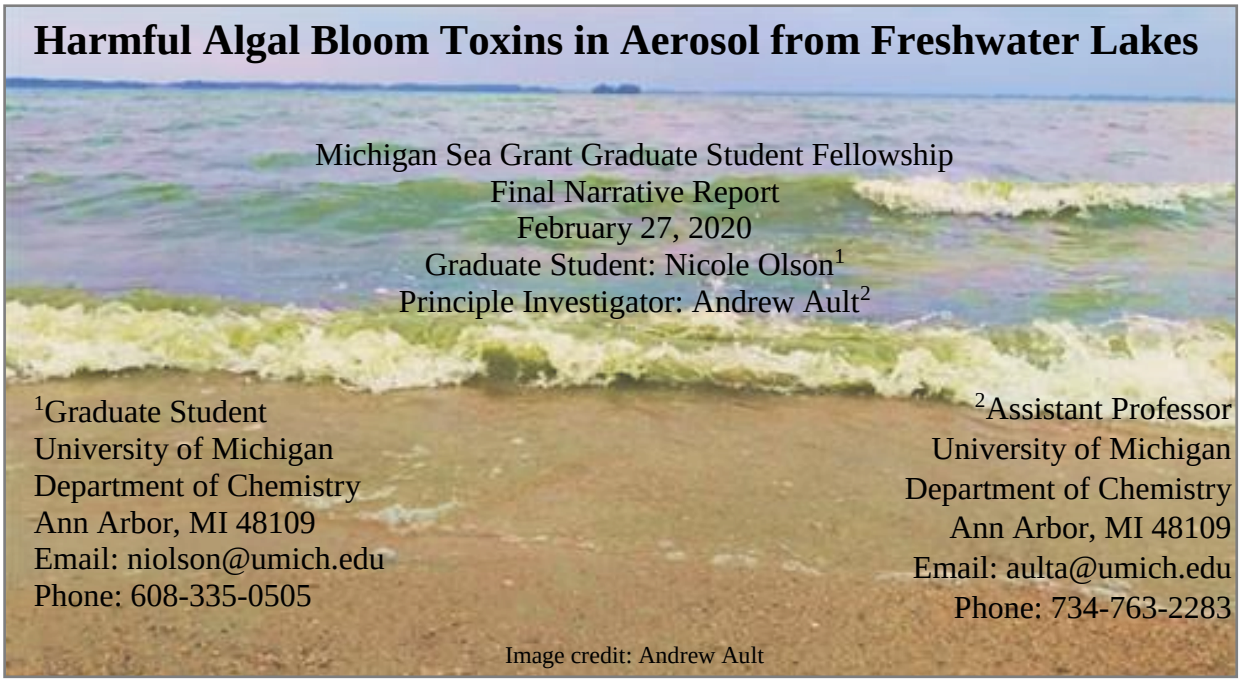
# Harmful Algal Bloom Toxins in Aerosol from Freshwater Lakes



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## Abstract

Harmful algal blooms (HABs) caused from blue-green algae in freshwater environments produce toxins, including microcystins, which are harmful to human health. HAB frequency and intensity are increasing globally with greater agricultural runoff and a warming climate, raising questions about unexpected routes of exposure. Lake spray aerosol (LSA) particles created from waves and recreational activity on freshwater lakes have been identified chemically in prior studies, but little is known regarding the potential for HAB toxins to be incorporated into LSA and transferred to the atmosphere. In this study, freshwater samples were collected from two lakes in Michigan: Mona Lake during a severe HAB with high microcystin concentrations (> 200 µg/L) well above the EPA recommended "do not drink" level (1.6 µg/L), and Muskegon Lake without a HAB (< 1 µg/L microcystin). Microcystin toxins were identified in freshwater and aerosol particles generated from Mona Lake by liquid chromatography tandem mass spectrometry. Enrichment of hydrophobic microcystins, such as microcystin-LR, was observed in aerosol particles relative to bulk freshwater. We show that hydrophobic microcystin congeners partition to the air-water interface of bubbles passing through the HAB from wave activity and are then transferred to the aerosol phase after bubble bursting. This study suggests that the relative amounts of toxins present in the aerosol phase are distinctly different than those present in the water column. As HABs increase in a warming climate, understanding and quantifying the emissions of toxins into the atmosphere is crucial for evaluating the health consequences of HABs.

**Keywords:** harmful algal bloom, lake spray aerosol, microcystin, toxin, freshwater

## Project Summary

Background

The frequency and intensity of harmful algal blooms (HABs) from blue-green algae in freshwater are increasing globally due to increased nutrient runoff, warmer global temperatures, and longer growing seasons.[1,2] HABs emit toxins, such as microcystins, that negatively impact human and animal health and, in extreme cases, can lead to death. Over 200 types of microcystins have been identified so far,[3] necessitating a deeper scientific understanding of their mechanisms of transport to fully understand their impacts on health. Scientific understanding of the ingestion risk of microcystins is fairly well-understood, but we lack understanding of the health impacts of inhaled microcystin droplets. Laboratory studies examining the toxicity of microcystin-containing aerosolized droplets in mice have shown ten times higher sensitivity to inhaled microcystin compared to orally ingested microcystin,[4,5] suggesting the toxic effects from exposure to aerosolized microcystin likely occurs at lower doses than for microcystin ingestion. This raises questions and concerns regarding unexpected exposure for populations living near or downwind of HABs, occupations that interact with HABs, and recreational users on HAB-impacted water. Characterizing the aerosolization of HAB toxins in freshwater environments is crucial for understanding the impacts of toxin inhalation on public health.

Lake spray aerosol (LSA) is produced by freshwater wave breaking and bubble bursting. LSA are generated in a size range important for inhalation exposure, with diameters < 2.5 μm making it possible to inhale these particles.[6] Recent work on which I was a co-author[7] showed that LSA generated from HABs contained greater organic and biological material than LSA generated during non-HAB conditions, demonstrating the incorporation of biological material from HABs into LSA. However, little is known regarding the incorporation of toxins from freshwater HABs into LSA. Ambient studies observed microcystin in LSA emitted from small, inland lakes in Michigan.[8,9] Similarly, laboratory studies identified microcystin-containing droplets produced from freshwater bubble bursting.[10] These studies demonstrated that freshwater toxins can become aerosolized, however they determined microcystin concentrations by using enzyme-linked immunosorbent assays (ELISA), a measurement technique that is unable to distinguish between the types of aerosolized microcystins. Additional questions remain about the relationship between freshwater toxins versus aerosolized toxin concentrations, and whether the various types of microcystins are aerosolized with the same efficiency.

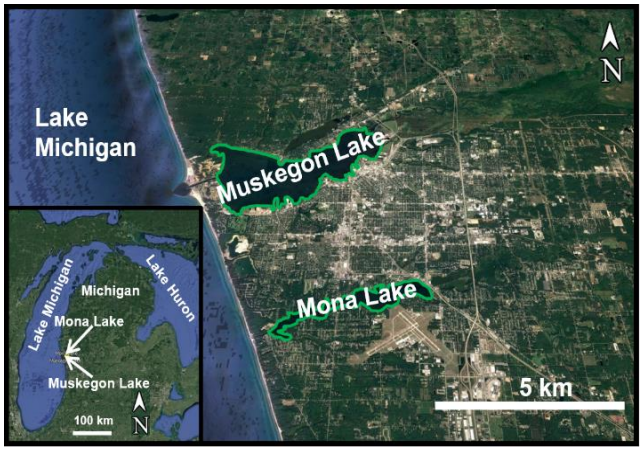


**Figure 1.** Map of Mona and Muskegon Lakes located in Western Michigan, with inset showing location of inland lakes relative to the surrounding Great Lakes. Map obtained from Google Earth.

Experimental Design and Methods

In this study, freshwater was collected in Michigan from Mona Lake during a HAB and high microcystin concentrations and Muskegon Lake in which

the microcystin concentrations were below EPA recommended levels (**Figure 1**). Freshwater samples were analyzed for the presence of blue-green algae and microcystin toxins, after which LSA was generated in the laboratory using a custom made LSA generator.[11] ELISA kits measured total microcystin concentrations in freshwater samples. For comparison, 12 types of microcystins were also measured using the liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) method developed by our collaborators at Wayne State University.[12] The LC-MS/MS method allows for the quantification of each type of microcystin based on calibration with commercially available microcystin standards. Individual aerosol particles were also chemically analyzed with an aerosol time-of-flight mass spectrometer (ATOFMS).

Results and Conclusions

Eight types of microcystins were detected in the freshwater samples from Mona Lake. Of the eight microcystins detected in the freshwater, seven were also detected in LSA particles, demonstrating that toxins are emitted through the aerosolization process in freshwater environments. However, the types of microcystin were not transferred from freshwater to the aerosol phase uniformly, leading to greater enrichment of hydrophobic microcystins in aerosol particle samples. In our experiments, microcystin-LR and microcystin–LA were more concentrated than other microcystins in aerosol particles. These types of microcystin contain the amino acid leucine, a hydrophobic amino acid.[13-16] Conversely, the amino acid arginine is significantly more hydrophilic,[14-16] and the congener with this structure, microcystin–RR, was significantly less enriched in the aerosol relative to freshwater. Our results suggest the hydrophobic microcystins partition to the air-water interface of bubbles passing through the HAB and are then transferred to the aerosol phase after bubble bursting (**Figure 2**). We also observed an enhancement in the concentration of ultrafine aerosol particles with an increase in freshwater HAB concentration, leading to aerosol production at smaller sizes that are more readily inhaled.

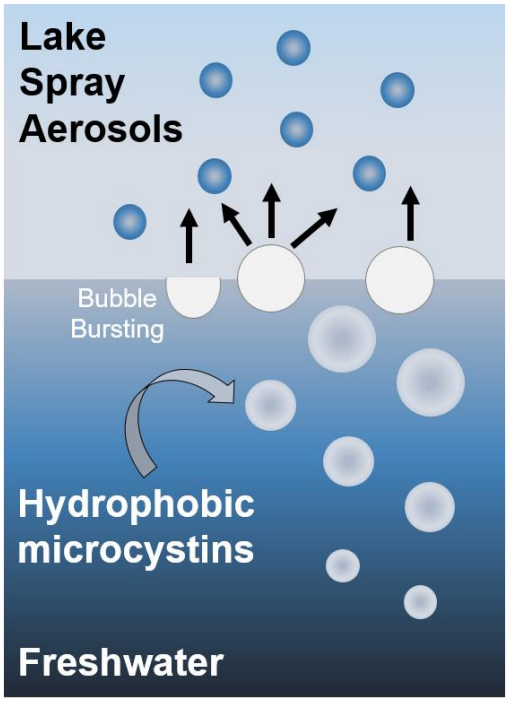


**Figure 2.** Schematic showing the aerosolization of hydrophobic microcystins from bubble bursting in freshwater environments.

This study demonstrates the emissions of microcystins within freshwater-derived aerosol particles and suggests that the relative amounts of toxins present in the aerosol phase are distinctly different than those present in the water column. This project has resulted in multiple presentations at local and national meetings, and a scientific manuscript currently under review. Overall, this project highlights the potential exposure risks for populations near or downwind of HABS.

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