two-way ANOVA, $n = 24$, $p = 0.65$) (Figure 27). In this case, there were no significant differences in BMAA concentrations in either the radish leaves or

taproots between either tunnel types or reservoir water types (three-way ANOVA, $n = 24$, $p_{\text{tunnel}} = 0.90$, $p_{\text{water}} = 0.27$), even when the two treatment factors were considered separately. For leaves across the four treatments, radish leaves from the filtered tunnel/lake water treatment (Treatment 3) had the highest dry weight BMAA concentration of $6157.66 \pm 368.14 \text{ ng g}^{-1}$ and leaves from the filtered tunnel/tap water treatment (Treatment 4) had the lowest with $3291.96 \pm 1034.81 \text{ ng g}^{-1}$ (Table 13). Radish taproots from the open tunnel/lake water treatment (Treatment 1) had the highest BMAA concentration with $3630.47 \pm 1801.18 \text{ ng g}^{-1}$ DW and those from the open tunnel/tap water treatment (Treatment 2) had the lowest with $2656.03 \pm 572.13 \text{ ng g}^{-1}$ DW.

Table 13. Free BMAA concentrations with standard error from lettuce and radish tissue samples exposed to lake cyanotoxins at four treatment levels for three weeks.

Plant	Tissue Type	Treatment #	Treatment Description	Dry Weight Free BMAA Concentration (ng g⁻¹)
Lettuce	Leaves	1	Open Tunnel - Lake Water	10970.31 ± 1960.80
Lettuce	Leaves	2	Open Tunnel - Tap Water	6691.41 ± 2382.51
Lettuce	Leaves	3	Filtered Tunnel - Lake Water	7913.85 ± 2847.90
Lettuce	Leaves	4	Filtered Tunnel - Tap Water	4673.97 ± 979.07
Lettuce	Stems	1	Open Tunnel - Lake Water	3505.21 ± 314.91
Lettuce	Stems	2	Open Tunnel - Tap Water	4515.01 ± 328.76
Lettuce	Stems	3	Filtered Tunnel - Lake Water	3581.27 ± 529.49
Lettuce	Stems	4	Filtered Tunnel - Tap Water	4472.68 ± 809.78
Lettuce	Roots	1	Open Tunnel - Lake Water	405.06 ± 77.42
Lettuce	Roots	2	Open Tunnel - Tap Water	440.80 ± 47.85
Lettuce	Roots	3	Filtered Tunnel - Lake Water	474.99 ± 139.91
Lettuce	Roots	4	Filtered Tunnel - Tap Water	886.65 ± 317.17
Radish	Leaves	1	Open Tunnel - Lake Water	4665.18 ± 426.00
Radish	Leaves	2	Open Tunnel - Tap Water	4725.74 ± 1358.57
Radish	Leaves	3	Filtered Tunnel - Lake Water	6157.66 ± 368.14
Radish	Leaves	4	Filtered Tunnel - Tap Water	3291.96 ± 1034.81
Radish	Taproots	1	Open Tunnel - Lake Water	3630.47 ± 1801.18
Radish	Taproots	2	Open Tunnel - Tap Water	2656.03 ± 572.15
Radish	Taproots	3	Filtered Tunnel - Lake Water	3219.95 ± 1710.37
Radish	Taproots	4	Filtered Tunnel - Tap Water	3387.95 ± 287.17

Concentration of Deposited MCs Aerosols Rinsed from Leaf Surfaces

At the end of the three-week field experiment, radish leaves had a higher concentration of microcystins than lettuce leaves overall at $0.41 \pm 0.03 \text{ pg cm}^{-2}$ for radishes and $0.25 \pm 0.02 \text{ pg cm}^{-2}$ for lettuce (two-way ANOVA, $p = 3.61\text{e-}06$). However, deposited MCs aerosol concentrations were not different between the two tunnel types for lettuce or radish leaves (two-way ANOVA, $p = 0.72$) (Figure 28). With an overall average of $113.12 \pm 9.30 \text{ pg cm}^{-2}$, deposited BMAA aerosol concentrations were approximately 275 times higher than the deposited MCs aerosols, but they were not significantly different between plant types or tunnel types (two-way ANOVA, $n = 24$, $p_{\text{plant type}} = 0.26$, $p_{\text{tunnel type}} = 0.25$) (Figure 29).

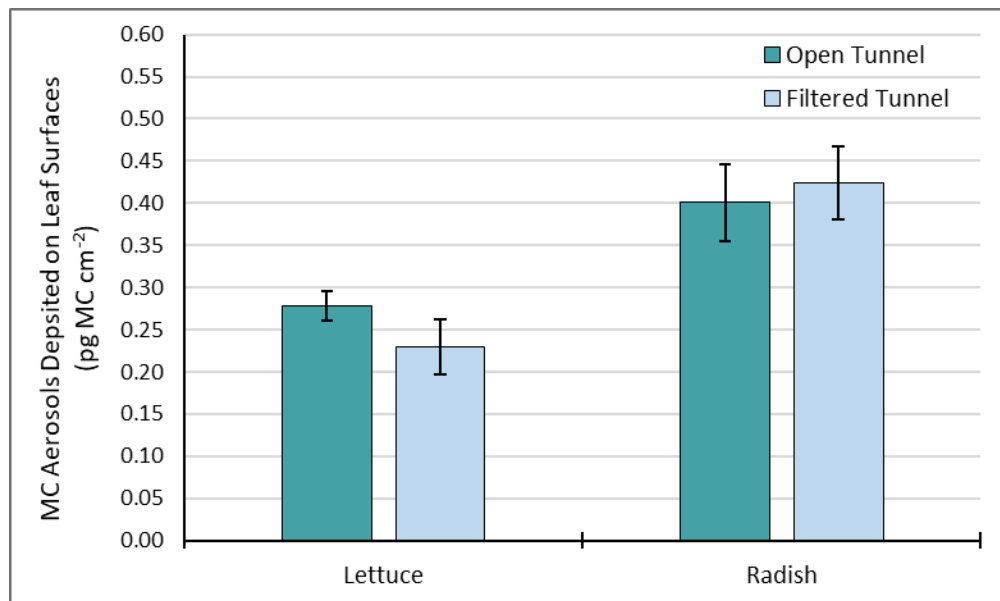


Figure 28. Deposited MCs aerosols on leaf surfaces of lettuce and radishes in open vs filtered tunnels. Overall, deposited MCs concentrations were higher on radish leaves than lettuce leaves (two-way ANOVA, $n = 46$, $p = 3.61\text{e-}06$), and tunnel type was not statistically significant (two-way ANOVA, $n = 46$, $p = 0.72$).

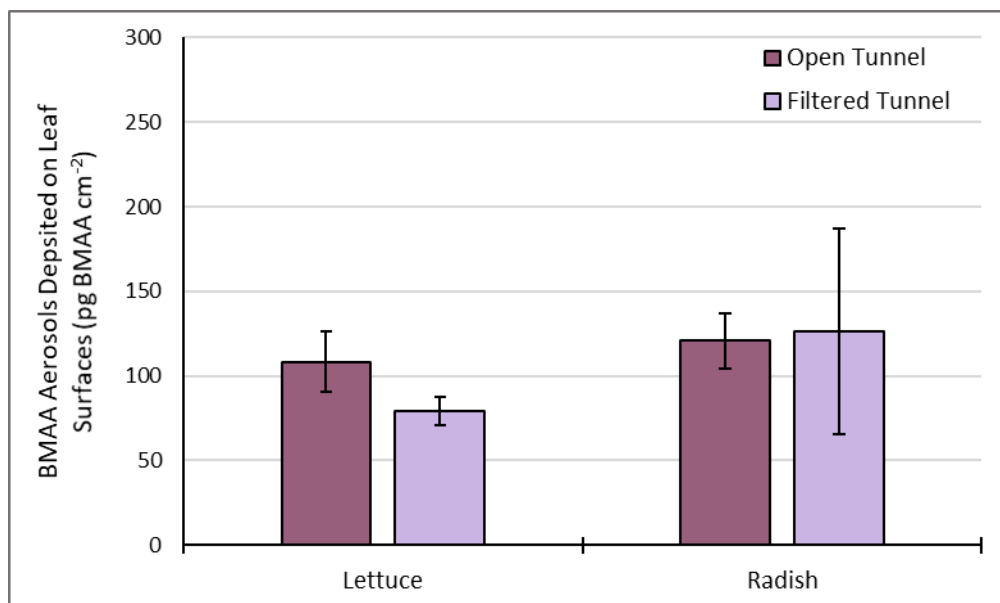


Figure 29. Deposited BMAA aerosols on lettuce and radish leaf surfaces at the end of the three-week field experiment. Deposited BMAA aerosol concentrations were not significantly different between plant types or tunnel types (two-way ANOVA, $n = 24$, $p_{\text{plant type}} = 0.26$, $p_{\text{tunnel type}} = 0.25$)

Lake Aerosol Tunnel Exclusion Experiment

The Lake Attitash phytoplankton community in 2017 was similar to what was observed in 2016. Once again, the Cyanophyceae dominated the $> 50 \mu\text{m}$ net phytoplankton, this time at 66.6% followed strongly by the Bacillariophyceae (diatoms) at 30.0%. *Lynby sp.* and *Dolichispermum sp.* were the two main cyanobacteria present in 2017 at 54.3% and 44.6%, respectively, and *Melosira sp.* was the only genera of diatom observed. Fluorometric analysis of the whole lake water and the filtered $< 50 \mu\text{m}$ lake water samples indicated that the cyanobacteria community was dominated by small cyanobacteria species, which represented 62.9% of the overall phycocyanin fluorescence. The whole lake water samples collected during the three 2017 sampling events had an average MCs concentration of $18.1 \pm 1.4 \text{ ng L}^{-1}$, and an average BMAA concentration of $600.4 \pm 57.8 \text{ ng L}^{-1}$ (Table 14).

Table 14. Whole, unfiltered lake water toxin and fluorometry results and < 50µm filtered lake water fluorometry results collected during the three sampling events on September 24th, October 2nd, and October 8th, 2017.

Water Type	Parameter	Sept 24 th	Oct 2 nd	Oct 8 th	Average ± SE
Whole Lake Water	Microcystins (ng L ⁻¹)	16.3	16.9	22.1	18.4 ± 1.4
	BMAA (ng L ⁻¹)	645.6	698.9	456.6	600.4 ± 57.8
	Phycocyanin (µg L ⁻¹)	6.4	9.9	10.6	9.5 ± 0.9
< 50 µm Lake Water	Phycocyanin (µg L ⁻¹)	3.4	8.4	5.3	5.6 ± 0.6

In the open tunnel, particulate microcystins aerosols were detected on CLAM filters with an overall average concentration of $2.28 \pm 0.47 \text{ pg m}^{-3}$, and dissolved microcystins aerosols were detected in both the methanol and water traps at $9.78 \pm 1.63 \text{ pg m}^{-3}$ and $7.10 \pm 1.43 \text{ pg m}^{-3}$, respectively. Though the concentration of dissolved MCs aerosols was higher in the methanol trap than the water trap, this difference was not statistically significant (one-way ANOVA, $p = 0.24$). Overall, the average total concentration of MCs aerosols present during the day in the open tunnel was 10.72 pg m^{-3} . For the HEPA filtered tunnel, particulate MCs aerosols were detected on CLAM filters with an average concentration of $3.12 \pm 0.57 \text{ pg m}^{-3}$ and dissolved MCs aerosols were detected in the methanol traps at $14.67 \pm 2.50 \text{ pg m}^{-3}$ for a total average daytime MCs aerosol concentration of 17.79 pg m^{-3} . Both the particulate aerosols from the filters and the dissolved MCs aerosol levels in the methanol traps were higher in the filtered tunnel than the open tunnel, though neither difference was significant (one-way ANOVA, $p = 0.28$, and $p = 0.12$, respectively) (Figure 29).

BMAA aerosol concentrations from the open tunnel were several orders of magnitude higher than MCs in the particulate aerosol filter and dissolved aerosol trap samples. Particulate BMAA aerosols from filters had an average concentration of $546.05 \pm 84.45 \text{ pg m}^{-3}$, and

dissolved BMAA aerosols from water traps had an average concentration of $630.86 \pm 87.94 \text{ pg m}^{-3}$, for a total average daytime BMAA aerosol concentration of $1176.91 \text{ pg m}^{-3}$.

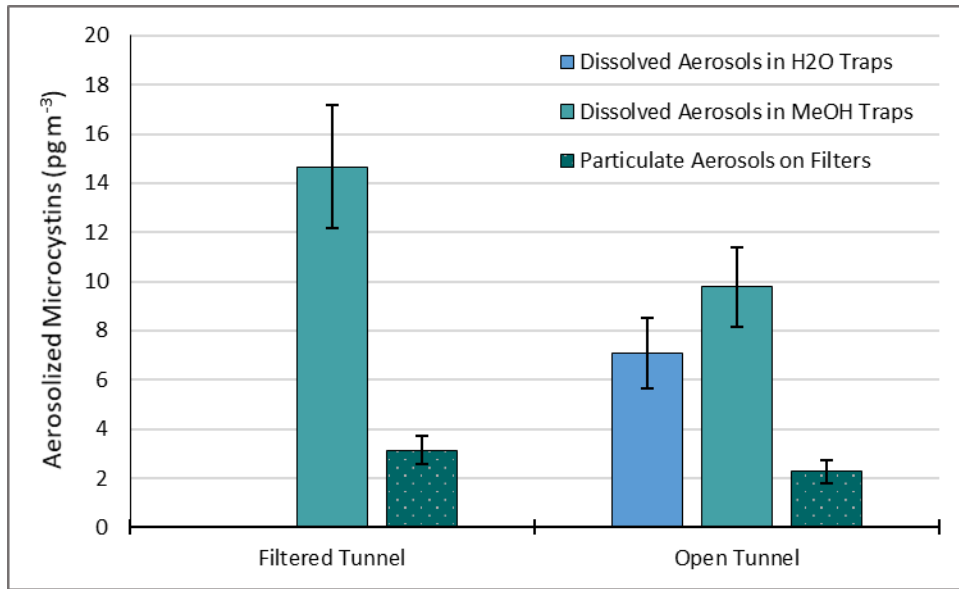


Figure 30. Aerosolized microcystins concentrations from open and sealed treatment tunnels detected in particulate form on filters as well as dissolved form from liquid traps. Within the open tunnel, MCs detected in the methanol traps were not significantly higher than the water traps (one-way ANOVA, $p = 0.24$). The dissolved MCs aerosol concentration from the filtered tunnel methanol traps was not significantly higher than the methanol traps in the open tunnel (one-way ANOVA, $p = 0.12$), and the particulate MCs aerosol concentration from the filtered tunnel was not significantly higher than the open tunnel (one-way ANOVA, $p = 0.28$).

DISCUSSION

Lake Attitash and Experimental Treatment Conditions

In the summer of 2016, the conditions of Lake Attitash as well as those inside the experimental tunnels were favorable for our experiment to investigate the uptake of the lake-derived cyanobacteria toxins β -methylamino-L-alanine and Microcystins by lettuce and radishes via exposure to lake water and aerosols. Throughout the three-week experiment in August, Lake Attitash exhibited an average chlorophyll- α level of $11.6 \mu\text{g L}^{-1}$, total nitrogen level of $607.7 \mu\text{g L}^{-1}$, and total phosphorus level of $23.4 \mu\text{g L}^{-1}$, placing it at the low end of the eutrophic trophic status. In past years, Lake Attitash has exhibited more eutrophic (and even hypereutrophic) conditions with cyanobacteria communities dominated by *Aphanizomenon sp.*, *Dolichospermum sp.*, and *Microcystis sp.* The $> 50 \mu\text{m}$ phytoplankton community present in the lake water used during this experiment was dominated by toxigenic genera of cyanobacteria, specifically a very thin type of *Lyngbya sp.*, (Figure 31) that had not been observed in previous deep-site epilimnetic phytoplankton samples in Lake Attitash. This is likely because the water used in the experimental reservoirs was collected off the dock in shallow water along the shoreline of the lake, and though most *Lyngbya* species are benthic (Komárek et al. 2013; Hudon et al. 2014), the wave action along the shoreline likely mixed these benthic *Lyngbya sp.* up into the water column where they were collected. Freshwater species of the *Lyngbya* genus are most recognized for producing a number of saxitoxin analogues (Onodera et al. 1997; Pearson et al. 2010), but they have also been found to produce anatoxin-a, cylindrospermopsin, deoxy-cylindrospermopsin, and microcystins (Carmichael et al. 1997; Smith et al. 2019; Poirier-Larabie et al. 2020). In addition, *Lyngbya sp.* extracts caused neuro- and hepatotoxicity in mice as well as cytotoxicity in

mammalian and fish cell lines (Teneva et al. 2003). Interestingly, the fluorometry results indicated that 92% of the phycocyanin observed in the whole lake water samples was from the < 50 μm fraction of phytoplankton. As cyanobacteria counts are generally based on large, bloom-forming species that can be identified without epifluorescence, our samples for phytoplankton identification were collected with a 50 μm net. As a result, these smaller organisms observed via fluorescence of the < 50 μm fraction were missed in the identification samples, but since they likely constitute the majority of the cyanobacteria biomass it would be interesting to include them in future samples to better understand the phytoplankton community and cyanotoxin dynamics of Lake Attitash. Subsamples of the < 50 μm fraction plankton samples could be saved for identification and enumeration under higher magnification than the standard > 50 μm samples, possibly using an epifluorescence microscope to determine the presence of phycocyanin and chlorophyll- α pigments to assist in identification.

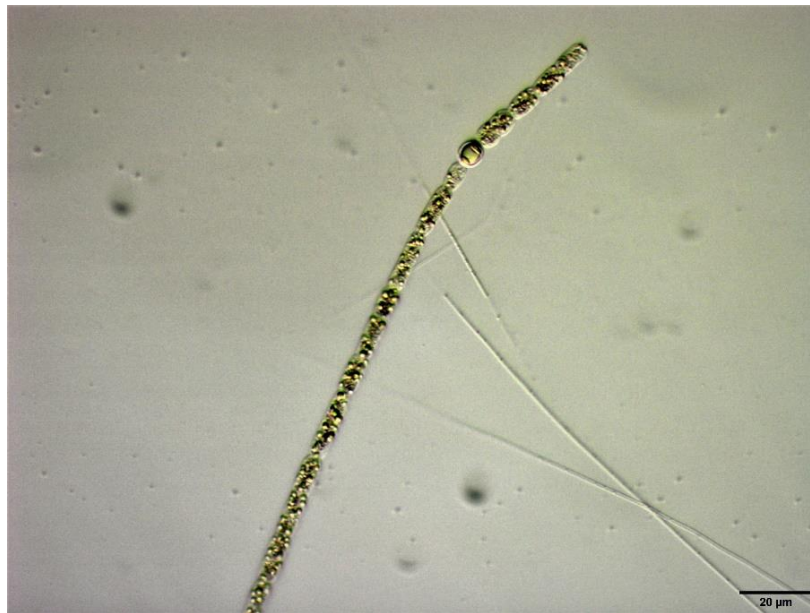


Figure 31. Image of several Lyngbya sp. (right) filaments next to a much larger filament of Dolichospermum sp. (left/center). This Lyngbya sp. was the dominant cyanobacteria observed in the Lake Attitash > 50 μm phytoplankton samples. This type of Lyngbya sp. was very thin and almost colorless with a sheath that was hollow at each end of the trichome.

The concentration of microcystins in the lake water during the 2016 field experiment was relatively low with an overall average of 78.9 ng L⁻¹ and the highest concentration of MCs seen during the last week of the experiment at 106.4 ng L⁻¹. Though these levels were low compared to worldwide reports of lake water MCs ranging from 0.04 – 150,000 ug L⁻¹ (EPA 2015b), they were moderate to high for the region (NEIWPCC 2010), and averaged five times than the summer statewide MCs average of 15.6 ng L⁻¹ observed in the neighboring state of NH (Haney 1999).

BMAA concentrations in the lake water were much higher than MCs, with the highest reading observed seen during the last week of the experiment at 584.5 ng L⁻¹, and an overall average concentration of 350 ng L⁻¹. It is difficult to compare the whole lake water BMAA levels observed in this study to others reported in the literature as there are few reports of BMAA concentrations in whole lake water, and each report uses different methods of sample collection and analysis. For example, Al-Sammak et al. (2014) measured BMAA levels in near surface grab samples collected during the summer months of 2009 and 2010 in nine highly eutrophic Nebraska reservoirs with a range of BDL – 25300 ng BMAA L⁻¹ and an overall average concentration of 11800 ng BMAA L⁻¹. This average is 34 times higher than the average BMAA concentration we observed, but it is possible that some or all of the samples they reported as below detectable limit were comparable to our observations as the reported detection limit for their HPLC-FD method was 5000 ng L⁻¹, or 14 times that of Lake Attitash. Using a novel method to derivatize cyanotoxins with dansyl chloride before analysis via ultra-high performance liquid chromatography, Roy-Lachapelle et al. (2015) found BMAA in four lakes in Québec, Canada with concentrations ranging from 10 – 2000 ng L⁻¹ and an overall average of 585 ng L⁻¹, a value much closer to the levels observed in Lake Attitash. As one of the few examples of

BMAA concentrations measured using the ELISA method as we did for our samples, Wiltsie et al. (2018) reported BMAA levels over the course of 15 months from the highly eutrophic B. Everett Jordan Reservoir, NC, ranging from BDL to 23450 ng L⁻¹, with an average of 10750 ng L⁻¹. This average BMAA concentration is 30 times higher than what we observed in Lake Attitash in 2016. Interestingly, the values reported by Wiltsie et al. (2018) represent only the extracellular toxins present in the lake water once the plankton were removed via 0.7 µm filtration. It can be assumed that these already high toxin concentrations would be higher had these samples extracted the toxins potentially present within the phytoplankton cells as well. Lastly, average seasonal BMAA concentrations measured using the ELISA method from three sites in Lake Winnipeg, Canada ranged from 390 ng L⁻¹ to 960 ng L⁻¹ (Pip et al. 2016), very similar to what was observed in Lake Attitash in 2016. This was the only study to report samples collected in a similar fashion to this study, from a system with a similar trophic state, and using the same analysis technique. Overall, the BMAA concentrations observed in whole water from Lake Attitash during the summer of 2016 were up to 34 times lower than those observed in more productive reservoirs across the US (Al-Sammak et al. 2014; Wiltsie et al. 2018), but were comparable to some readings observed from more similar, less eutrophic systems in Québec, Canada (Roy-Lachapelle et al. 2015) and Lake Winnipeg, Canada (Pip et al. 2016).

In addition to direct exposure to cyanotoxins through the lake water in the hydroponic reservoirs, the plants were also exposed to aerosolized BMAA and MCs released from the lake surface. In 2016 a MCs aerosols value of 8.13 ± 1.50 pg m⁻³ was recorded from August 8th of that year, and in 2017 the presence of both BMAA and MCs in aerosols emitted from Lake Attitash was confirmed in aerosol filter and liquid trap samples with average concentrations of 1176.91 pg BMAA m⁻³ and 10.7 pg MCs m⁻³. Since the phytoplankton communities were very

similar in both years, it is likely that both BMAA and MCs aerosols were also emitted from Lake Attitash during the 2016 experiment.

Conditions in the experimental tunnels and reservoirs were also suitable for the growth of the lettuce and radish plants. Despite the differences in design between the open-ended tunnels and the sealed tunnels fitted with HEPA filters, temperature and light levels did not differ between them, which helped to ensure consistent growth rates in the two tunnel types. In order to expose plants to lake water that most accurately reflected the ongoing conditions in the lake, water in the reservoirs was replaced weekly to refresh the biological assemblage, and the hydroponic reservoirs were wrapped with foil to block light and prevent further growth of phytoplankton. However, because light could not enter the hydroponic reservoirs, the phytoplankton community in the lake water reservoirs degraded over time each week, as indicated by the fluorometry results of the drained reservoir water. With one exception, water drained from all the reservoirs each week had no detectable levels of phycocyanin or chlorophyll- α , an indication that the phytoplankton had degraded during the previous week, which demonstrated the importance of weekly water replacement.

Microcystins also decreased in the reservoir water by the end of each week. Interestingly, the total percent of MCs remaining in the lake water reservoirs compared to the amount originally in the fresh lake water increased each week. At the end of the first week, only 13.5% of the MCs initially present in the lake water reservoirs remained in the drained reservoir water. At the end of the second week this percentage increased to 32.5%, and at the end of the final week it increased again to 47.9%. Fresh lake water MCs concentrations were similar for the first and second weeks at 67.6 ng MCs L⁻¹ and 62.7 ng MCs L⁻¹, respectively, where the third week had a significantly higher MCs concentration at 106.4 ng MCs L⁻¹. It is interesting that despite

the similar initial MCs concentrations, there was a higher percentage of MCs remaining in the drained reservoir water at the end of the second week compared to the first, suggesting changes in growth and uptake rates during these two weeks as the plants increased in size. It is possible that the plants reached an MCs saturation concentration during the second week as observed in another prolonged exposure study (Cordeiro-Araújo et al. 2016), which would have resulted in lower uptake rates of MCs and more MC left in the reservoir water.

The aeration system used to maintain healthy oxygen levels in the reservoirs was another route through which cyanotoxins could have entered the treatment reservoirs and then been detected in the drained reservoir water samples. For example, MCs were not detected in the fresh tap water that went into the treatment reservoirs, but MCs were detected in the drained tap water reservoir at the end of each week. The drained tap water reservoirs had average MCs concentrations of 4.9 ng MCs L⁻¹, 10.0 ng MCs L⁻¹, and 17.4 ng MCs L⁻¹ for the first, second, and third weeks, respectively. The presence of these MCs in the drained tap water reservoirs is likely the result of accumulation of water-soluble toxins through the aeration system or depuration by the plant roots. For future hydroponic studies involving the exposure of plants to aerosolized cyanotoxins from lakes, it would be better to utilize the Kratky passive hydroponic method (Kratky 2004) to grow the plants as it doesn't require circulation or aeration of the water and the hydroponic reservoirs could remain sealed throughout the experiment, which would prevent exposure to aerosolized toxins. It would not, however, prevent accumulation of toxins in reservoir water due to root depuration.

BMAA was detected in the fresh tap water for all three weeks at levels that were not significantly different from what was measured in the lake water reservoirs (Two-Way ANOVA, n = 18, p = 0.19) (Figure 18). Source water for the town of Amesbury, MA includes surface

water sources including the Powwow River and Lake Attitash itself, especially in the fall during drawdown of the lake (Bunker et al. 2010). BMAA is not removed through boiling (Banack et al. 2006), and since traditional water treatment practices do not effectively remove extracellular dissolved contaminants (Chen et al. 2018), it is possible that the BMAA levels detected in the tap water could have originated from surface water sources, including Lake Attitash itself. As another example of the complications that can arise during a complex field study, the presence of BMAA in the tap water treatment reservoirs suggests that future studies should test source water thoroughly prior to running experiments. It also highlights the need to test for BMAA in drinking water when surface waters are utilized in the supply chain.

Cyanotoxins in Greenhouse and Start Seedling Samples

Both BMAA and MCs were detected in the lettuce and radish seeds and seedlings, however the amounts in these samples were miniscule compared to the total toxin in the final experimental plants and did not contribute significantly to the experimental toxin concentrations. Both the lettuce and radish seeds tested had BMAA concentrations of $1100.7 \pm 26.1 \text{ ng g}^{-1} \text{ DW}$ and $7749.8 \pm 5920.9 \text{ ng g}^{-1} \text{ DW}$, respectively. The concentrations of microcystins in the lettuce and radish seeds were $1.1 \pm 0.3 \text{ g MC g}^{-1} \text{ DW}$ and $4.6 \pm 1.0 \text{ g MC g}^{-1} \text{ DW}$, respectively. This was an interesting discovery, as it is likely that the plants that produced these seeds were unintentionally exposed to these cyanotoxins before they were harvested. It is possible that the parent plants were irrigated with water containing cyanotoxins, but it is also known that cyanobacteria and cyanotoxins can be found terrestrially on bare rock, in soils, and even in desert crusts (Meeks 1998; WHO 1999; Richer et al. 2015).

To determine the contribution of the pre-existing toxins in the seeds and seedlings to the overall toxin concentrations in the final experimental plants, total toxins and relative proportions

were calculated using available weights of the seeds, seedlings, and adult plants. For lettuce, the seeds and seedlings each contained less than one tenth of a percent of the total BMAA and MCs present in the final harvested plants (Table 15). For radishes these percentages were higher, but the total amount of BMAA and MCs in the seeds and seedlings still accounted for less than one percent of the final toxin amounts (Table 15).

Table 15. Total BMAA and MCs present in lettuce and radish seeds, seedlings, and final harvested plants. Relative percentages were calculated using available seed, seedling, and final plant tissue weights to determine the potential toxin contribution from the seeds and seedlings to the amount of toxins found in the final plant samples

	Average Total BMAA (ng)	% of Final Plants	Average Total MCs (ng)	% of Final Plants
Lettuce Seeds	1.05	0.001	0.001	0.001
Radish Seeds	78.13	0.10	0.05	0.03
Lettuce Seedlings	43.99	0.02	0.13	0.07
Radish Seedlings	155.67	0.20	0.31	0.19
Final Lettuce Leaves + Stems	205360.97	-	197.24	-
Final Radish Leaves	77884.12	-	165.69	-

Collection of Lake-Derived Cyanotoxin Aerosols Utilizing Liquid Traps

During the 2017 aerosol tunnel experiment, three CLAM units were used to investigate aerosol concentrations in an open and filtered treatment tunnel. In contrast to the CLAM unit used during the 2016 experiment, these CLAMs were modified to collect both particulate aerosols on the glass fiber filters as well as dissolved fraction aerosols that passed through the filters by utilizing a liquid trap. Drawing air through the GFF filter and then a deionized water or 80% methanol trap allowed us to collect more free toxins that were not intracellular or bound to any type of particle, giving a more representative reading of total aerosol toxin concentrations. MCs aerosols were detected in the filter and trap samples from the two methanol-trap CLAM units that were placed in the open tunnel as well as the water-trap CLAM unit placed in the

filtered tunnel. The average particulate and dissolved MCs concentrations from all filter and trap samples were $2.60 \pm 0.37 \text{ pg m}^{-3}$ and $10.16 \pm 1.21 \text{ pg m}^{-3}$ of air, respectively, for a total MCs aerosol concentration of 12.76 pg m^{-3} .

Using the second CLAM unit in the open-ended tunnel, BMAA aerosols were detected on the glass fiber filters at $546.05 \pm 84.45 \text{ pg m}^{-3}$ of air, and dissolved BMAA aerosols were detected in the water traps at $630.86 \pm 87.94 \text{ pg m}^{-3}$ of air for a total concentration of $1176.91 \text{ pg m}^{-3}$. This is the one of the first reports of quantifiable BMAA aerosols, and the first report of concurrent MCs and BMAA aerosol concentrations. Previous to this research, particulate BMAA aerosols were collected during the summers of 2015 and 2016 from Hartbeespoort reservoir in South Africa at concentrations that ranged from 6-39 pg L^{-1} ($0.006\text{-}0.039 \text{ pg m}^{-3}$) (Scott et al. 2018). In addition, Banack et al. (2015) detected BMAA in a single aerosol sample collected from Lake Mascoma in New Hampshire, but it was below the limit of quantification used in their study. It is probable that Banack et al. (2015) did not effectively collect the majority of cyanotoxin aerosols present because they used high volume total suspended particle collectors with filters that had an effective pore size of $100 \text{ }\mu\text{m}$, which likely would have missed many aerosol particles as well as dissolved toxins. By collecting samples directly on the shoreline, using combusted glass fiber filters with a pore size of $0.3 \text{ }\mu\text{m}$, adding the liquid traps, concentrating our liquid samples 20x, and utilizing the ELISA method, this made it possible for us in this study to detect the BMAA aerosols.

Utilizing the liquid traps in the CLAM units also allowed us to better understand the composition of lake-derived cyanotoxin aerosols by collecting the dissolved and particulate fractions separately. We found that dissolved cyanotoxins did in fact pass through the glass fiber filters into the liquid traps, and that more toxins were in the dissolved form in those traps than in

particulate form on the filters. BMAA aerosols were only collected in the open tunnel using the CLAM with water traps, with an average concentration of 630.86 pg BMAA m⁻³ in the traps and 546.05 pg m⁻³ on the filters. For MCs aerosols in the open tunnel, the methanol and water traps collected 4.5 and 3.1 times more MCs than the filters, respectively. In the filtered tunnel, the methanol trap samples had an MCs concentration 4.7 times higher than the filter samples (Table 16). Overall, dissolved MCs aerosols were 4.0 times higher than particulate, and dissolved BMAA aerosol concentrations were 1.2 times higher than particulate aerosols.

Table 16. Microcystins and BMAA aerosols collected using CLAM units with added liquid traps in an open and sealed/filtered tunnel. One CLAM unit was placed inside the sealed/filtered tunnel with 80% methanol used in the liquid trap. Two CLAM units were placed in the open-ended tunnel, one with 80% methanol in the trap and the other with DI water. Filter MCs values from both CLAM units in the open-ended tunnel were averaged.

	CLAM 1	CLAM 2	CLAM 3
Tunnel	Sealed/Filtered	Open-ended	Open-ended
Trap Type	80% Methanol	80% Methanol	Water
Filter MCs (pg m ⁻³)	3.12 ± 0.57	2.28 ± 0.47	2.28 ± 0.47
Trap MCs (pg m ⁻³)	14.67 ± 2.50	9.78 ± 1.63	7.10 ± 1.43
Total MCs (pg m⁻³)	17.79	12.06	9.38
Filter BMAA (pg m ⁻³)	-	-	546.05 ± 84.45
Trap BMAA (pg m ⁻³)	-	-	630.86 ± 87.94
Total BMAA (pg m⁻³)	-	-	1176.91

In addition to information on the composition of aerosols, the use of two different liquids in the traps provided information on how to effectively collect MCs and BMAA aerosols. Though the methanol traps in the open tunnel had a slightly higher MCs concentration than the water traps, this difference was not significant (one-way ANOVA, p = 0.24), indicating that the use of water in the liquid traps of the CLAM units could be a useful method to sample for both microcystins and BMAA aerosols simultaneously using a single CLAM unit. Cyanotoxin aerosol collection methods reported do not use liquid traps to help collect dissolved toxins (Cheng et al. 2007; Backer et al. 2008; Wood and Dietrich 2011; Banack et al. 2015; Murby and

Haney 2016; Langley 2019), and it is very likely that these studies missed a large proportion of the cyanotoxin aerosols that were present, however, the present study was by no means a thorough investigation of the efficiencies of different collection liquids in the traps, and further research is required to determine the best aerosol collection method for each toxin.

Deposition of Cyanotoxin Aerosols on Lettuce and Radish Leaf Surfaces

At the end of the field exposure study investigating the accumulation of lake-derived cyanotoxins in lettuce and radishes, the leaves from all plants in each treatment were rinsed to remove any remaining deposited cyanotoxin aerosols. This rinse water was tested for both MCs and BMAA to determine if aerosolized toxins deposited on leaf surfaces at the end of the three weeks could be removed by rinsing and detected, and if so, if there were differences between the plant types and treatment tunnels. Imaging software was used to determine the average surface area of the different plant types in each treatment, and along with leaf weights, these values were used to calculate the amount of toxin present per square centimeter of leaf tissue.

The overall average amount of rinseable MCs deposited on the lettuce and radish leaves was 0.252 ± 0.020 pg cm⁻², and 0.412 ± 0.031 pg cm⁻², respectively. Relative to total toxin present both on the surface of the leaves and bound within the tissues, these values represent only 1.8% of the total MCs associated with the lettuce leaves and 1.3% for the radish leaves. In addition, rinseable BMAA was detected on lettuce leaves at a concentration of 0.103 ± 0.014 ng cm⁻² and 0.124 ng cm⁻² on radish leaves. Again, these values only represented a small fraction of the total toxin associated with the plants, at 0.70% for the lettuce and 0.80% for the radish leaves. Suffice it to say the amounts of free MCs and BMAA that could be rinsed off of the surface of the lettuce and radish leaves at the end of the three-week experiment were minimal

compared to the amount of toxins found within the leaves, but they were present in measurable amounts.

The amount of rinseable surface microcystins and BMAA detected in these samples was not significantly different between the open and filtered tunnels, which is not surprising since it was determined that the HEPA filters did not remove particulate or dissolved aerosolized cyanotoxins from the air that entered the tunnels. Interestingly, the rinse water from the radish leaves had more MCs than lettuce leaves, but BMAA levels were not significantly different between the two plants. It is likely that the hairy texture of the radish leaves facilitated the collection of more microcystins in comparison to the smooth surface of the lettuce leaves. The small molecular weight of the amino acid BMAA (118 g mol^{-1}) versus the peptide microcystin-LR (995 g mol^{-1}) could explain why there was no difference in deposited BMAA levels between lettuce and radishes. Despite the presence of hairs on the radish leaves, the BMAA molecules might have been too small to be trapped by them in significant amounts as may have occurred with the microcystins.

Though it is interesting that the radishes had more microcystins on their leaf surfaces than the lettuce in this study, and it has been well established that the physical properties of leaf surfaces are important in controlling the absorption of agrochemical compounds (Santier and Chamel 1998), the persistence of cyanotoxins on leaf surfaces in particular is not well understood. It is likely that the cyanotoxins were absorbed into the leaf tissues as reported in aquatic plants (Contardo-Jara et al. 2013), and for other substances including water, pesticides, and air pollutants (O'Dell et al. 1977), but published research on the uptake of cyanotoxins through leaf surfaces is minimal. Codd et al. (1999) observed visible *Microcystin sp.* cells on the surface of lettuce leaves that had been irrigated with water from a pond with a dense, toxigenic

cyanobacteria bloom, but they did not investigate the potential absorption of toxins specifically through the leaves. It appears that the leaves of different plants may also differ in their wettability and absorption properties. In a study of the effects of irrigation water containing MCs at a concentration of 2.1 mg L⁻¹ MCs on ryegrass, clover, canola, and lettuce plants, Crush et al. (2008) reported the presence of MCs in lettuce and clover shoots and the absence of MCs in ryegrass and canola shoots. They noted that the water ran off the leaves of the ryegrass and canola plants without wetting the surfaces, where the lettuce and clover leaves were visibly wetted (Crush et al. 2008), suggesting that MCs were absorbed from the irrigation water through the leaf surfaces of the lettuce and clover plants. In the present study, however, the lettuce and radish leaves were exposed to cyanotoxins via aerosols, not irrigation water. A more thorough investigation of the fate of aerosolized cyanotoxins deposited on leaf surfaces is necessary to fully understand their role in aquatic and agricultural systems.

Accumulation of Lake-Derived Cyanotoxins in Lettuce and Radishes

All lettuce and radish tissue samples were analyzed for BMAA and MCS, but because of an unknown interaction between the sample material and the ELISA method, radish root samples could not be read. The ELISA kits used for MCs and BMAA use a competitive toxin-labeled horseradish peroxidase, where free (unbound) toxin present in the tissue sample competes with this horseradish peroxidase for binding sites on the plate. It is likely that an enzyme present in the radish uptake root samples interfered with this peroxidase, as it is known that the ELISA method might not accurately measure toxins in samples with complex matrices (Qian et al. 2015). To date, there are no reports of BMAA concentrations in radishes, and only one other study tested for MCs in radish roots (Mohamed and Al Shehri 2009). Interestingly, Mohamed and Al Shehri (2009) successfully detected MCs in the uptake roots of radishes collected from

fields irrigated with well water contaminated with cyanotoxins and reported an average fresh weight MCs concentration of 0.23 ug g^{-1} . Mohamed and Al Shehri (2009) used the same ELISA kit from the same manufacturer as this study, but they used a different extraction method that involved a much longer sample extraction time in methanol, which required their samples to be evaporated to dryness and reconstituted in a buffer solution for analysis. In this study our samples were extracted in water over a much shorter period, and though they were concentrated, they were not evaporated to dryness and reconstituted before analysis. It is possible that the longer methanol extraction with full evaporation could have inactivated bio-active compounds present in the radish root tissue samples, allowing the ELISA analysis to be successful where our samples were not readable. This, along with the lack of reports of BMAA analysis of radish tissues indicate the need for further research to determine the differences between the extraction methods in different tissue types for MC analysis, and if the BMAA analysis would be successful for radish roots with a different extraction method.

Overall, averaged across all four treatments, microcystins were detected in lettuce plants at 7.16 ng g^{-1} DW in the leaves, 5.65 ng g^{-1} DW in the stems, and 3.59 ng g^{-1} DW in the roots. For the radishes in this study, the overall average microcystin concentrations were 10.39 ng g^{-1} DW in the leaves and 3.83 ng g^{-1} in the taproots, the taproot being the part of the plant that is normally consumed. Assuming a serving size of 80g for adults and 40g for children (Lee et al. 2017b), one serving of lettuce leaves would contain 20.4 ng of MCs for an adult and 10.2 ng for a child. Using the recommended daily intake level of $0.04 \text{ } \mu\text{g}$ per kg of bodyweight (WHO 1999), these amounts would equal to only 0.9% and 1.0% of the daily intake limits for adults and children, respectively. Assuming a serving size of 15 g for adults and 7.5 g for children (Lee et al. 2017b), one serving of radish taproots would contain 3.07 ng of MCs for an adult and 1.53 ng

for a child, which represent less than 1% of the total daily intake recommended by the World Health Organization (WHO 1999). These results are not of immediate acute health concern, and far lower than previous reported MCs concentrations in lettuce and radishes. However, it has been demonstrated that long term, chronic exposure to lower concentrations of MCs is also linked with negative health effects (Chen et al. 2009; Li et al. 2011; Zhao et al. 2020). In addition, it was expected that the plants in the present study would have relatively low final toxin concentrations as they were exposed to much lower concentrations of MCs than in previous reports. This study demonstrates that accumulation of MCs can occur in plants exposed to low levels of toxins that could occur in many lakes considered to be ‘clean’, not just highly impacted hypereutrophic systems, and that plants grown in these systems could function as a low-level dietary source of consistent cyanotoxin exposure.

There are several studies that reported the accumulation of MCs in lettuce after exposure via a wide variety of growth conditions and toxin concentrations (Codd et al. 1999; Crush et al. 2008; Mohamed and Al Shehri 2009; Wang et al. 2011; Bittencourt-Oliveira et al. 2016; Cordeiro-Araújo et al. 2016; Lee et al. 2017b; Levizou et al. 2017; Cao et al. 2018, 2019; Chia et al. 2019), but only a few used known natural surface waters as their source of cyanotoxins as we did for this research (Codd et al. 1999; Crush et al. 2008; Levizou et al. 2017; Cao et al. 2018), and all of these reported much higher final toxin concentrations than what was observed in the present study. Of the studies that utilized natural surface waters, reported exposure concentrations and durations were much higher than the present study, and none utilized hydroponic systems. For radishes, only two studies have reported MCs concentrations; one of which measured field-grown radishes grown with an unidentified toxin exposure concentration and duration (Mohamed and Al Shehri 2009), and the other measured radishes grown in soil

under laboratory conditions with a much higher exposure concentration and for a longer period of time (Table 17) (Levizou et al. 2020).

A direct comparison of lettuce and radish MCs concentrations between previous reports and the results of this study is therefore difficult due to the variation in growth conditions, length of toxin exposure, cyanotoxin sources, cyanotoxin exposure concentrations, and analysis methods used. However, in an effort to compare our results to previous reports, the average wet weight to dry weight ratios of the lettuce and radish tissues from our results were used to convert fresh weights found in the literature to dry weight concentrations (Table 17). Overall, these studies found MCs concentrations many times higher than what was observed in this study, likely due at least in part to the much higher exposure concentrations and timing used. Also, their plants were grown in soil systems instead of hydroponically, thus exposing them to very different growth conditions than in this study. There are no reports that directly compare MCs concentrations in hydroponically grown plants to those grown in soil systems, so we do not know the extent to which this could play a part in cyanotoxin uptake by plants, and this topic should be researched further as a possible method to reduce cyanotoxins in the food supply. Overall, the MCs concentrations in our treatment water and the final tissue samples were dramatically less than other results published so far, demonstrating that even low levels of exposure can result in MCs accumulation in produce irrigated with water containing microcystins.

Table 17. Examples from the existing literature of microcystin accumulation in lettuce and radishes after exposure to surface or ground irrigation water with natural levels of microcystins. Reported values were averaged where necessary and converted to dry weight microcystins concentrations in ng g^{-1} using the wet to dry weight ratios calculated from the samples collected in this study for comparison.

Authors	Year	Sample Type	Reported MCs Concentrations	Fresh or Dry Weight	MCs Concentrations Converted to ng g^{-1} Dry Weight
Chia et al	2019	Exposure Water	Unknown	-	-
		Lettuce Leaves	$2.41 \mu\text{g g}^{-1}$	Fresh Weight	67200.6
Codd et al	1999	Exposure Water	$3.23 \mu\text{g g}^{-1}$	DW (Scum)	-
		Lettuce Leaves	$1.155 \mu\text{g g}^{-1}$	Dry Weight	1155
Levizou et al	2017	Exposure Water	1810 ng L^{-1}	-	-
		All Lettuce Leaves	281 ng g^{-1}		7834.3
		All Lettuce Roots	409 ng g^{-1}		11847.8
		2-Leaves Stage: - Lettuce Leaves	260 ng g^{-1}	Fresh Weight	7242.3
		2-Leaves Stage: - Lettuce Roots	290 ng g^{-1}		8405.8
Levizou et al	2020	Exposure Water	3760 ng L^{-1}	-	-
		All Radish Leaves	255.2 ng g^{-1}		4399.6
		All Radish Taproots	420.2 ng g^{-1}		7868.4
		2-Leaves Stage: - Radish Leaves	226.3 ng g^{-1}	Fresh Weight	3901.7
		2-Leaves Stage: - Radish Taproots	287.7 ng g^{-1}		5387.6
Mohammed & Al Shehri	2009	Exposure Water	Unknown	-	-
		Radish Leaves: (Max Value)	$0.23 \mu\text{g g}^{-1}$		3965.5
		Radish Taproots	$1.2 \mu\text{g g}^{-1}$	Fresh Weight	22471.9
		Radish Roots	$0.36 \mu\text{g g}^{-1}$		8219.2
This Study	-	Exposure Water	78.9 ng L^{-1}	-	-
		Lettuce Leaves	7.16 ng g^{-1}		-
		Lettuce Roots	3.59 ng g^{-1}		-
		Radish Leaves	10.39 ng g^{-1}	Dry Weight	-
		Radish Taproots	3.83 ng g^{-1}		-

Despite the initial discovery of BMAA as a toxin in the food web of Guam, where the bioaccumulation of this toxin from cycads into fruit bats resulted in the high rate of ALS/PDC in the native Chamorro people there (Cox et al. 2003; Banack et al. 2010), very little research on the bioaccumulation of BMAA in other terrestrial plants has been conducted. In this study, BMAA was detected in lettuce at overall average dry weight concentrations of 7562.4 ng g⁻¹ in leaves, 4018.5 ng g⁻¹ in stems, and 551.9 ng g⁻¹ in roots (Table 18). BMAA was also detected in radishes at overall average dry weight concentrations of 4710.1 ng g⁻¹ in leaves and 3223.6 ng g⁻¹ in taproots (Table 18). To date, no studies have reported accumulation of BMAA in radishes, though Esterhuizen-Londt and Pflugmacher (2019) report detectable levels of BMAA in lettuce roots as well as scallion roots and shoots (Table 18). A few other studies have also investigated the accumulation of BMAA in wheat (Contardo-Jara et al. 2014, 2018), as well as watercress and carrots (Niyonzima 2010). As with MCs, BMAA concentrations reported for crop plants in the literature have been summarized to provide a frame of reference to the concentrations reported by the present study (Table 18).

Interestingly, BMAA concentrations reported in crop plants are much more similar to what was observed in this study compared to the dramatically higher MCs concentrations reported in the literature. In fact, Esterhuizen-Londt and Pflugmacher (2019) detected BMAA in lettuce roots using LC-MS/MS analysis at 400 ng g⁻¹ dry weight after exposing plants grown in a field to irrigation water with BMAA at a concentration of 50 µg L⁻¹ (50,000 ng L⁻¹) for 60 days, a value very similar to what was observed in the lettuce roots of the present study, despite the much higher toxin exposure concentration. In addition, the BMAA concentrations observed in the shoot and leaf samples of this study were much higher than previous studies, with the concentrations found in watercress seedlings by Niyonzima (2010) as the only exception.

Table 18. Reported BMAA concentrations in crop plants from the existing literature. All values were converted to units of ng g⁻¹ DW for comparison; Young wheat samples assumed 90% water content; mature wheat samples assumed 40% water content. * = Values not reported in text and estimated from figures. For the Contardo-Jara et al. and Niyonzima studies, only values from last day of experiment and 100 µg/L treatment were reported here.

Author & Exposure Concentration	Year	Sample Type	Free BMAA Concentration	Bound BMAA Concentration	Total BMAA Concentration (ng g ⁻¹ DW)
* Esterhuizen-Londt & Pflugmacher 50,000 ng L ⁻¹	2019	Lettuce Shoots	BDL	BDL	BDL
		Lettuce Roots	400 ng g ⁻¹ DW	BDL	400
		Scallion Shoots	600 ng g ⁻¹ DW	BDL	600
		Scallion Roots	3200 ng g ⁻¹ DW	BDL	3200
* Niyonzima 100,000 ng L ⁻¹	2010	Watercress Seedlings	100 ng g ⁻¹ DW	10000 ng g ⁻¹ DW	10100
		Carrot Seedlings	1200 ng g ⁻¹ DW	1800 ng g ⁻¹ DW	3000
Contardo-Jara et al. 100,000 ng L ⁻¹	2014	Young Wheat Shoots	BDL	100 ± 15 ng g ⁻¹ FW	1000 ± 150
		Young Wheat Roots	BDL	* 24 ng g ⁻¹ FW	* 240
Contardo-Jara et al. 100,000 ng L ⁻¹	2018	Mature Wheat Shoots	BDL	22 ± 19 ng g ⁻¹ FW	36.7 ± 31.7
		Mature Wheat Roots	BDL	25 ± 2 ng g ⁻¹ FW	41.7 ± 3.3
		Mature Wheat Grains	BDL	217 ± 150 ng g ⁻¹ FW	361.7 ± 250
This Study 350 ng L ⁻¹	2021	Lettuce Leaves	7562.4 ng g ⁻¹ DW	-	7562.4
		Lettuce Stems	4018.5 ng g ⁻¹ DW	-	4018.5
		Lettuce Roots	551.9 ng g ⁻¹ DW	-	551.9
		Radish Leaves	4710.1 ng g ⁻¹ DW	-	4710.1
		Radish Taproots	3223.6 ng g ⁻¹ DW	-	3223.6

Once again, direct comparisons between these reports and the results of the present study are difficult due to differences in reported units, analysis methods, growth medium, exposure concentrations, exposure durations, as well as plant types and ages. One major difference between the present study and previous reports is the use of hydroponics instead of a soil-based system. It is possible that BMAA could have bound to soil particles in the studies conducted by Esterhuizen-Londt and Pflugmacher (2019) and Contardo-Jara et al. (2014, 2018), which would have reduced the potential for those plants to uptake BMAA from the soil. Other differences include plant types, exposure concentrations, timing of exposure, and growth duration between studies. For example, Niyonzima (2010) exposed carrot and watercress seeds to three concentrations of BMAA for 7 days in hydroponic agar and harvested the resulting seedlings at different intervals for toxin analysis. Contardo-Jara et al. (2014) examined wheat seeds exposed to BMAA in petri dishes for only four days, but then grew these seeds in a controlled environment in pots with soil for a further 28 days as well. In a separate study Contardo-Jara et al. (2018), investigated BMAA accumulation in fully mature, 205-day old wheat plants after exposure throughout the entire growth cycle. As with their earlier study, these wheat plants were grown in pots with soil under controlled light and temperature conditions (Contardo-Jara et al. 2018). Esterhuizen-Londt and Pflugmacher (2019) analyzed 60-day old lettuce and scallion plants grown in pots with soil after exposure throughout the entire growth period in a controlled environment, laboratory setting. In this study, our lettuce and radish plants were not intentionally exposed to BMAA until they were 18 days and 10 days old, respectively, and were then exposed in a hydroponic system for only 21 days.

It is known that plants can generally absorb amino acids from soil through roots (Tegeger and Rentsch 2010; Svennerstam et al. 2011), but depending on the type of plant and the stage of

growth, uptake rates of BMAA as well as distribution of free and protein-associated BMAA is different (Esterhuizen-Londt and Pflugmacher 2019). Amino acids are used to form new proteins for growth at different rates, and BMAA has been observed to become metabolized very quickly in aquatic macrophytes (Downing et al. 2015), which could limit the total amount of detectable BMAA in a plant tissue sample. Overall, this study demonstrated the uptake of BMAA by crop plants in a system with a much lower BMAA concentration in the irrigation water than previous studies. In addition, due to the many differences between the few studies that have examined BMAA accumulation in crop plants, it is evident that further research on this subject is necessary to better understand the potential risks involved.

Differences in Cyanotoxin Accumulation Between Plant Tissue Types

Microcystins and BMAA concentrations were measured in each part of the lettuce and radish plants at the end of the lake-derived cyanotoxin exposure field study from all treatments, then averaged for an overall representation of toxin concentrations in each tissue type (Figure 20, Figure 24). The concentration of BMAA found in the lettuce leaves was significantly higher than the lettuce stems, which in turn had a higher concentration of BMAA than the lettuce roots. Microcystin accumulation in lettuce was similar, where the MCs concentration in the leaves was higher than in the roots, but in this case, the average amount of MCs was not significantly different between the leaves and the stem of the lettuce. In radishes, the average concentration of BMAA found in the leaves was higher than what was found in the taproots, but this difference was not significant. However, the concentration of MCs in the radish leaves was significantly higher than what was found in the radish taproots.

Only a few other studies reported higher MCs concentrations in leaf tissues of crop plants compared to root or shoot tissues as found in the lettuce and radishes of this study; these include

tomato plants (Gutiérrez-Praena et al. 2014), carrots (Levizou et al. 2020), and dill (Mohamed and Al Shehri 2009). In contrast, most research reports higher MCs concentrations in root tissues compared to shoot or leaf tissues for lettuce (Crush et al. 2008; Mohamed and Al Shehri 2009; Wang et al. 2011; Lee et al. 2017b; Cao et al. 2018) and radishes (Mohamed and Al Shehri 2009; Levizou et al. 2020), as well as many other crop plants (Järvenpää et al. 2007; Peuthert et al. 2007; Crush et al. 2008; Mohamed and Al Shehri 2009; Corbel et al. 2016; Lee et al. 2017b).

To date, no reports of BMAA accumulation in radishes could be found, and only one study has investigated the accumulation of BMAA in the different tissues of lettuce (Esterhuizen-Londt and Pflugmacher 2019). Like the majority of MC accumulation reports, Esterhuizen-Londt and Pflugmacher (2019) found a higher BMAA concentration in the lettuce roots than the lettuce leaves; they also found the same trend in scallions under the same experimental conditions. The only other reports to examine BMAA accumulation in different plant parts report contrasting results for the same study organism, likely due to the difference in age of the plants between the two studies. After exposure to $10 \mu\text{g L}^{-1}$ BMAA, Contardo-Jara et al. (2018) found similar concentrations of BMAA in the roots and shoots of mature (205-day old) wheat (*Triticum aestivum*), but in another study, Contardo-Jara et al. (2014) found a higher BMAA concentration in the shoots of young (28-day old) wheat plants (*Triticum aestivum* L. “Taifun”), compared to the roots exposure to $100 \mu\text{g L}^{-1}$ BMAA.

One explanation for the higher toxin concentrations in the lettuce and radish leaves found in this study could be the hydroponic system used for this research, where the others used soil-based systems. In our hydroponic system, the roots of the lettuce and radish plants were continuously exposed to the reservoir water, which could have resulted in increased transpiration rates since the plants were not water limited as occurs periodically in a soil-based system.

Transpiration is known to play a large role in the uptake and accumulation of soil pollutants in plant tissues (Gutiérrez-Praena et al. 2014) and would have concentrated the toxins in the leaves as water evaporated out through stomatal openings at a higher rate. Exposure to aerosolized toxins could be another explanation for the higher MCs concentrations in the lettuce and radish tissues exposed to the air in comparison to the root tissues. Particulate and dissolved aerosols deposited on the surfaces of leaves could be absorbed through these surfaces as observed for other air pollutants (O'Dell et al. 1977). Previous studies that report toxin concentrations for different tissues of the same plant were primarily conducted under laboratory conditions, where aerosolized toxins would be unlikely to occur. The present study is the first to examine aerosols as a source for cyanotoxin accumulation in crop plants, and this extra source of exposure combined with the effects of higher transpiration rates could certainly result in a higher MCs concentration in the upper portions of the plants, though further research with more plant types, realistic toxin concentrations in irrigation water, as well as aerosols is necessary to better understand the role of aerosolized toxins in agricultural systems.

Potential Contribution of Cyanotoxin Aerosols to Plant Toxin Concentrations

In the early Fall of 2017, an experiment was run to investigate whether the HEPA filters had prevented cyanotoxin aerosols from entering the sealed treatment tunnels. During this second experiment, conditions were similar to those recorded in the summer of 2016 including phytoplankton community composition as well as toxin and phycocyanin concentrations (Table 19). The small differences observed in phycocyanin and toxin concentrations between the two experiments can be explained by seasonal timing, which affected the composition and quality of the phytoplankton present. The second experiment was run later in the growing season at the time of year in New England when cyanobacteria populations generally start to decline, which

explains the lower overall cyanobacteria composition and phycocyanin levels and could affect toxin generation rates. As conditions in Lake Attitash were similar in 2016 and 2017, we can infer that aerosol levels were similar as well.

Table 19. Differences in the average phycocyanin, microcystins, and BMAA concentrations and the phytoplankton community in Lake Attitash during the initial lake cyanotoxin exposure field experiment conducted in the summer of 2016 and the aerosol exclusion experiment run in the early fall of 2017.

	2016	2017
Phycocyanin ($\mu\text{g L}^{-1}$)	30.7	9.5
Microcystins (ng L^{-1})	78.9	18.4
BMAA (ng L^{-1})	350.0	600.4
Phytoplankton Composition:	-	-
Cyanobacteria (% Overall)	72.3	66.6
Lyngbya (% of Cyanos)	63.5	54.3
Dolichospermum (% of Cyanos)	17.1	44.6

Filter and liquid trap samples collected using the CLAMs indicated that both particulate and dissolved cyanotoxins were present as aerosols in the HEPA sealed treatment tunnel (Table 16). Interestingly, the MCs aerosol concentrations from both the filter and trap samples were slightly higher (not significant, one-way ANOVA, $p = 0.28$, and $p = 0.12$) in the filtered tunnel than the open tunnel. This small increase could be due to the fact that the fans of the HEPA filters were constantly forcing new air through the sealed tunnel, and if the HEPA filters were not effectively removing the particulate or dissolved aerosols, this would essentially have acted as a constant supply of fresh aerosols from off the lake, even during very calm times of the day with very little airflow in the open-ended tunnel. BMAA aerosols were not collected in the filtered tunnel, but like MCs, both particulate and dissolved were detected in the open tunnel. Because of this and the detection of both particulate and dissolved MCs aerosols in the HEPA filtered tunnel, it is expected that BMAA aerosols also entered the filtered tunnel. Overall, the

use of the CLAMs in the open and sealed tunnel indicated that aerosolized cyanotoxins did not differ significantly between the two tunnels, and that neither dissolved nor particulate cyanotoxin aerosols were removed by the of HEPA filtration system used.

The presence of aerosols in the sealed and filtered treatment tunnel during the 2017 experiment indicated that plants in the four treatment tunnels of the 2016 plant experiment were all exposed to similar levels of cyanotoxin aerosols. This explains why the concentrations of MCs in the lettuce leaves and stems from the filtered tunnel with tap water reservoirs (control) did not differ from those grown in the open tunnel with tap water reservoirs (two-way ANOVA, $n = 36$, $p = 0.068$). Further, despite having lake water as an additional source of MCs, the final concentrations of MCs in the lettuce leaves and stems from the two lake water treatments did not differ from those in the tap water treatments. This indicates that exposure to aerosols was likely the main source for cyanotoxin accumulation for these tissues. Additionally, lettuce roots had a lower overall average MCs concentration than the leaves and stems, and in contrast to the lettuce leaf and stem samples, lettuce roots from the lake water treatments did have significantly higher MCs concentrations than the tap water treatments (one-way ANOVA, $n = 12$, $p = 0.019$). For the root tissues, the reservoir water was the only direct route of toxin exposure because they were submerged below the lids of the reservoirs and not exposed to aerosols. This lack of exposure to aerosols could explain why the toxin concentration in the roots was lower than the leaves and stems as well as why the roots from lake water treatments had a lower MCs concentration than roots from the tap water treatments.

Radish taproots displayed a similar pattern to the lettuce leaves and stems with no significant differences in MCs concentrations between the four treatments overall (one-way ANOVA, $n = 12$, $p = 0.074$) or the two tunnel types (two-way ANOVA, $n = 12$, $p = 0.97$).

Interestingly, MCs concentrations were significantly higher in radish taproots from the tap water treatments than those from the lake water treatments. Though seemingly counterintuitive, it is possible that the higher concentration of MCs in the lake water could have stimulated faster enzymatic metabolism (Pflugmacher et al. 1998) and subsequent storage of the then-modified toxin molecules in vacuoles (Cao et al. 2019) of the toxin in the taproot tissues in these treatments. In addition to the higher concentration of MCs observed in the taproots from tap water treatments, radish leaves from the open tunnel/tap water treatment had significantly higher MCs concentration than leaves from the other three treatments. This could be further evidence of the importance of aerosols in this system, as the MCs concentration in these leaves was higher than the other treatments, despite the lack of MCs in the hydroponic reservoirs of this treatment. Overall, the presence of cyanotoxin aerosols in the tunnels fitted with the HEPA filters and the lack of differences in MCs concentrations between the tap water and lake water treatments indicate that air-borne cyanotoxins, the majority of which are in the dissolved fraction, may contribute to the contamination of leafy crops such as lettuce and radishes. These results highlight the risk for the accumulation of cyanotoxins in crops grown in close proximity to surface waters from aerosols alone, not just as a result of direct irrigation with cyanotoxin-containing water.

Implications of Lake-derived Cyanotoxin Transference to Terrestrial Plants

Overall, the average concentrations of both BMAA and MCs in the plant tissues of this research were relatively low compared to previously reported concentrations. A single adult-size serving of lettuce leaves from this research would contain 20.4 ng of MC, and a single serving of radishes would contain 3.07 ng; these values represent only 0.9% and 0.1% of the recommended adult total daily intake level of 0.04 µg MC per kg of body weight set by the WHO. The amount

of toxin found in our plant samples was low in comparison to this acute exposure limit, but this limit reflects the point at which a person would start to experience immediate health effects from short term exposure to a high concentration of microcystins; it does not reflect the risk of long term exposure to low levels of MCs. Though a similar acute intake limit has not been established for BMAA, it has been demonstrated that exposure to both these toxins at lower levels and over longer periods of time is linked to negative health effects including tumor promotion, liver damage, non-alcoholic liver disease (Nishiwaki-Matsushima et al. 1991; Humpage and Falconer 1999; Chen et al. 2009; Zhang et al. 2015), and neurodegenerative diseases (Cox et al. 2009; Banack et al. 2014, 2015; Regueiro et al. 2017).

Because of the risks associated with long term, low level exposure to both MCs and BMAA, it is crucial to understand the routes through which these toxins can transfer to and accumulate outside of their initial lake ecosystems. One surprising aspect of this research was the detection of both MCs and BMAA in the lettuce and radish seeds. These toxin levels only represented a tiny fraction of what was measured in the plants at the end of our experiment, but the fact that they were present in these seeds suggests the potential for trans-generational effects after exposure to MCs and BMAA. Cyanotoxins originating from lakes and deposited in seeds can then spread to new locations where these seeds grow into new plants or are consumed directly by humans or wildlife. Many food products are seeds themselves and make up a large proportion of diets worldwide such as corn, rice, wheat, and other grains. In addition, many other commonly consumed foods are young seedlings that could retain the toxins from the original seed such as baby lettuce, alfalfa sprouts, or mung bean sprouts. The MCs and BMAA concentrations detected in lettuce and radish seeds, seedlings, and mature plants in this research add to a growing understanding of the many ways through which cyanotoxins can enter our food

supply. This is especially relevant as both BMAA and MCs have been detected in many different types of plants, and they have also been detected in many other consumables including drinking water, fish, mollusks, crustaceans, and even quail eggs and chickens (Lahti et al. 2001; Ibelings and Chorus 2007; Chen et al. 2009; Mohamed and Al Shehri 2009; Brand et al. 2010; Yen et al. 2011; Mulvenna et al. 2012; Al-Sammak et al. 2014; Banack et al. 2014; Andersson et al. 2018; Kim and Rydberg 2020). It is apparent that these toxins are entering our food supply system from many different sources, and though the concentration of toxins in one source may not be very high, as was seen in this research, the overall combined exposure could be potentially much higher when all sources are considered. This is especially true for individuals or wildlife that live near surface waters with substantial toxigenic cyanobacteria populations, as the inhalation of aerosolized cyanotoxins is an additional potential route of exposure in these locations.

In addition, since both BMAA and MCs often co-occur with other environmental toxins, including other cyanotoxins (Metcalf et al. 2008; Faassen et al. 2009; Yen et al. 2011; Al-Sammak et al. 2014), there is the potential for synergistic effects of exposure to multiple toxins at once. For example, (Lobner et al. 2007) showed that BMAA levels as low as 10 μM can cause further damage to neurons if these cells were previously damaged by other toxic agents, where BMAA alone did not cause damage unless at a concentration of 1 mM or higher. Rush et al. (2012) also found that low levels of BMAA and methylmercury together caused both neurotoxicity and reduced levels of the antioxidant glutathione where little or no effect was observed with these toxins individually. Lastly, the simultaneous exposure to MCs and linear alkybenzene sulfate (LAS), a common surfactant used in cleaning products, resulted in the enhanced accumulation of this cyanotoxin in lettuce seedlings (Wang et al. 2011). This is

particularly relevant to eutrophic systems as they are more likely to experience LAS pollution, and LAS has been found to increase the risk of toxic *Microcystis aeruginosa* blooms by improving both *M. aeruginosa* growth and microcystins production (Wang et al. 2015). This indicates that irrigation water from systems with LAS pollution could cause higher MCs accumulation rates in crops, which could further increase the amount of toxins being transported from lake ecosystems into terrestrial systems. Overall, the fact that the concurrent exposure of lake-derived cyanotoxins with other environmental toxins can have an even more detrimental effect on exposed plants and animals further exemplifies the need to understand the many routes of exposure to these toxins on a day-to-day basis; even if the levels present in each source are low, their combined concentrations could be damaging.

Two key findings from this research that highlight importance of understanding how cyanotoxins travel from water bodies out into the surrounding environment include the detection of cyanotoxin aerosols emitted from a lake with relatively low toxin concentrations, and transference of these aerosolized toxins into nearby lettuce and radish plants. Previous research investigating cyanotoxins in crops has focused so far on the application of irrigation water containing these toxins to the plants as the only source of cyanotoxin exposure. In this study, aerosolized BMAA and MCs were detected and quantified, and we observed that these aerosols likely contributed to the overall accumulation of toxins in the lettuce and radish plants. We determined from the 2017 experiment that all treatments were equally exposed to aerosolized toxins, and it was because of this that we determined their importance in this system. Despite the presence of MCs in the lake water treatment reservoirs as an additional source of toxins in these treatments, the lettuce leaves and stems from the lake water treatments did not have higher MCs concentrations than those from the tap water treatments. This indicated that aerosols were likely

a major source of cyanotoxin for the tissues that were exposed to the air. The only tissue where MCs concentrations were higher in samples from the lake water treatments compared to the tap water treatments was the lettuce roots, which were not exposed to aerosolized toxins, were in direct contact with the lake water in the reservoirs, and represented only a small fraction of the overall biomass of the lettuce plants. Unfortunately, a similar trend could not be observed for BMAA due to the presence of BMAA in the tap water treatments at an equivalent concentration to the lake water treatments.

This accumulation of aerosolized cyanotoxins generated from a lake with moderate to low levels of cyanotoxins indicates the need for further research into the significance of aerosols as a potential source of cyanotoxins in food crops as well as the potential impacts of these toxic aerosols emitted from lakes on the health of humans and surrounding ecosystems. Some research has been conducted on cyanobacteria aerosols in recent years, but the processes behind the generation of cyanobacteria aerosols is not yet well understood. For example, some studies have quantified particulate MCs aerosols (Cheng et al. 2007; Backer et al. 2008; Wood and Dietrich 2011; Murby and Haney 2016; Langley 2019) and detected BMAA aerosols generated by freshwater lakes (Banack et al. 2015), and others have found relationships between lakes with cyanobacteria blooms or poor water quality and diseases such as ALS (Caller et al. 2009) and non-alcoholic liver disease (Zhang et al. 2015). More recently, research has been done on the factors that regulate emission of particulate cyanotoxin aerosols as cells from lakes in New Hampshire (Langley 2019). In addition, Carter and Haney (2020) found that approximately 85% of the total collected BMAA and MCs aerosols from Lower Mill Pond in Brewster, MA were in the soluble form ($< 0.3\mu\text{m}$); a proportion that is similar to what was observed in this study for dissolved MCs aerosols (79.7%), but much higher than what we observed for dissolved BMAA

aerosols (53.6%). This in particular highlights the importance of the soluble fraction of aerosols that have not been considered in previous research. These studies begin to bring light to the topic of cyanobacteria aerosols, and the fact that the dissolved portion of these aerosols could be a more important factor than the particulate toxins, however there are many aspects that we have yet to understand.

For example, as cyanobacteria aerosols have the potential to be a significant source of toxins in agricultural crops, it is crucial to understand how far they can travel from the lake they are emitted from; something that likely depends on prevailing wind speed and direction, as well as how long cyanotoxin aerosols can persist in the environment before breaking down. It has been observed that other aerosolized organisms and materials can travel long distances before they are deposited via gravitational settling (Dueker et al. 2012), but as of yet no research has focused on how far cyanotoxin aerosols can travel from their original environment. It is crucial to understand how far cyanotoxin aerosols can potentially travel in order to identify agricultural areas that would at risk of exposure to them.

Our results indicate that this aerosol pathway could be a significant source of cyanotoxin exposure to farm fields and natural areas in systems with low or moderate cyanobacteria populations, something that has not been considered previously. Aerosols could be an even more important factor in agricultural areas that rely on small enriched irrigation ponds, as well as those that lie on the margins of large bodies of water with chronic cyanobacteria blooms such as the Imperial Valley in California (Carmichael and Li 2006) and the southern Laurentian Great Lakes areas in Wisconsin, Michigan, and Ohio (Miller et al. 2017) where crops are grown in close proximity to their surface water sources. In these cases, the risk of exposure to cyanotoxins from lakes is twofold; from the lake water itself as irrigation water, and from the aerosols emitted

from the lake. Surface waters accounted for 52% of total crop irrigation withdrawals in the US in 2015 (Dieter et al. 2018), with many fields adjacent to their irrigation sources. In Europe, surface water is also a major source for agricultural irrigation, ranging from 85% in Greece to about 70% in Spain, Italy, and France, and 26% in France (Lee et al. 2017a). As surface waters play such a key role in food production systems worldwide, it is essential to further understand the routes of cyanotoxin accumulation in crops exposed to irrigation water and aerosols derived from these water bodies.

Many farming-intensive areas around the world are located on eutrophic bodies of water that have chronic cyanobacteria blooms. These areas use the lake water containing cyanobacteria to irrigate their crops, but even if it were possible to find an alternate source of irrigation water, it would not be possible to prevent exposure to aerosolized cyanotoxins if the fields are within the fallout area of the lake. In the past, eutrophic water bodies with cyanobacteria blooms were considered isolated, self-contained problems that only became an issue when humans, livestock, or wildlife entered the lake margin. It is now becoming apparent that the cyanobacteria toxins generated within lakes can be transported out into the environment and other systems both through the water itself as irrigation water and as aerosolized toxins, and this systemic environmental connectivity between lakes and their surroundings must be recognized moving forward.

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