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Detection of Microcystin Aerosol Particles

Alexis D. Dabney

Detection of Microcystin Aerosol Particles

By

Alexis D. Dabney

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Accepted by:

Geoffrey I. Scott, Director of Thesis

John Ferry, Reader

Eric Vejerano, Reader

Tracey L. Weldon, Vice Provost and Dean of the Graduate School

ABSTRACT

Harmful Algal Blooms (HABs) are a growing global concern due to their increased prevalence and magnitude, frequency, and duration in affecting both aquatic ecosystems and human health (Brooks et al. 2015). In a series of experiments, lab-generated aerosol particles containing Microcystis and Microseira were captured using filters to analyze for concentrations of Microcystin, Saxitoxin, and Lyngbya toxins. The solutions were found to contain aerosols but did not contain detectable levels of Microcystin, Saxitoxin, and Lyngbya toxins following analysis by HPLC-MS. While the techniques for generating aerosols were shown to be effective, the generation and analysis of toxins did not produce the expected results. The lack of toxin detection likely resulted from the lower level of toxin in the algae and/or the length of time air samples were extracted. Future research is proposed to consist of experiments in the lab with increased exposure times and in the field with naturally generated aerosols during periods of major bloom events.

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CHAPTER 1: INTRODUCTION

1.1 Harmful Algal Blooms

Harmful Algal Blooms (HABs) are a growing global concern due to their increased prevalence and magnitude, frequency, and duration (Brooks et al. 2015). While HABs in both salt water and freshwater are detrimental to environmental health, the growing impacts of freshwater HABs appear to acutely threaten environmental quality more significantly than conventional chemical contaminants of concern (Brooks et al. 2015). Unlike marine HABs which are primarily a public health threat in seafood and contact recreation, freshwater HABs are mainly a concern in contact recreation and drinking water and airborne exposure to toxins in people residing along lakes and streams.

A particular class of HABs causing significant ecosystem health impacts to aquatic life, wildlife and pets, as well as human public health effects in freshwater is cyanobacteria, otherwise known as blue-green algae. Blooms of cyanobacteria are known to produce a host of problems related to water quality including lower dissolved oxygen concentrations, reduced aesthetics, limited recreational use, taste and odor issues, and human, aquatic life and animal health impacts on livestock and wildlife due to toxin production (Brooks et al. 2015). Cyanotoxins, the toxins produced by cyanobacteria, are composed of a variety of different compounds that are detrimental to humans and wildlife

(Murby and Haney 2015). Due to the environmental health risks and the increasing prevalence of these HAB events, there has been a subsequent increase in awareness of cyanobacterial blooms and the risks posed by the blooms and the toxins they produce (Murby and Haney 2015).

A particular genus associated with cyanobacterial HABs is *Microcystis* and several species within this genus including *Microcystis aeruginosa.* *Microcystis* is a non-nitrogen-fixing cyanobacteria with the ability to form large colonies (World Health Organization (WHO), 1999). These colonies are distributed non-homogeneously throughout the water column due to the unique adaptation of buoyancy regulation (WHO, 1999). With this ability, *Microcystis* cells can find optimal light conditions to promote growth, making them less sensitive to high light intensities (WHO 1999). When colonies are present at the top of the water column, wind may often stir surface waters, causing multiple colonies to aggregate and form stable scum (Figures 1.1 and 1.2) (WHO 1999). The danger of *Microcystis* blooms and the formation of scums lies in the production of the cyanotoxin microcystin. *Microcystis* produces variations of this toxin, linking the cyanobacteria to hepatotoxic blooms worldwide (WHO 1999). Microcystin is a hepatotoxin, specifically a cyclic heptapeptide (Walsh et al. 2008). The cyanotoxin has been found in multiple types of cyanobacteria, including Anabaena, Microcystis, Oscillatoria, Nostoc, and Anabaenopsis (WHO 1999). More than 90 molecular variants of microcystin toxin have been identified, with the most common variant being Microcystin-LR (Cheng et al. 2007). A concerning quality of microcystin is its stability, having an environmental half-life of 10 weeks in aquatic ecosystems. However, though certain factors may increase the rate of breakdown including, high temperature (> 40°C) and

extremely low or high pH (< 1 or > 9) (Anadón et al. 2015). Due to the widespread presence of microcystin, the toxin is one of the most well-studied of those produced by cyanobacteria (Murby and Haney 2015). In a study conducted on the reports gathered through the Harmful Algal Bloom-related Illness Surveillance System (HABISS), microcystin was the most common toxin identified in state-reported samples that were tested for toxins, as it was identified in 82% of freshwater samples and 80% of total samples tested (Figure 1.3 and Table 1.1) (Backer et al. 2015). Microcystin is also one of the most frequently identified cyanotoxins in human and livestock cyanobacteria poisonings, with the most common variant being Microcystin-LR (Cheng et al. 2007).

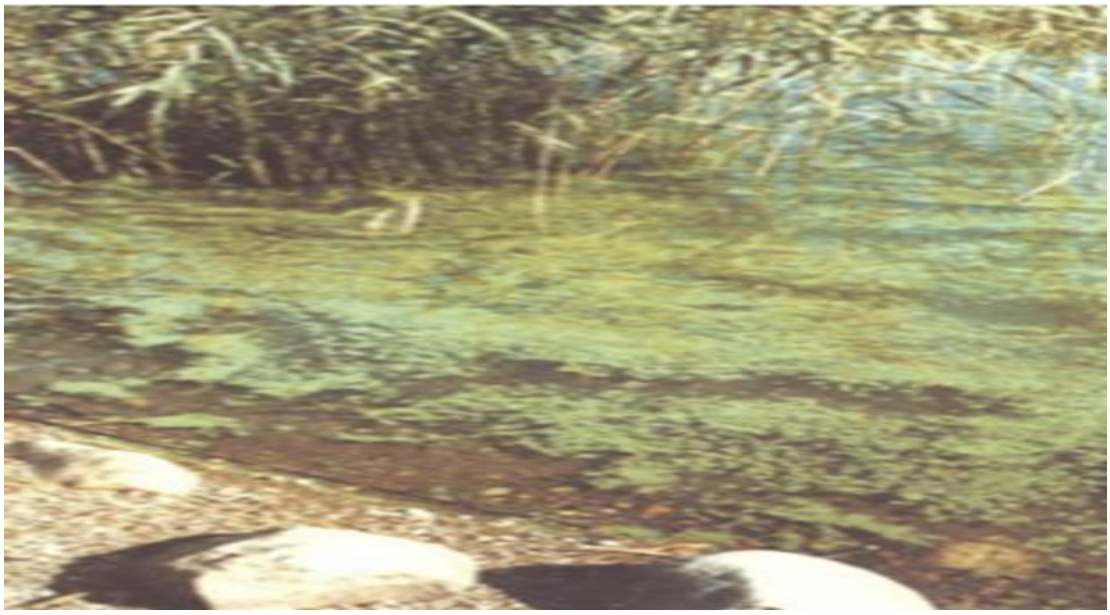

Figure 1.1 A bloom of Microcystis sp. on the surface of a body of freshwater (WHO 1999)

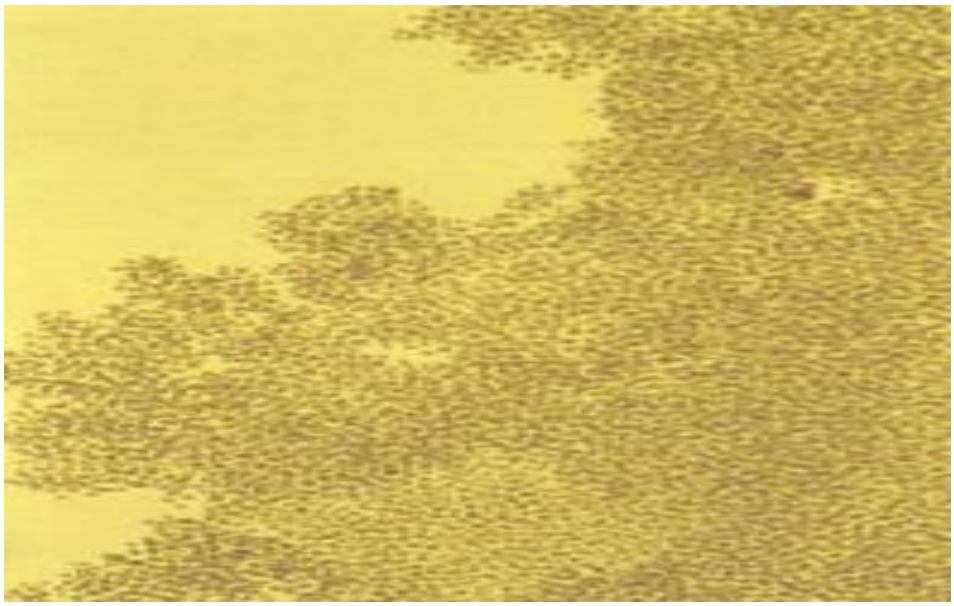

Figure 1.2 Micrograph of Microcystis aeruginosa (WHO 1999).

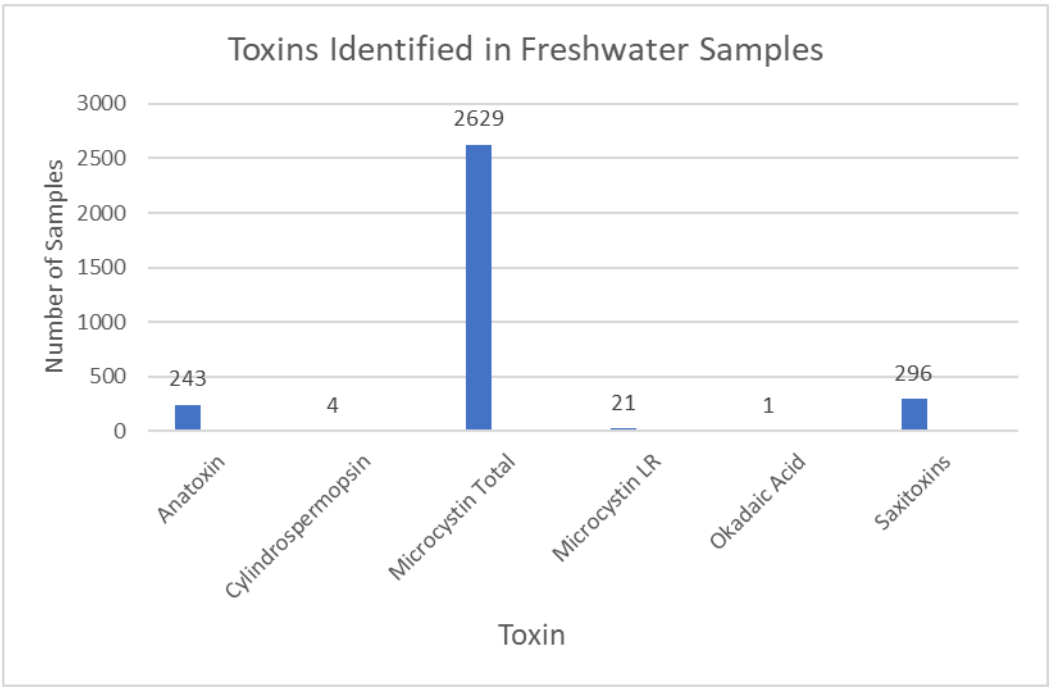

Figure 1.3 *Toxins Identified in Freshwater Samples.* Six toxins were identified in the freshwater samples reported to the HABISS by state contributors.

Table 1.1 Percentage of each cyanobacterial toxin identified in state-reported samples taken from fresh, brackish, marine, and unknown water bodies as reported by CDC in HABISS (Backer et al. 2015).

Toxin	Water Type				
	Fresh	Brackish	Marine	Unknown	Total (%)
Anatoxin	243	2	0	1	246 (7)
Azaspiracid	0	0	1	0	1 (<1)
Brevetoxoins	0	3	0	0	3 (<1)
Cylindrospermopsin	4	0	0	0	4 (<1)
Domoic Acid	0	0	31	0	31 (1)
Karlotoxins	0	3	1	0	4 (<1)
Microcytins Total	2629	35	2	10	2676 (81)
Microcytsin LR	21	0	0	0	21 (1)
Okadaic Acid	1	2	0	0	3 (<1)
Saxitoxins	296	1	11	3	311 (9)
Unidentified Toxin	0	1	0	0	1 (<1)
Total	3194	47	46	14	3301

1.2 Toxicity of Microcystin Toxins

The toxicity of microcystin varies between the different molecular forms of the toxin. Depending on the variant of microcystin, the LD50 (by intraperitoneal injection in mice) can range from 25 to < 100,000 μg/kg of body weight (Walsh et al. 2008). Microcystin-LR is not only the most common variant of microcystin identified in reported blooms but was also the most toxic to rodents (Walsh et al. 2008). Wang et al. (2020) have reported that LF, LY, & LA isomers are more toxic than the LR form to the human ovary, as LF, LY and LA isomers were endocrine disruptors to the human ovary, adversely affecting ovarian hormone secretion, follicle ovulation and development, and oocyte development. This high toxicity is partially due to the level of hydrophobicity caused by the molecule containing both an L-leucine and an L-arginine in the two L-form amino acid positions (Walsh et al. 2008). Another structural factor key to the elevated toxicity of Microcystin-LR is the ADDA portion of the molecule, with just a

stereochemical change in the side chain greatly reducing toxicity (Figure 1.4 and Table 1.2) (Walsh et al. 2008).

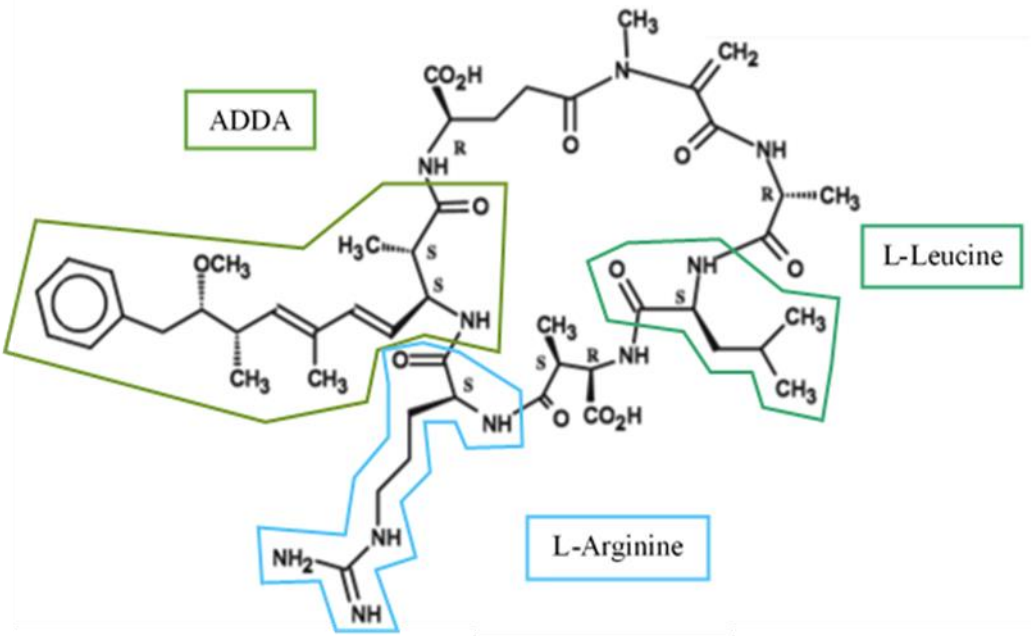

Figure 1.4 A molecule of Microcystin-LR with the three groups key to toxicity. L-Leucine (teal) and L-Arginine (light blue) give the toxin its high hydrophobicity. ADDA (green) is the key structure to its high toxicity.

Table 1.2 Eight variants of microcystin with molecular weights, toxicity, and organisms by which they are produced. Toxicity is based on intraperitoneal injections in mice. Adapted from WHO 1999.

Microcystin Variant	**Molecular Weight**	**Toxicity LD50 (µg/kg)**	**Organism**
MCYST-LA	909	50	*M. aeruginosa, M. viridis*
MCYST-AR	952	250	*Microcystis sp.*
MCYST-LR	994	50	*M. aeruginosa, A. flos-aquae, M. viridis*
MCYST-LY	1,001	90	*M. aeruginosa*
MCYST-FR	1,028	250	*Microcystis sp.*
MCYST-RR	1,037	600	*M. aeruginosa, M. viridis, Anabaena sp.*
MCYST-YR	1,044	70	*M. aeruginosa, M. viridis*
MCYST-WR	1,067	150-200	*Microcystis sp.*

Current guidelines vary for the limit of Microcystin-LR in drinking water. The World Health Organization (WHO) and Health Canada recommend a maximum concentration of 1 μg/L, with a maximum concentration of < 20 μg/L for recreational use (Carmichael and Boyer 2016). The United States Environmental Protection Agency (USEPA) has established health advisory guidelines for drinking water of 0.3 μg/L for children 6 years old or younger and 1.6 μg/L for anyone older than 6 years of age for drinking water (Carmichael and Boyer 2016), with an EPA advisory level of 9 ug/L for contact recreation (EPA). Recreational advisory levels may also vary from state to state.

As a hepatotoxin, microcystin appears to target the liver in humans and animals. However, the toxin has been found in the small intestine and kidneys after oral exposure and subsequent absorption by the ileum and nephrons, respectively (Peterson and Talcott 2013). Microcystin works by inhibiting protein phosphatase enzymes in liver cells, particularly serine-threonine protein phosphatases 1 and 2A (Walsh et al. 2008). During normal function, these enzymes remove phosphate from hydroxyl groups of serine and threonine, two amino acids that are constituents of specific proteins, in what is usually a reversible process (Walsh et al. 2008). When microcystin binds to specific biochemical sites within these protein phosphatase enzymes, the removal of phosphate groups from the amino acids can no longer take place, and the proteins targeted by this process will accumulate an excess of phosphate (Walsh et al. 2008). Due to the unique nature of the serine-threonine protein phosphatases 1 and 2A, microcystin can disrupt certain cytoskeletal components and an accompanied rearrangement of filamentous actin (Peterson and Talcott 2013). This means that hepatocytes deform when exposed to microcystin, with the structure of the liver being destroyed (Walsh et al. 2008), which

may lead to liver fibrosis. At lower doses, microcystin has been shown to cause a slow progression of liver and kidney failure, while higher doses have led to intrahepatic hemorrhages, liver necrosis, and shock (Peterson and Talcott 2013). It should be noted, however, that the effects of microcystin are not limited to death by liver failure or shock (Walsh et al. 2008). Suptapa et al (2019, 2020) and Albadrani et al (2019,2020) have shown that the LR form causes significant inflammation in the liver and kidney, leading to liver fibrosis, alterations in the gut microbiome and inflammation of the blood-brain axis. In addition, exposure to weanling mice at contact recreational standard levels (9 ug/L) resulted in the liver's priming for Non-Alcoholic Liver Disease development later in life (Suptapa et al., 2020) and may lead to liver cancer. Epidemiology studies from China found a significant correlation between blood levels of microcystin and hepatocellular carcinoma (Zheng et al. 2017).

Microcystin, specifically Microcystin-LR, has also been shown to act as a "promoter of tumor growth" and has been labeled as a "possible carcinogen" (Walsh et al. 2008). In 2002, Health Canada classified Microcystin-LR as a Group IIIB carcinogen, meaning it could be carcinogenic to humans but there was inadequate data on humans and limited evidence in experimental animals (Carmichael and Boyer 2016). A few years later, in 2006, the International Agency for Research on Cancer also labeled Microcystin-LR as "possibly carcinogenic", with solid evidence to suggest mechanistic carcinogenicity (Carmichael and Boyer 2016). Other effects of microcystin include mitochondrial alterations, free radical formation, intracellular calcium level alterations, and oxidative stress, contributing to hepatotoxicity and apoptosis (Peterson and Talcott 2013).

Microcystin poisoning is a frequent source of livestock poisoning, with *Microcystis aeruginosa* being the most common species identified (Walsh et al. 2008). The scums usually form in eutrophic, freshwater bodies in late summer and the toxin accumulates in concentrations high enough to cause microcystin poisoning (Walsh et al. 2008). In livestock, symptoms usually include small hemorrhages throughout the abdomen, which fills with a yellow fluid, and massive liver damage (Walsh et al. 2008). Liver failure is typically the cause of death within 24 hours of exposure, though it can take weeks for mortality to occur (Walsh et al. 2008).

Like animals, humans may be affected by microcystin by consuming contaminated water, a direct source, or contaminated food, an indirect source (Murby and Haney 2015). In the HABISS data collected by Backer et al. (2015), microcystin was associated with 54% of non-food-related illness in humans that were reported in association with cyanobacteria exposure. Respiratory symptoms have also been reported in humans in association with the recreational use of freshwater bodies containing blue-green algae, leading to studies of microcystin aerosols (Cheng et al. 2007). A significant concern is atmospheric aerosol levels of Microcystin. There are currently no advisory levels for atmospheric concentrations of Microcystin due to a lack of data on the topic.

1.3 Atmospheric Levels of HAB Toxins

Through a wave mechanism driven by wind, aerosols are formed when bubbles of trapped air rise to the surface of the water before bursting and forming jet and film droplets (Cheng et al. 2007). Microorganisms, including algae, that are concentrated in surface water as films or other forms can be ejected into the air by these droplets, transferring anything from salts to bacteria to algae into the air (Cheng et al. 2007). The

study of the potential aerosolization of microcystin is relatively new. However, recent findings indicate that cells may be transported from lakes as aerosols containing high concentrations of cyanotoxins, including microcystins (Murby and Haney 2015). With the inhalation of microcystin being shown to be 10 to 50 times higher than that of oral toxicity, information on microcystin and aerosol characterization is key to learning the potential for exposure to inhaled microcystin (Walsh et al. 2008).

Research conducted in 2007 on characterizing aerosols that contained microcystin used both a field study and laboratory study to determine whether microcystin could be transferred from water to air via jet/film droplets and whether the concentration of microcystin aerosols produced by these droplets could be detected (Cheng et al. 2007). The laboratory study confirmed that cyanotoxins, including microcystin, could be transferred from water to air via a bubble-bursting process, with the microcystin containing droplets showing a bimodal size distribution with a mass median aerodynamic diameter of 1.4 and 27.8 μm (Figure 1.5) (Cheng et al. 2007). The field study confirmed that very low levels of aerosolized microcystin could be detected in areas containing blue-green algae (Cheng et al. 2007). Microcystin concentrations ranged from 0.02 to 0.08 ng/m^3 in samples collected through high-volume and personal samplers (Cheng et al. 2007).

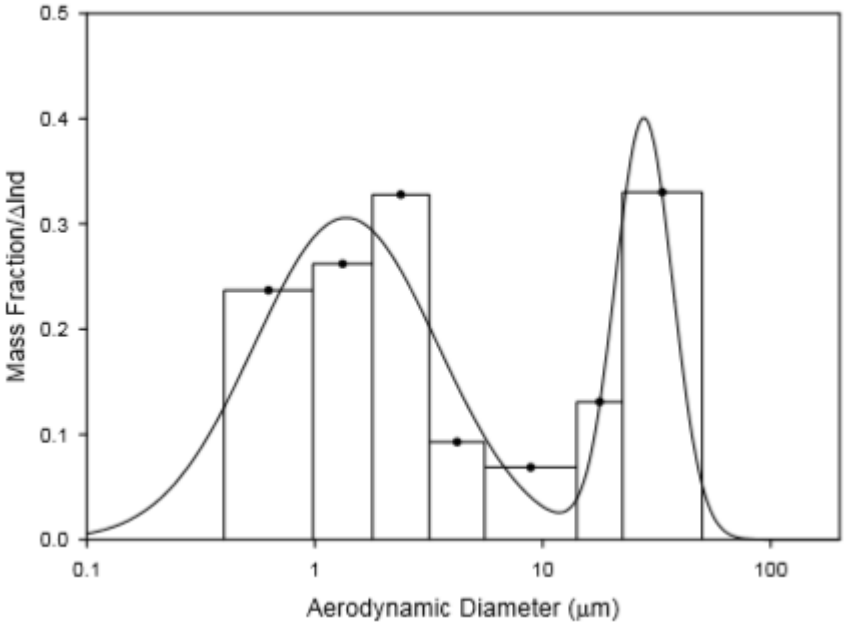

Figure 1.5 The laboratory-produced aerosolized microcystin droplet bimodal size distribution with mass median aerodynamic diameters of 1.4 and 27.8 μm (Cheng et al. 2007).

A more recent study in 2015 focused on methods in the field and laboratory that could be used to monitor cyanobacteria and microcystin aerosols (Murby and Haney 2015). The techniques used in this study were designed to estimate cyanobacteria and microcystin aerosols directly above the lake surface (Murby and Haney 2015). Aerosols in both the laboratory and field were collected through light-weight personal air monitoring pumps with filters from the field being tested for microcystin aerosols by an enzyme-linked immunosorbent assay (ELISA) to determine in situ toxicity directly above the surface water (Figures 1.6) (Murby and Haney 2015). It should be noted that the production of aerosols in this study was not enhanced by mechanical agitation other than the process of air exchange due to the vacuum pump samplers. The aerosols were most likely produced by small-scale water turbulence, evaporative processes, and proximity of potentially aerosolized cells to the water surface (Murby and Haney 2015). The data

from all samples showed microcystin concentrations ranging from <13 pg/m^3 to 384 pg/m^3 (Murby and Haney 2015). An important conclusion from this study is that these concentrations are likely to represent the surface water emissions of cells (Murby and Haney 2015). It should also be noted that the methods detailed within this study could be used for public health, with the concentrations of aerosols being used to estimate potential recreational exposure (Murby and Haney 2015).

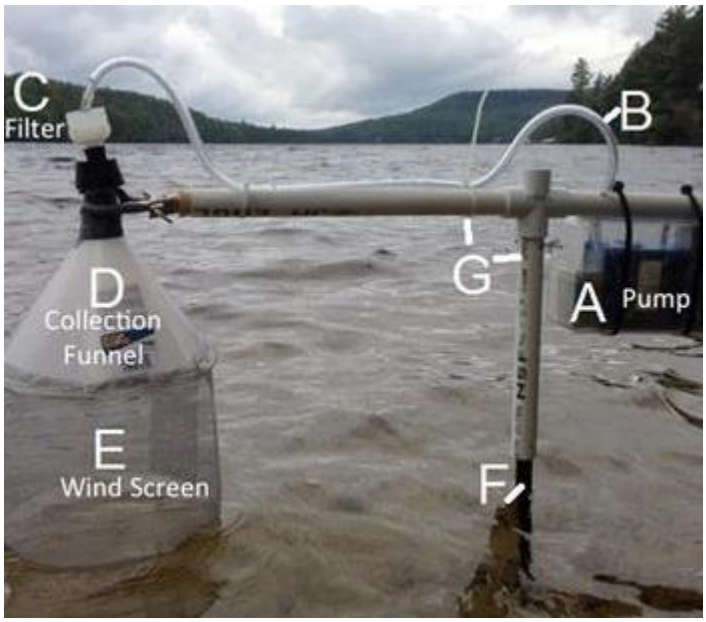

Figure 1.6 Portable device for field sampling near shorelines composed of a personal air monitoring vacuum pump connected to a collection funnel and filter via PVC pipe and anchored to the lake bottom with an iron rod.

As this review of the literature has shown, there is limited data on aerosol formation of cyanobacterial toxins and more research is clearly needed on measuring of HAB toxins in aerosols.

CHAPTER 2: STATEMENT OF PURPOSE, OBJECTIVES, AND HYPOTHESIS TESTED

2.1 Statement of Purpose

This research seeks to examine the potential for cyanobacteria to produce aerosolized toxins which may pose a significant public health threat to humans near HAB blooms.

2.2 Objectives

- Collect and analyze water samples to determine the presence of microcystin in the water bodies sampled for HABs
- Collect and analyze samples of aerosolized algae droplets to determine the existence of Microcystin within aerosol droplets
- Analyze collected data as well as data from other resources to determine a relative abundance of Microcystin and other toxins within cyanobacterial aerosols.

2.3 Hypothesis Tested

- Null Hypothesis: Microcystin levels in aerosols will be detectable using precision analytical chemistry methods at concentrations ranging from 12 pg/m^3 to 384 pg/m^3.
- Alternative Hypothesis: Microcystin will not be at detectable levels.

CHAPTER 3: MATERIALS AND METHODS

3.1 Lab Experiments with Harmful Algal Blooms

A series of experiments were conducted in the lab, using different HAB toxin samples, including Microcystis and *Microseira* (formerly Lyngbya) *wollei.* The purpose of the first preliminary experiment was to establish a method for creating aerosols within a controlled setting. A solution of tap water with a salt concentration of 50 mg/L of NaCl was used. The solution was placed in a beaker with an air pump to stir the water and create aerosols, with the setup being placed in a Lunaire environmental chamber. A vacuum pump was hooked up to an aerodynamic particle electrostatic classifier to analyze the size and concentration of created aerosols.

The second preliminary experiment was conducted but modified the method of aerosol creation. A liter of solution with an indigo trisulfonate concentration of 15 mg/L, concentration of 50 mg/L of NaCl, and chlorella cells was run through a nebulizer to create aerosols. The nebulizer was placed into a chamber with a vacuum pump hooked to an aerodynamic particle electrostatic classifier to analyze particle concentration (Figure 3.1). A second run was then completed with the vacuum pump connected to a filter holder housing a 25 mm Whatman glass fiber filter. The setup was run for 30 minutes before collecting the filter and storing it in tinfoil in a freezer to be analyzed at a later time.

Figure 3.1 Nebulizer under bell jar hooked to vacuum pump and filter to collect aerosols for later analysis.

The next aerosols were run using *Microseira* (formerly *Lyngbya) wollei* samples taken from Dr. John Ferry's lab. A freeze-dried and live sample of Microseira was allowed to sit in 1L deionized water with 50 mg NaCl/L for 30 minutes. The samples were then nebulized under a fume hood for 30 minutes with a vacuum pump connected to an aerodynamic particle electrostatic classifier. The same solutions were then nebulized for 30 minutes with a filter holder housing a 25 mm Whatman glass fiber filter.

The final set of aerosols was created using a solution of 1 L deionized water, 37 mg of freeze-dried Microcystis, and 58 mg salt (NaCl). The solution was allowed to sit for 30 minutes before being aerosolized. The solution was nebulized under a fume hood for 30 minutes with a vacuum pump connected to an aerodynamic particle electrostatic

classifier. The same solution was then nebulized for 30 minutes with a filter holder housing a 25 mm Whatman glass fiber filter.

3.2 Chemical Analysis of Harmful Algal Bloom Toxins

The filter from the indigo trisulfonate and chlorella aerosols was stored in a freezer for six days before being analyzed via UV-vis spectroscopy at 600 nm. Two different plates were run with 0.2 micron-filtered and unfiltered samples. The filters collected from both the freeze-dried and live Microseira aerosols and the Microcystis aerosols were analyzed for microcystin and saxitoxin concentrations.

CHAPTER 4: RESULTS

The aerosols from the solution containing only NaCl produced a particle concentration of 8.31 x 10^3 particles/cm^3, with a mean particle size of 71.0 nm and a mode of 51.4 nm (Figure 4.1). The aerosols from the solution containing indigo trisulfonate, NaCl, and chlorella produced a particle concentration of 2.73 x 10^5 particles/cm^3 (Figure 4.2). The aerosols from the solution containing the live Microseira had a concentration of 3.98 x 10^5 particles/cm^3 (Figure 4.3). The freeze-dried Microseira aerosols produced a concentration of 2.88 x 10^5 particles/cm^3 (Figure 4.4). The final solution of Microcystis aerosols had a concentration of 4.75 x 10^5 particles/cm^3.

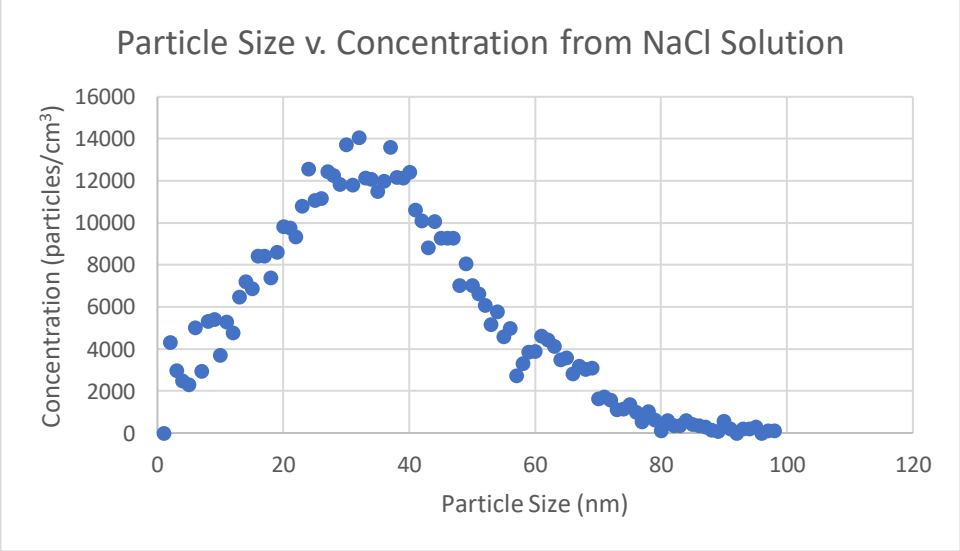

Figure 4.1 Particle size (nm) versus concentration (particles/cm^3) data from the NaCl solution aerosols collected using scanning mobility particle sizer

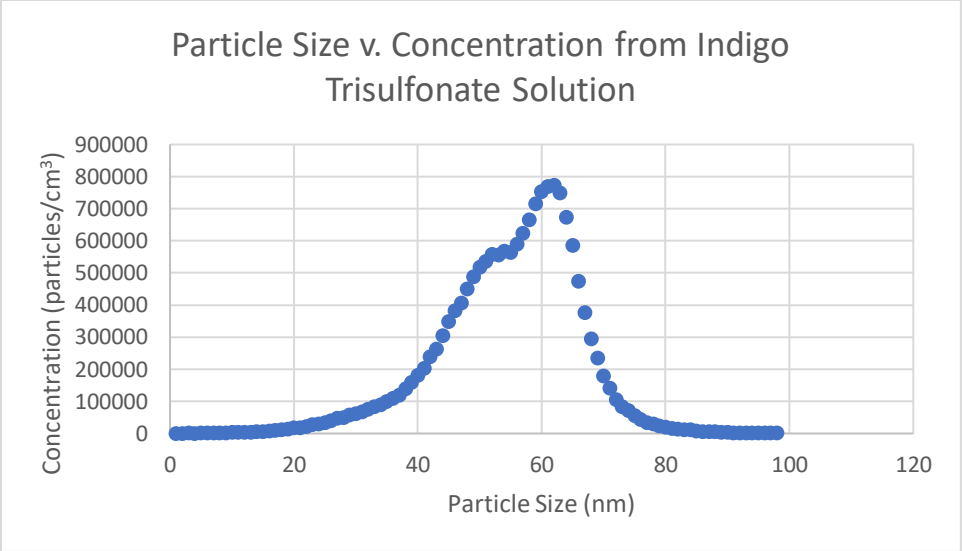

Figure 4.2 Particle size (nm) versus concentration (particles/cm^3) data from indigo trisulfonate/chlorella solution aerosols collected using the SMPS.

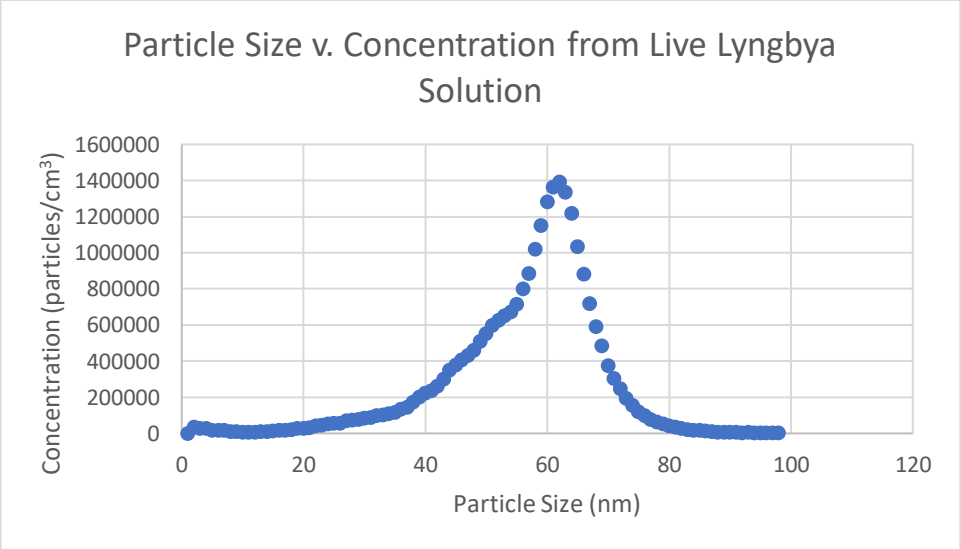

Figure 4.3 Particle size (nm) versus concentration (particles/cm^3) data from live Microseira solution aerosols collected using the SMPS.

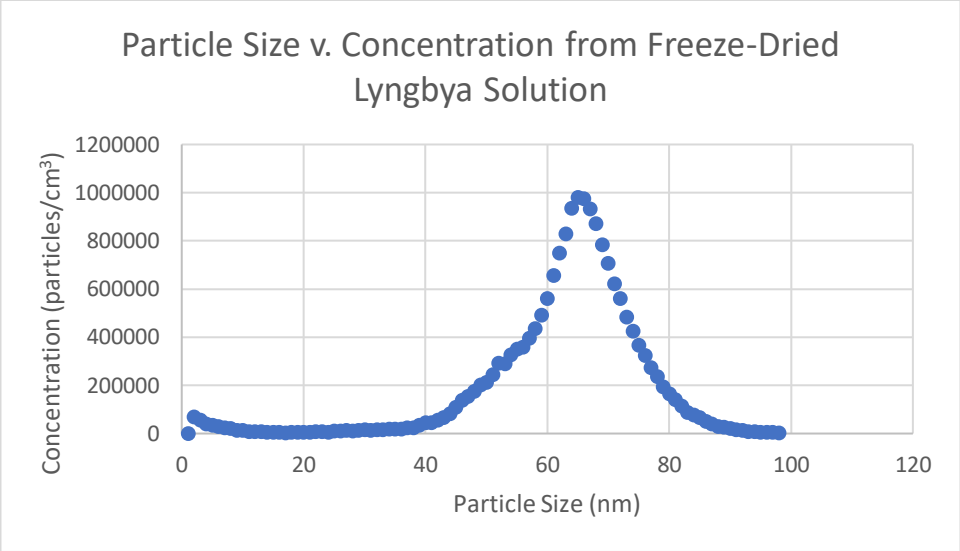

Figure 4.4 Particle size (nm) versus concentration (particles/cm^3) data from freeze-dried Microseira solution aerosols collected using SMPS.

The results from the UV-Vis spectroscopy run on the indigo trisulfonate/chlorella solution indicated that there were no detectable levels of the dye. The results from analysis of the Microseira filters and Microcystis filter indicated that there were no detectable levels of Microseira toxins (e.g., Saxitoxin) and microcystin toxins detected.

CHAPTER 5: DISCUSSION

The particle concentration for each aerosol solution was found to be 1 to 9 x 10^5 particles/cm^3, except for the first solution of NaCl. This result is likely due to the nebulizer not being used to produce the aerosols in the first experiment. NaCl was used to generate test aerosol and to serve as the benchmark for aerosol generation.

While aerosols were detected, no dye or toxins were detected in the filters used to collect aerosols using the vacuum pump. This could be due in part to the time between taking the sample and analyzing the samples, as well as the stability of the indigo trisulfonate dye and algal toxins. Another possible explanation is that toxin levels were too low to be detected. While the amounts of algae mass were measured and weighed, the actual level of toxin in the algae was unknown. This means that they could have been well below the 100 µg/L concentration of Microcystin, as used in the Cheng et al. (2007) study. An improvement on the study could be to take water samples before and after the test, to see if microcystin was present in the solution being aerosolized (Cheng et al., 2007).

The increased occurrence of HAB toxins represents a new class of air pollution that may increase population vulnerability to respiratory illnesses such as asthma and when considered with other air pollutants such as diesel particulates may increase effects such as COPD and heart disease. Airborne exposure to HAB toxins has been receiving greater attention as recent studies have correlated certain human illnesses such as

amyotrophic lateral sclerosis with cyanobacterial exposure, such as microcystin and beta methyl-aminoalanine (BMAA) in New England lakes (Tobick et al., 2014). Cyanobacteria are known to produce acute and chronic HAB toxins that have human health effects, including microcystins, cylindrospermopsins, lyngbyatoxins, anatoxins, lipopolysaccharide endotoxins and beta methyl-aminoalanine (BMAA) (Codd et al. 2005, Tobick 2014). Blood levels of microcystin have been highly correlated with increased occurrence of hepatocellular carcinoma and was synergistically increased with high levels of alcohol consumption and underlying liver disease and obesity (Zheng et al, 2017).

In 2020, Jang et al. conducted a study on the influence of sunlight, ozone, and OH radicals on the degradation of Microcystin-LR. The estimated lifetime of Microcystin-LR in the presence of sunlight and typical ambient ozone concentrations during the day was 1 hour and 41 minutes (Jang et al. 2020). This study suggests that another reason for toxins not being at detectable level is the exposure to light for an extended period of time.

During a red tide exposure associated with *Karenia brevis* in Sarasota County FL, research has shown that Brevetoxins are dissolved into aerosol particulates and may be transported > 6.4 km inland, away from bloom conditions at the coast (Backer et al. 2003). During these events hospital emergency department diagnoses increased by 19% for pneumonia, 40% for gastrointestinal illnesses, and 54% for respiratory illnesses compared to a period when the red tide was not present in the area (Cheng et al. 2005;

Hoagland et al., 2009). The costs for hospital visits associated with respiratory illness during a red tide event alone in Sarasota County alone ranged between $0.5 to $4 million (Kirkpatrick et al., 2010).

In a more recent 2018-2019 HAB event in Florida, both marine and freshwater HABs were observed lasting approximately 19 months and causing beach closures, affecting contact recreation and shellfish harvesting. These blooms have also led to an increase in exposure to airborne HAB toxins, including Brevetoxin in marine waters and Microcystin in brackish/freshwater areas (Philips et al, 2020, Rosen et al., 2018).

In 2018, Microcystis blooms were identified around Cape Coral, Florida, in the Caloosahatchee River, lasting for several months. According to the Florida Department of Environmental Protection (FLDEP), measured microcystin concentration reached 46μg/L on September 17, 2018, compared to the 20 μg/L set as the "high risk" concentration for acute health effects by the WHO. After researchers from Florida Gulf Coast University ran a series of field tests with air samplers, it was determined that low but measurable levels of cyanotoxin could be measured more than one mile inland from the bloom and, in fact were measured 25 miles away (NOAA. NCCOS, 2019). More importantly, the air sampler used had different size passages, with larger openings on the top and openings on the bottom that simulate a human lung. The largest pore sizes would be like the nasal passages and the mouth, then the pharynx, then down into the bronchi, with the last three pore sizes simulating the alveoli. Microcystins were measured in all the size fractions indicating the potential for exposure deep into the lung. FGCU researchers also noted a compound produced by cyanobacteria, beta-Methylamino-L-

alanine (BMAA) was also present in their air samples, which has been linked to some degenerative neurological diseases.

As this discussion has shown, certain HAB toxins can be aerosolized, absorbed onto particulates and transported distances away from the bloom location. Schaffer et al (2020) measured nasal swabs in FL residents for microcystin. Results indicated that 95% of the 121 participants who provided nasal swabs had microcystin concentrations above the lower detection limit with a mean concentration of 0.61 ppb (+/- 0.75 ppb). There were significant differences ($p < 0.01$) in mean nasal microcystin concentrations between individuals with direct HAB contact with impacted waters (mean = 0.77 ppb +/- 0.88 ppb) compared to those with no recent HAB contact (mean = 0.37 ppb +/- 0.49 ppb). Also, higher nasal concentrations were observed among occupationally exposed individuals. In addition, nasal microcystin concentrations varied significantly over time and location of exposure to the bloom, concordant with microcystin concentrations measured in water samples. These results clearly suggest that inhalation of aerosols may be an important pathway for exposure to microsystin concentrations as nasal concentrations were generally highest during periods when concentrations in the surrounding waters peaked. Further research is needed to characterize the public health implications of exposure to airborne HAB toxins. In this and other studies the sampling periods were much longer than in our study, which may partly explain our inability to measure Microcystin and Lyngbya toxins. However, we were able to sample aerosols, and thus our methods may be effective if we increase the sampling time to enable a larger mass fraction of the toxin to be collected. Future studies will be conducted to test this hypothesis.

Table 5.1 Summary of Detection Methods, Concentration Ranges, and Major Findings from Three Studies

Study	Detection Method	Concentration Range	Major Findings
FGCUN NOAA, 2019	Air Samplers with different size filters simulating the human lung	Microcystin, Low but Measurable	Microcystin aerosols were detected inland as far as 25 miles away from HABS
Kirkpatrick et al. 2010. Backer et al. 2003 Chen et al. 2005; Hoagland et al. 2009	High-Volume Air Samplers	Brevetoxin, 0.06 – 38 ng/m^3 (between two sampling sites)	Leaving the beach does not necessarily discontinue environmental exposure to brevetoxin aerosols. Measured as far as 6.4 km inland Hospital emergency department diagnoses increased by 19% for pneumonia, 40% for gastrointestinal illnesses, and 54% for respiratory illnesses
Fleming et al. 2007.	Questionnaire and Spirometry	N/A	Measurable adverse changes in lung function were observed in response to exposure to aerosolized Florida red-tide toxins in asthmatic subjects

CHAPTER 6: FUTURE RESEARCH

Future research would primarily focus on collecting samples in the field around local bodies of water where microcystin-producing blooms have been detected. Such bodies of water would include Lake Whelchel, Bear Creek (Figure 7.1), and the Anne Springs Close Greenway, all located in South Carolina. Blooms of *Microcystis wesenbergii* and *Microcystis sp.* have been detected in all bodies of water by SCDHEC over the past two years with detectable microcystin levels. Future research would focus on producing aerosol samples from blooms with detectable microcystin levels to see what concentrations could be detected in aerosols.

Figure 6.1 Microcystis bloom at Bear Creek reservoir in Lancaster, South Carolina on July 25, 2020.

Future studies should include longer sampling times for collection of air samples, as well as perhaps advanced methods of layered filters as were used by Dr. Mike Parson of Florida Gulf Coast University in sample Microcystin blooms in south Florida. These layered filters attempt to simulate the human lung and provide insight into the degree of respiratory exposure may occur during HAB events. The increased exposure times will allow collection of more toxin mass which would hopefully result in detectable levels of HAB toxins being measured. During the 2018-2019 HAB event in Florida, only low level bakcground levels of Microcystin were detected, which required long sampling time, much longer than was used in these experiments. It is possible to determine the length of time needed for toxin detection certain information is known, and a worksheet

has been created with these formulas for future research (see Appendix A).

In addition, other studies using nasal swabs have found detectable levels of Microcystin, using ELISA methodologies for more rapid chemical analysis, which can be followed with more quantitative methods for confirmatory analysis (Schaffer et al. 2020). This methodology should be employed with other HAB toxins to evaluate its efficacy in future major HAB events.

Appendix A contains a worksheet of important factors that should be considered when future research on aerosolized HAB toxins is conducted. This is not an exhaustive list, but it does contain important physiochemical factors to consider in designing future experiments.

WORKS CITED

[WHO] World Health Organization. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring, and management. London: E & FN Spon.

Albadrani A, Sarkar S, Saha P, Bose D, Kimono D et al. A preclinical assessment of early life microcystin-linked inflammasome activation and risks of development of fatty liver disease phenotype and insulin resistance in adolescence, Environmental Toxicology and Pharmacology 2020 (In review)

Albadrani M, Seth RK, Sarkar S, Kimono D, Mondal A, Bose D, Porter DE, Scott GI, Brooks B, Raychoudhury S, Nagarkatti M, Nagarkatti P, Jule Y, Diehl AM, Chatterjee S. Exogenous PP2A inhibitor exacerbates the progression of nonalcoholic fatty liver disease via NOX2-dependent activation of miR21. Am J Physiol Gastrointest Liver Physiol. 2019 Oct 1;317(4):G408-G428.

Backer LC, L.E. Fleming, A. Rowan, Y-S. Cheng, J. Benson, R. Pierce, J. Zaias, J. Bean, G. Bossart, and D.G. Baden. 2003. Recreational Exposure to Aerosolized Brevetoxins During Florida Red Tide Events. *Harmful Algae* 2:19–28.

Backer, L. C., D. Manassaram-Baptiste, R. LePrell, and B. Bolton. 2015. Cyanobacteria and Algae Blooms: Review of Health and Environmental Data from the Harmful Algal Bloom-Related Illness Surveillance System (HABISS) 2007-2011. Toxins: DOI 10.3390/toxins7041048.

Brooks, B., J. Lazorchak, M. Howard, M. V. Johnson, S. L. Morton, D. Perkins, E. R. Reavie, G. I. Scott, S. A. Smith, and J. A. Steevens. 2015. Are Harmful Algal Blooms Becoming the Greatest Inland Water Quality Threat to Public Health and Aquatic Ecosystems. *Environmental Toxicology and Chemistry:* DOI 10.1002/etc.3220.

Carmichael, W. W. and G. L. Boyer. 2016. Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. Harmful Algae: DOI 10.1016/j.hal.2016.02.002.

Cheng YS, Zhou Y, Irvin CM, Pierce RH, Naar J, Backer LC, et al. 2005. Characterization of Marine Aerosol for Assessment of Human Exposure to Brevetoxins. Env. Hlth Perspect. 113:638-643.

Cheng, Y. S., Y. Zhou, C. M. Irvin, B. Kirkpatrick, and L. Backer. 2007. Characterization of Aerosols Containing Microcystin. Marine Drugs 5:136-150.

Codd G.A., L.F. Morrison , and J.S. Metcalf. 2005. Cyanobacterial toxins: risk managementfor health protection. *Toxicol Appl Pharmacol* 203:264–272.

Hoagland P, Jin D, Polansky LY, Kirkpatrick B, Kirkpatrick G, et al. 2009. The Costs of Respiratory IllnessesArising From Florida Gulf Coast Karenia Brevis Blooms. Environ Health Perspect. 117:1239-1243.

Jang et al. 2020. Atmospheric Progression of Microcystin-LR from Cyanobacterial Aerosols. Environmental Science & Technology Letters. 7(10):740-745. DOI 10.1021/acs.estlett.0c00464

Kirkpatrick B., Bean J.A., Fleming L.E., Kirkpatrick G., et. al. 2010. Gastrointestinal ER Admissions and Florida Red Tide Blooms (2010) Harmful Algae, 9 (1), pp. 82-86. O'Neil, J. M., Davis, T. W., Burford, M. A. & Gobler, C. J. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 14, 313–334 (2012).

Murby, A. L. and J. F. Haney. 2016. Field and laboratory methods to monitor lake aerosols for cyanobacteria and microcystins. Aerobiologia: DOI 10.1007/s10453-015-9409-z.

NOAA, National Centers for Coastal Ocean Science 2019. Exploring Airborne Health Risks from Cyanobacteria Blooms in Florida: https://coastalscience.noaa.gov/news/study-explores-airborne-health-risks-from-cyanobacteria-blooms-in-florida/

Phlips, E.J., S. Badylak, N.G. Nelsonamd K. E. Havens. 2020. Hurricanes, El Niño and harmful algal blooms in two sub-tropical Florida estuaries: Direct and indirect impacts. Scientific Reports 10:1910; https://doi.org/10.1038/s41598-020-58771-4.

Puschner, B. and C. Moore. 2013. Cyanobacteria. In: Peterson, M. E. and P. A. Talcott, editors. Small Animal Toxicology. 3rd ed. London: Elsevier Publications. p. 533-540.

Rosen, B. et al. 2018. Understanding the effect of salinity tolerance on cyanobacteria associated with harmful algal blooms in Lake Okeechobee, Florida. Scientific Investigations Report 2018-5092. U.S. Dept. Of Interior, U.S. Geological Service, Reston Virginia, USA. https://doi.org/10.3133/sir20185092.

Sarkar S, Saha P, Seth RS, Mondal A et al. Environmental microcystin co-exposure-induced higher intestinal and circulatory lactate causes NOX2 activation and is key to ectopic fibrotic pathology in murine model of fatty liver disease. *Comparative Physiology and Biochemistry*, omparative Biochemistry and Physiology Part C, Volume 238: December 2020, 108852020 .

Sarkar, S., D. Kimono, M. Albadrani, R. Seth, P. Busbee, H. Alghetaa, D.E. Porter, G.I. Scott, B. W. Brooks, M. Nagarkatti, and P. Nagarkatti. 2019. Environmental microcystin targets the microbiome and increases the risk of intestinal inflammatory pathology via NOX2 in underlying murine model of Nonalcoholic Fatty Liver Disease" submitted to *Scientific Reports* 9, 8742. https://doi.org/10.1038/s41598-019-45009-1. (June 2019 Published After Year 1 Report)

Schaefer, A.M., L. Yrastorza, N. Stockley, K. Harvey, N. Harris, R. Grady, J. Sullivan. M. McFarland and J. S. Reif. 2020. Exposure to microcystin among coastal residents during a cyanobacteria bloom in Florida. Harmful Algae 92:101769. doi: 10.1016/j.hal.2020.101769.

Stewart, I. and I. R. Falcon. 2008. Cyanobacteria and Cyanobacterial Toxins. In: Walsh, P. J., S. L. Smith, L. E. Fleming, H. M. Solo-Gabriele, and W. H. Gerwick, editors. Oceans and Human Health: Risk and Remedies from the Seas. London: Elsevier Publications. p. 271-296.

Torbick et al. 2014. Mapping amyotrophic lateral sclerosis lake risk factors across northern New England. *International Journal of Health Geographics* 13:1;http://www.ij-healthgeographics.com/content/13/1/1.

van der Merwe, D. 2015. Cyanobacterial (Blue-Green Algae) Toxins. In: Gupta, R. C. editors. Handbook of Toxicology of Chemical Warfare Agents. 2nd ed. London: Elsevier Publications. p. 421-429.

Wang Y, Xu J, Stanley JE, Xu M, Brooks BW, Scott GI, Chatterjee S, Zhang Q, Zelinski MB, Xiao S. A closed vitrification system enables a murine ovarian follicle bank for high-throughput ovotoxicity screening, which identifies endocrine disrupting activity of microcystins. *Reprod Toxicol.* 2020 Apr;93:118-130. doi: 10.1016/j.reprotox.2020.01.009. Epub 2020 Feb 1.

Zheng et al, 2017. Hepatology 66(5):1519-1528. doi: 10.1002/hep.29310

Zheng, C., H. Zeng , H. Lin, J. Wang, X. Feng , Z. Qiu, J.-A. Chen, J. Luo, Y. Luo, Y. Huang, L. Wang, W. Liu, Y. Tan, A. Xu, Y. Yao, and W. Shu. 2017. Serum Microcystin Levels Positively Linked With Risk of Hepatocellular Carcinoma: A Case-Control Study in Southwest China. Hepatology 66(5):1519-1528. doi: 10.1002/hep.29310

APPENDIX A – WORKSHEET FOR FUTURE RESEARCH

1. Type of Cyanobacteria

_____

2. Toxin Produced

_____

a. Molecular Weight

_____

b. Log KOW

_____

c. Henry Law Constant

_____

3. Algal Material Used in Experiment (Live, Air Dried, Freeze Dried)

_____

a. Mass of Material in Grams

_____

b. Measured Toxin Level in Material

_____

c. Lower Limits of Detection

_____

d. Instrumentation

_____

4. Aeration Experiments

a. Solution and Volume

_____

b. Exposure Time

_____

c. Flow Rate

_____

d. Source of Particle Generation (Air Pump, Nebulizer, Other)

_____

e. Filter Used for Particle Collection (Filter Size)

_____

e. Output of Particle Data (Units and Particle Size)

_____

f. Chemical Analysis Method (HPLC/MS, ELISA, Other)

_____

g. Lower Limit of Detection (ug/M3 or PPB, Other)

_____

5. Conversion Between PPB and ug/M3

Concentrations of gaseous pollutants is sometimes given in units of mass per volume, and at other

times in ppb or ppm.

ppb (v) is parts per billion by volume (i.e., volume of gaseous pollutant per 109 volumes of ambient

air).

µg/m3 is micrograms of gaseous pollutant per cubic meter of ambient air.

The conversion assumes an ambient pressure of 1 atmosphere and a temperature of 25 degrees

Celsius.

The general equation is:

µg/m3 = (ppb)*(12.187)*(M) / (273.15 + °C)

where M is the molecular weight of the gaseous pollutant.

An atmospheric pressure of 1 atmosphere is assumed.

Estimation for Microcystin:

Here is the formula for the calculation:

ug/m3 = (ppb) X (12.187) (Molecular Weight)/ (273.15 C + degrees C of sample)

Substituting what we Know where

3.7 pg/M3 = 0.0037 ng/M3 = Detection Limit = 0.0000037 ug/M3

30 degrees = water temperature

Molecular Weight of Microcystin = 995.2

Thus

0.00000037 ng/m3 = (ppb) (12.187) (995.2) / (273.15C + 30)

0.00000037 ng/m3 = (ppb) (12, 128.5)/303.15

303.15 X 0.00000037 ng/m3 = (ppb) (12,128.15) (Multiplying both sides by 303.15)

0.00112 ng/m3 = (ppb) (12, 128.15)

0.00112 / 12,128.15 = ppb

9.23 X 10-8 = ppb = 0.0000000923 ug/m3

9230 X 10-5 = parts per trillion (pico grams) = 0.000923 ng/m3