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Aerosolized Cyanobacterial Harmful Algal Bloom Toxins: Microcystin Congeners Quantified in the Atmosphere

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Notes

The authors declare no competing financial interest.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c03297.

DNA extraction and qPCR setup, a compilation of additional congeners found at GLSM (Table S1); description of the determination of D-Asp^3-MC-HtyR structure; details on the identification of D-Asp^3-MC-HtyR congener at GLSM (Figures S1–S10); and size-resolved ambient aerosolized MC collected in the aeration treatment and clarifier rooms of the Celina Water Plant (Figure S11) (PDF)

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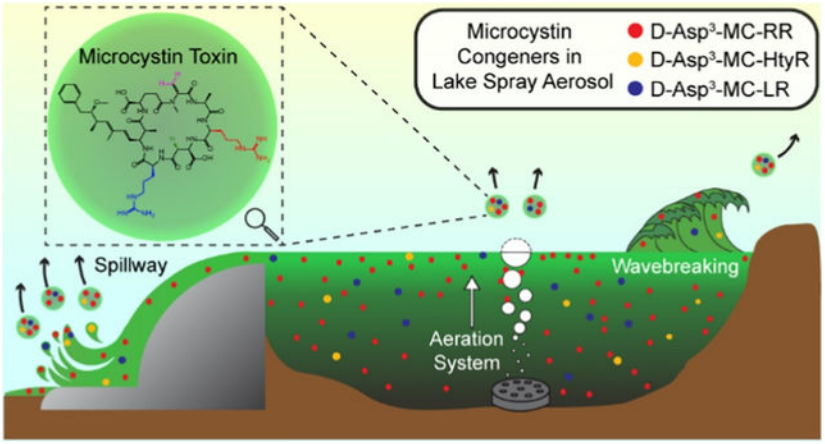
Abstract

Cyanobacterial harmful algal blooms (cHABs) have the potential to adversely affect public health through the production of toxins such as microcystins, which consist of numerous molecularly distinct congeners. Microcystins have been observed in the atmosphere after emission from freshwater lakes, but little is known about the health effects of inhaling microcystins and the factors contributing to microcystin aerosolization. This study quantified total microcystin concentrations in water and aerosol samples collected around Grand Lake St. Marys (GLSM), Ohio. Microcystin concentrations in water samples collected on the same day ranged from 13 to 23 μg/L, dominated by the D-Asp^3-MC-RR congener. In particulate matter <2.5 μm ($PM_{2.5}$), microcystin concentrations up to 156 pg/m^3 were detected; the microcystins were composed primarily of D-Asp^3-MC-RR, with additional congeners (D-Asp^3-MC-HtyR and D-Asp^3-MC-LR) observed in a sample collected prior to a storm event. The PM size fraction containing the highest aerosolized MC concentration ranged from 0.44 to 2.5 μm. Analysis of total bacteria by qPCR targeting 16S rDNA revealed concentrations up to 9.4×10^4 gc/m^3 in aerosol samples (≤3 μm), while a marker specific to cyanobacteria was not detected in any aerosol samples. Concentrations of aerosolized microcystins varied even when concentrations in water were relatively constant, demonstrating the importance of meteorological conditions (wind speed and direction) and aerosol generation mechanism(s) (wave breaking, spillway, and aeration systems) when evaluating inhalation exposure to microcystins and subsequent impacts on human health.

Graphical Abstract



Keywords

cyanobacteria; bloom aerosolization; lake spray aerosol; mass spectrometry; particulate matter toxins; aerosolized bacterial rDNA

INTRODUCTION

Cyanobacterial harmful algal blooms (cHABs) can pose a serious health risk to nearby communities due to the plethora of toxins produced by a variety of cyanobacteria.[1–4] Certain cyanobacteria (e.g., *Microcystis* sp. and *Planktothrix* s.p.) produce microcystins (MCs), a class of toxins linked to negative health effects, including hepatotoxicity through inhibiting enzymes that regulate phosphorylation.[5] In an infamous 1996 event, patients undergoing treatment at a dialysis clinic in Caruaru, Brazil, were exposed to MCs in the water supply, which resulted in 52 deaths from acute liver failure.[6] More recently, in 2014, an intense cHAB in Lake Erie introduced high MC levels into the Toledo water treatment facility, leading to unsafe drinking water for multiple days.[7] Based on these and other events, cHABs and MCs have received greater attention as bloom intensity has increased on the Great Lakes,[8] and their levels could continue to increase as temperatures warm in a changing climate.[9,10]

MCs are heptapeptides with cyclic structures and are differentiated by their amino acid compositions, with over 300 congeners identified to date.[11] Most MC research has focused primarily on the MC-LR congener, distinguished by leucine and arginine, in positions 2 and 4, respectively.[12] Congener toxicity differs by orders of magnitude based on differences in the MC structures. For example, the median lethal dose (LD_{50}) of MC-LR (50 μg/kg) is an order of magnitude lower than MC-RR (500 μg/kg).[13] While it has been established that MCs can efficiently bind to protein phosphatase type 2A in the liver, Ikehara et al.[14] found a weak correlation between congener-specific inhibitory concentrations and their cytotoxicity to human hepatocytes, indicating the need to further explore the health impacts of MCs. Due to the growing body of literature on the toxicity of MCs, the EPA has set a health advisory limit for MC-LR at 1.6 μg/L for drinking water consumption.[15] However, there is a high diversity of MC congeners possible in any bloom, and, given the range of congener toxicities, bloom-to-bloom toxicity can fluctuate wildly.[16–18]

Many studies on the health effects of MCs have solely focused on MC exposure via ingestion, with far fewer studies focusing on other routes of exposure, like inhalation.[19] Fitzgeorge et al.[20] found that mice had a greater sensitivity to MC-LR when introduced through intranasal instillation (LD_{50} of 250 μg/kg) than gastric intubation (3000 μg/kg). Another study found that direct exposure to aerosolized MC-LR can be lethal at an even lower concentration, with an LD_{50} of 43 μg/kg.[21] Human respiratory tissue exposed to MC-LR can change the expression of certain genes associated with inflammation and cancer.[22,23] Therefore, it is critical to understand the different exposure pathways and risks MCs pose to human health.[24,25]

During cHABs, organic material in the water can be aerosolized during the generation of lake spray aerosol (LSA) from freshwater.[26,27] The process of LSA generation is similar to the more well-documented formation of sea spray aerosol (SSA), where wind-driven wave breaking traps air within the water column, and when the air bubbles rise to the surface and burst, spray aerosol is emitted into the atmosphere.[28–32] Multiple studies have shown that biological material from aquatic systems is emitted into the atmosphere through the bubble bursting process[33,34] and that the concentrations of the biological material found in aerosol

can be enriched by several orders of magnitude relative to bulk water samples.[26,35–37] Once emitted, LSA particles can be transported further inland,[32,38,39] and they have been observed at cloud heights and incorporated into cloudwater.[40] The aerosolization process generates particulate matter less than 2.5 μm in diameter ($PM_{2.5}$),[28,32] posing a potentially serious health concern due to its ability to deposit deep in the human respiratory system.[41]

Aerosolized MCs and other cyanotoxins have been detected in aerosol samples collected near cHABs,[42–46] but little is known about the factors that contribute to their emissions. In samples analyzed by Wood and Dietrich[45] that contained detectable aerosolized MC (or nodularin), the airborne concentrations ranged from 0.2 to 16.2 pg/m^3, but they were not found to be correlated with wind speed. However, Backer et al.[47] studied an algal toxin called brevotoxin and found that elevated onshore winds led to higher concentrations of aerosolized brevotoxin, alongwith increased reports of symptoms in the respiratory tract of beach-goers. Onshore and offshore wind directions can greatly influence the ambient aerosolized marine algal toxin, with Kirkpatrick et al.[48] noting that some of the lowest concentrations of aerosolized brevotoxin were found in samples collected when wind directions were offshore. Plaas et al.[24] observed aerosolized cyanobacteria DNA that correlated with the fetch of the body of water, but did not find a statistical correlation with onshore winds. Therefore, more research is needed to elucidate the relationship between environmental factors and aerosolized MCs in order to better assess their exposure risks.

A complicating factor for understanding blooms and their potential for generating aerosolized MCs is the underlying biology of the bloom. While *Microcystis* is the most studied contributor to cHABs, *Planktothrix* sp. has been documented in 38% of cHABs within the U.S.[49] However, the congeners produced from *Planktothrix* sp. have not been characterized in the U.S., in contrast to a few studies in other countries.[50–53] Enzyme-linked immunosorbent assays (ELISA) kits[42–45] have been the most commonly used approach to measure MC concentrations, but are unable to determine the specific congener concentrations. In contrast, high-resolution mass spectrometry (HRMS) enables the identification of congeners for which there are no commercially available standards. Despite the ubiquity of *Planktothrix* sp. blooms, only one study[54] has investigated the MC congeners produced from *Planktothrix* sp. with HRMS in the U.S., and considerable uncertainty remains about the congener profile in many freshwater lakes.

In this study, we utilized HRMS to identify the MC congener profile at Grand Lake St. Marys (GLSM) in Ohio, a hypereutrophic lake that has been primarily driven by *Planktothrix* sp.[55–58] The persistent cHABs at GLSM have led MC concentrations to be consistently above the EPA exposure guidelines (1.6 μg/L for drinking water[15] and 8 μg/L for recreational exposure[59]) during the summer month,[60] but their emissions to the atmosphere within aerosols have previously not been probed. Water samples were collected at multiple locations around the lake and show that the lake is dominated by three congeners (two of which have no commercially available standards). Aerosolized MCs were also quantified in $PM_{2.5}$ and size-resolved samples, and congener concentrations were compared between air and water samples. Aerosolized MCs were observed at sites around GLSM, with higher concentrations detected adjacent to a spillway and near aeration systems. Both airborne MC toxins and bacterial gene (16S rDNA) were observed primarily in particles <3

μm. MC concentrations peaked in the accumulation mode (0.1–1.0 μm), the size range of aerosols with the longest atmospheric lifetimes. The aerosolization of MCs from a cHAB observed in this study under different aerosol-generating conditions demonstrates the need to improve the understanding of MC inhalation exposure so that risk can be assessed more accurately.

METHODS

Study Site.

GLSM is the largest inland lake (roughly 15 km × 4 km) in the state of Ohio and extends between the cities of St. Marys and Celina, Ohio (Figure 1A).[55] Between 80 and 90% of the land surrounding GLSM is used for agricultural purposes, leading to a nutrient-rich watershed that drains directly into the lake.[55] The nutrient-rich runoff and shallow depth of the lake (averagedepth = 1.5–2.1 m) facilitate the growth of cyanobacteria, leading to consistently elevated concentrations of MCs.[57] Monitoring of MCs in GLSM between 2009 and 2021 using ELISA kits has demonstrated that their concentration peaks in late spring and the middle of the fall, but the concentrations remained at unsafe level throughout the summer months.[60] An important driver to the persistent cHABs at GLSM between 2009 and 2021 is the temperature, with an average water temperature of 25.8 °C and the highest recorded was 32 °C.[60] The hypereutrophic state of the lake during this 2019 field study was described in Hanlon et al.,[61] with the concentration of total phosphorus between 0.27 and 0.39 mg/L and phycocyanin concentrations between 600 and 800 ppb.

The field study was conducted between 8/5/2019 and 8/9/2019. Water and aerosol samples were collected at the Wright State University-Lake Campus (WSU-LC) on the beach (next to the boat dock) and the porch of a student housing building where waves were observed on the adjacent shoreline (Figure 1B,C). To determine the spatial variability of the MC toxins in water and aerosol, other areas sampled included a central area of the lake, the spillway located on the western side of GLSM, and Prairie Creek on the south side of the lake (Figure 1D–F). The different locations have different mechanisms that generate bubbles that can lead to LSA production (Figure 1G). At the spillway, excess water from the lake is discharged into Beaver Creek to prevent flooding. The water cascading over the spillway entrains air and the resulting bubbles emit particles into the atmosphere via the established bubble bursting mechanism (or via impaction against concrete at the bottom of the spillway when water levels are low).[28,31,33,62] Additionally, aeration systems directly generate bubbles into the water column that later burst at the surface, ejecting particles into the atmosphere. Aerosol sampling was also conducted at the City of Celina Water Plant, specifically in an isolated indoor location that was piloting the aeration treatment of cHAB water. The combination of a large cHAB, wind-driven wave breaking, and other localized factors (spillway and aeration systems) makes GLSM an ideal location for studying the aerosolization of MCs.

Wind Measurements.

Wind speed and direction measurements from 8/5/2019 to 8/9/2019 were taken from Weather Underground, station KOHCELIN4, which is located less than 5 km west from

the WSU-LC on the western shore of GLSM. Meteorological data from the station was confirmed via cross-checking with observational notes from the field, with specific focus on a high wind speed and storm event (8/6/2019).[63] Wind speed and direction measurements were recorded with 5 min time resolution and were averaged into 60 min intervals. While the wind speed measurements were not taken at the sampling locations, which could potentially be influenced by the topography (though the region is very flat), these data are consistent with trends in noncontinuous data from Bilyeu et al.[64] and González-Rocha et al.[63] collected on the shoreline of the WSU-LC. Satellite imagery is from the 2019 Ohio National Agriculture Imagery Program and retrieved from the NOAA Office of Coastal Management (https://naip-usdaonline.hub.arcgis.com/).

Water Sample Collection.

Water samples at GLSM were collected in 8 L plastic (low-density polyethylene, LDPE) carboys on 8/6/2019. To see if there were variations in the concentrations of MCs in different areas around the lake, samples were collected from the spillway, Prairie Creek, central area of the lake, as well as thebeach and boat dock right next to the beach of the WSU-LC shoreline (Figure 1). A fluorometer (AquaFluor, Turner Design) was used to measure the phycocyanin concentration in water samples as a proxy for cyanobacteria concentration.[65] Due to a calibration issue with the fluorometer, relative concentrations are reported. Following EPA method 544,[66] whichaims to determine the total concentrations of MC congeners in a sample through cell lysis, the entire containers of water samples were subjected to three freeze–thaw cycles, thoroughly mixed, and a 1.5 mL aliquot was used to quantify MCs via liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Aerosol Sample Collection and Extraction.

Four different aerosol samplers were used in this study (Table 1), two of which probed airborne MCs and the other two to quantify airborne bacterial genetic material (16S rDNA). For MC analysis, the URG $PM_{2.5}$ cyclone impactor collected aerosol particles below 2.5 μm on a single filter. In contrast, the TSI cascade impactor collected aerosol particles that were size-resolved onto five separate filters. For 16S rDNA, size-resolved aerosol particles were collected onto six separate filters, whereas the Bobcat air samples collected particles below 10 μm onto one filter.

Aerosol samples for toxin (MC) analysis were collected at multiple locations (Table 1) on land and on a boat around the GLSM using a $PM_{2.5}$ cyclone (URG Corp., Model 2000–30ED) coupled with a single-stage impactor (URG Corp., Model 2000–30FV). The single-stage impactor was operated at 3 L/min, impacting particles smaller than 2.5 μm onto glass fiber filters (Whatman, 1.2 μm pore size). Additionally, two 5-stage cascade impactors (TSI, Model 130) were used to sample size-resolved particles for MC analysis, one on the beach (<1 m from the shoreline) and one on the porch of a housing unit (~30 m from the shoreline) at the WSU-LC. The cascade impactors sampled at 100 L/min, impacting aerosol onto prebaked quartz fiber filters for stages with d_{50} size cuts of 2.5, 1.4, 0.77, 0.44, and 0.25 μm. Filter extraction was based on procedures previously used to measure aerosolized MCs.[35,45,67] The difference in power demand between the $PM_{2.5}$ cyclone impactors and the TSI cascade impactors dictated the sampling locations. The pump for the cyclone impactors

was more compatible with the portable battery packs and allowed for sampling at locations like the spillway, whereas the pumps for the TSI cascade impactors hadsignificant power draws, making them incompatible with portable battery packs. Thus, TSI cascade impactors were primarily limited to the beach and porch at the WSU-LC. The filters underwent freeze–thaw cycling 3 times prior to the extraction procedure, where they were fully submerged in 5 mL of 70% methanol (Thermo Scientific, Optima LC-MS grade) and 30% water (Milli-Q, 18.2 MΩ·cm) solution and sonicated for 15 min. The supernatant was poured into a separate vial, and the procedure was repeated two more times. Afterward, the supernatant was filtered through a 0.22 μm nylon syringe filter (Thermo Scientific, Choice). The sample was dried under N_2 and reconstituted in water (Thermo Scientific, Optima LC-MS grade) for LC-MS/MS analysis.

Aerosol samples for bacterial genetic analysis were collected using a six-stage Andersen cascade impactor (Tisch Environmental) and a PM_{10} Bobcat high-volume sampler (ACD-200 Bobcat, InnovaPrep LLC). The flow rates of the samplers were 28.3 and 200 L/min, respectively. The samplers were located side by side on the beach (<1 m from the shoreline) with their inlets elevated ~0.5 m above the ground. With the Andersen cascade impactor, samples were collected onto 90 mm polycarbonate filters, which were autoclaved prior to sampling. With the Bobcat high-volume sampler, samples were collected on an electret filter provided by the manufacturer. Immediately after sampling, the filters were unloaded from the cascade impactor, placed inside clean Petri dishes, and stored in a refrigerator. The Bobcat samples were eluted onsite using 6 mL of buffer (0.075% Tween 20 and 25 mM Tris, InnovaPrep LLC), separated into 1 mL aliquots, and stored in a freezer. Field blank(s) were collected for each sampler. At the end of the sampling campaign, the samples were transported on ice to laboratories at Virginia Tech and stored at −20 °C.

Microcystin Quantification and Identification.

Water and extracted aerosol samples were analyzed for the following MC congeners with standards that were commercially available from Enzo Life Sciences: D-Asp^3-Dhb^7-MC-RR (supplied as D-Asp^3-MC-RR but tested to be D-Asp^3-Dhb^7-MC-RR),[54,68] MC-RR, nodularin, MC-YR, MC-HtyR, MC-LR, D-Asp^3-MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, and MC-LF. Microcystin congeners in the samples were quantified using an LC (Thermo Scientific, EQuan MAX Plus) coupled with a triple quadrupole mass spectrometer (Thermo Scientific, TSQ Altis). Samples were loaded onto an online concentrator column (Thermo Scientific, Hypersil GOLD aQ, 20 mm × 21 mm, 12 μm particle size) and then backflushed onto the analytical column (Thermo Scientific, Accucore aQ, 50 mm × 2.1 mm, 2.6 μm particle size). The analytes were eluted into the MS/MS system and quantified by using selective reaction monitoring. Further details on the LC-MS/MS parameters are described in prior publications by Birbeck et al. and Olson et al.[35,69]

An unknown congener was identified using nano-LC (Thermo Scientific, EASY-nLC 1200) coupled to a high-resolution mass spectrometer (Thermo Scientific, Orbitrap Exploris 120). The precolumn was equilibrated with 16 μL, and the analytical column was equilibrated with 5 μL at 700 bar. Two μL of sample was eluted with 5 μL loading phase through a Thermo Acclaim PepMap C18 3 μm × 75 mm × 2 cm trap column with 5 μL at 700 bar.

After equilibration and loading, chromatographic separation was conducted using a Thermo Acclaim PepMap C18 2 μm × 0.075 mm × 250 mm column with 0.1% formic acid in water (mobile phase A) and 80% acetonitrile, 20% water, and 0.1% formic acid (mobile phase B). The separation gradient was set to ramp from 5 to 30%B in 5 min and from 30 to 100%B in 20 min, and held at 100%B for 5 min. The sample syringe was then washed with 3 cycles 15 μL each of acetonitrile and water. The source was set to expect 6 s peaks with RunStart Easy-IC on. The source voltage was 1600 V, and the transfer tube was at 300 °C. The system was run in a targeted top 4 Data Dependent Analysis (DDA) in positive, centroid mode at 3000 resolution scanning from 500 to 1500 m/z at 70% RF. DDA MS2 was collected with an isolation width of 0.7 m/z using stepped collision energy 15, 45, 75 high collision dissociation (HCD) at 15 000 resolution, centroided, with a starting mass of 50 m/z. Intensity filter: 5E5, Data Exclusion: 1 scan in 2 s, Targeted MS2:1045.5353 and 1123.5492 m/z, most intense if no target found, Targeted Exclusion: set to ignore common backgrounds determined from blank runs, Apex Detection: 30%.

Bacterial Genetic Analysis.

Concentrations of total bacteria in aerosol samples were determined by quantifying portions of 16S rDNA with quantitative polymerase chain reaction (qPCR) using previously described methods.[70] Briefly, each polycarbonate filter from the Andersen cascade impactor was cut into eight smaller sections for ease of processing. These sections were later transferred into a 5 mL bead tube (DNeasy PowerWater Kit, Qiagen). Likewise, one aliquot of the eluate was taken from the Bobcat sampler and pipetted into the same type of bead tube. Samples were extracted using the DNeasy PowerSoil Kit (Qiagen) according to the manufacturer's protocol with the modifications described in the Supporting Information (SI). The forward primer was 1369F: 5′-GGTGAATACGTTCYCGG-3′ and the reverse primer was 1496R: 5′-GGWTACCTTGTTACGACTT-3′.[70,71] PCR reactions were conducted with primers for a common gene that represents total cyanobacteria (CYAN-108F: 5′-ACGGGTGAGTAACRCGTRAG-3′, CYAN-377R: 5′-CCATGGCGGAAAATTCCCC-3′).[72] Additional details of the PCR assays and reactions can be found in SI. Autoclaving the filters prior to use did not remove all genetic material, as low levels of 16S rDNA were detected in the field blanks. Concentrations significantly higher than in the field blanks, determined by the Student's t test ($p < 0.05$), were reported, and concentrations not significantly different from that of the field blanks were defined as "Not Detected".

RESULTS AND DISCUSSION

Composition of Microcystins across Water Samples Collected from Grand Lake St. Marys.

Analysis of the MC profile at GLSM from all water samples collected revealed that >98% of the composition MC concentrations is composed of three MC congeners: putative D-Asp^3-MC-RR (isomer of standard D-Asp^3-Dhb^7-MC-RR), putative D-Asp^3-MC-HtyR (isomer of standard MC-YR), and D-Asp^3-MC-LR (Figure 2). Retention time shifts, chemical derivatization, and the qualifier and quantification transition ratio were used to determine that two of the three dominant MCs were isomers of the standards. When examining the peak present for the standard D-Asp^3-Dhb^7-MC-RR, the putative D-Asp^3-MC-RR was

observed to have a retention time 0.07 min earlier in the chromatogram than D-Asp^3-Dhb^7-MC-RR. Less obvious but consistent, putative D-Asp^3-MC-HtyR eluted 0.01 min later than standard MC-YR. Standard chemical derivatization (thiol Michael Addition) was used to determine if the MC position 7 amino acid is Dba or Dhb. Dha is very reactive, and Dhb is slightly reactive. The three MC congeners underwent the reaction, hence the conclusion Dha was in position 7 (see the Supporting Information). Finally, the quantification and qualification transition ion ratio used to validate the targeted MC-YR was 70%, whereas putative D-Asp^3-MC-HtyR was 50%. D-Asp^3-MC-LR was found to match the standard in retention time and quantification-qualification ion ratio. HRMS/MS analysis provided a putative identification of D-Asp^3-MC-HtyR and D-Asp^3-MC-RR (Figures S1–S10). Since the D-Asp^3-MC-RR and D-Asp^3-MC-HytR congeners found in the GLSM samples did not have commercially available standards, D-Asp^3-Dhb^7-MC-RR and MC-YR standards were used to quantify these congeners in the samples.

Individually quantified congeners in the GLSM water samples were summed to determine the total (composite) of 12 MC congeners quantified. Within the lake itself, samples collected at the spillway, beach, dock, and central and had total MC concentrations of 14.9, 12.8, 21.6, and 21.0 μg/L, respectively (Figure 2E). The phycocyanin concentrations at the spillway, beach, and dock were 0.65 ± 0.02, 0.98 ± 0.07, and 0.98 ± 0.02, respectively, relative to the concentration found in the central part of the lake (Figure 2E). However, the water sample collected at Prairie Creek had a lower relative phycocyanin concentration (0.25 ± 0.01) than samples collected from the lake (relative is provided due to a calibration issue), which was also reflected in the lower concentration of total MCs (3.1 μg/L) (Figure 2E). Some homes along Prairie creek used aeration systems to disrupt the cyanobacteria bloom, which could have contributed to the reduction in the cyanotoxin concentrations in the water sample.[73,74] However, other factors like local nutrient loading[75] may also contribute to the concentration of cyanobacteria. Overall, the composite concentration of MCs detected via LC-MS/MS in the water samples have the same congeners present at similar concentrations to the average concentration of 15.0 μg/L detected from drone water collection conducted at the same time period.[61]

Targeted LC-MS/MS analysis found that the most abundant MC congener in the water samples was D-Asp^3-MC-RR, which was detected in the spillway, Prairie Creek, beach, dock, and central samples at concentrations of 9.8, 2.6, 10.2, 16.2, and 16.1 μg/L, respectively (Figure 2E). D-Asp^3-MC-RR accounted for 76 ± 6% of the total concentration of MCs detected in each sample. Two other major MCs that were detected were D-Asp^3-MC-HtyR and D-Asp^3-MC-LR, which made up 14 ± 5 and 8 ± 1% of the total concentration of MCs in the water samples (Figure 2D). The three most abundant congeners observed and their respective concentrations align with parallel measurements conducted during a similar time period by Hanlon et al.,[61] with the exception of MC-YR, which was later identified as D-Asp^3-MC-HtyR (noted above). An additional seven congeners (MC-RR, MC-HtyR, MC-LR, MC-HilR, MC-LA, MC-LY, and MC-LF) were detected at significantly lower concentrations, accounting for <2% of the total MC in each sample (Table S1). Among those seven are some of the more well-studied congeners (e.g., MC-LR and MC-RR),[16] which were not present in high concentrations, emphasizing the need to study a broad suite of congeners. One of the underlying similarities between the three major congeners

detected in water samples was that the aspartic acid residues were demethylated (D-Asp^3). Other than the results published by Hanlon et al.,[61] there has been no peer-reviewed literature identifying this modification in the MC structure at GLSM. However, in He et al.[76,77] the authors found a discrepancy between the concentrations of MCs determined by LC-MS/MS and ELISA in water samples collected at lakes in Ohio, including GLSM. The authors noted that MCs detected in Ohio inland lakes can be demethylated per their communication with the Ohio EPA, but to our knowledge, these observations have not yet been published in a peer-reviewed journal. Consequently, those authors were unable to quantify MC congeners using their LC-MS/MS method. This specific D-Asp^3 substitution in MCs has been previously observed in other cyanobacteria blooms, including ones composed of mainly *Planktothrix* sp.[51,78,79] Furthermore, cHABs containing *Planktothrix* sp. are relatively common, having been reported in 38% of freshwater blooms in the U.S.[49] In fact, the cHAB at GLSM has historically been driven by *Planktothrix* sp., but can have minor contributions from *Microcystis* sp.[57,58] *Planktothrix* sp. was identified as the dominant species present during the 2019 bloom through optical microscopy. Minor variation in the structures of MCs, such as demethylation and other substitutions (such as Dhb^7), can alter their biological activities.[50,78,80,81] For example, Hoeger et al.[50] demonstrated the strong binding efficiency between D-Asp^3-MC-RR to protein phosphatase 1, with a half-maximal inhibitory concentration (IC_{50}) of 3.8 nM, comparable to the highly potent MC-LR (IC_{50} = 2.5 nM). In contrast, the commercially available D-Asp^3-Dhb^7-MC-RR standard has a much higher LC_{50} of 56.4 nM.[50] However, it was noted that for mice the LD_{50} of D-Asp^3-MC-RR via intraperitoneal exposure was comparable to D-Asp^3-Dhb^7-MC-RR at ~250 nmol/kg. Shimizu et al.[82] exposed D-Asp^3-MC-RR and D-Asp^3-E-Dhb^7-MC-RR to the hepatocytes of mice, and their IC_{50} values were >10 and 4.95 μg/L, respectively. The Shimizu results suggest that D-Asp^3-MC-RR is less cytotoxic than D-Asp^3-E-Dhb^7-MC-RR, while Hoeger et al. suggest similar toxicity. Thus, the toxicological data discussed above for MC's is seemingly contradictory with respect to the different degrees of bioactive properties based on congener structure. This demonstrates the need to thoroughly characterize the MCs produced during cHABs and to better understand their toxicities. This is especially relevant to GLSM, given that two of the three congeners with the highest concentrations at GLSM do not have commercially available standards.

Detection of Aerosolized Microcystins.

Aerosolized MC concentrations varied spatially across different locations around GLSM based on filters collected with the $PM_{2.5}$ impactor. The two $PM_{2.5}$ samples with the highest MC concentrations (156 and 97 pg/m^3) were collected on separate days near the spillway from the boat and on land, respectively (Figure 3). Only D-Asp^3-MC-RR was detected in those $PM_{2.5}$ samples, which is also the primary congener in the water samples. The higher $PM_{2.5}$ MC concentrations near the spillway are likely due to the waterfall when lake water passes over the spillway, a mechanism similar to the established wave breaking/bubble bursting mechanism for sea and lake spray aerosol generation.[30,33] Potential health effects related to dams, which have a similar mechanism of aerosolization, were observed in Caller et al.,[83] for individuals living near dams. Overall, based on the higher concentrations of aerosolized MCs detected near the spillway (compared to other samples), it is important to understand the contribution of the spillway to higher MC aerosol concentrations at GLSM.

At the center of the lake, aerosolized MCs were not detected, perhaps due to the relatively calm winds during sample collection shown in Figure 3 and Bilyeu et al.[64] Sampling on the porch right after the storm event on 8/6/2019 also did not produce detectable levels of MCs. Even though the average wind speeds were sustainably >4 m/s, the samplers were not directly downwind from the lake due to the wind direction coming from the west in the afternoon (Figure 3A), which is in agreement with the noncontinuous wind measurements reported in Bilyeu et al.[64] Wind speeds during the spillway sampling from boat and land samples were between 2.3 and 3.1 m/s, respectively (Figure 3A). There is outflow from the lake 20 m upwind of the land-based spillway collection site, which could have contributed to the aerosolized MC concentrations, even though the site was 70 m upwind from the spillway. The wind speeds during sampling periods were often below the minimum threshold (typically >6 m/s in marine[84] and freshwater[85] systems) observed for whitecap formation, indicative of bubble bursting. However, whitecap formation was observed on Lake Michigan in Slade et al.[32] during time periods when wind speeds were as low as 3.5 m/s. Overall, the significant emission of aerosolized MCs from GLSM can occur even below the wind speed threshold for LSA generation.

Interestingly, the Prairie Creek $PM_{2.5}$ sample was collected right before a storm event on 8/6/2019, when the wind speed measurement in Celina was as high as 6.5 m/s (Figure 3A). D-Asp^3-MC-RR was detected with a concentration of 23 pg/m^3 despite the lower concentration of MCs in the Prairie Creek water sample relative to lake water samples (Figure 3B,C). On Prairie Creek, wind-driven wave breaking was not observed due to the surrounding trees and homes, even though the average wind speed was higher during that sampling period. Independent of wind speed, a possible contribution to the aerosolization of MCs could be the commercial aeration systems used by the homes along the creek to disperse the cyanobacteria. Aeration systems function by pumping air to the bottom of the aquatic system and forming air bubbles that rise to the surface,[86] emulating the bubble bursting associated with LSA generation process. The influence of aeration systems on ambient aerosolized MC concentration is supported by aerosol samples collected within the city of Celina Water Plant, where a high concentration of MCs was found in the aeration treatment room (828 pg/m^3) (Figure S11). These results indicate that there are additional factors, besides wind-driven wave breaking, that have the potential to emit more MC-containing aerosol locally and contribute to community exposure risk.

Size-resolved aerosol samples were collected at the porch, beach, and spillway. Higher airborne MC concentrations were observed on 8/6/2019 (both porch and beach sites), which were attributed to higher wind speeds (6.5 m/s), a storm, and winds coming off the lake (Figure 4). The aerosol sampled on the porch right before the storm had a total D-Asp^3-MC-RR concentration of 13 pg/m^3 with the highest concentrations between 0.44 and 0.77 μm (Figure 4B,C). In the porch sample immediately after the storm (30 m from shore), aerosolized D-Asp^3-MC-RR was still detected (1 pg/m^3) with the mode shifting to larger sizes (0.77 to 1.4 μm). The lower concentration is likely due to the wind direction shifting from southwest (lake) to northwest (farms) as well as lower wind speeds (4.7 m/s).

A beach sample was also collected both before and after the storm event (sealed during the period of precipitation) with the cascade impactor. The same three highest MC congeners

found in the water were also detected in the aerosol sample (Figure 4B). The total concentration of aerosolized MCs found in the sample was 4 pg/m^3 and a mode of 0.77 to 1.4 μm (Figure 4E). The lower airborne MC concentrations at the beach might have been due to the sampling time including both higher MC concentration (before the storm) and lower concentration (after the storm) time periods, as observed for the porch samples. The change in meteorological conditions could also explain the lack of MCs detected in the size-resolved samples on 8/7/2019, when the winds (0.1 to 2.6 m/s) were calmer and came from a westerly direction (Figure 4A). From the samples that had detectable levels of MC, the size-resolved concentrations reveal that 94 ± 5% of the total concentrations of MCs were associated with particles <2.5 μm, indicating that the $PM_{2.5}$ sampler likely captured most airborne MC (Figure 3). Additionally, the size range of MCs detected in the aerosol sample is evidence that the MCs incorporated into LSA are likely extracellular because the cyanobacteria (*Planktothrix* sp.)[57] at GLSM form filaments that are tens to hundreds of micrometers long.[87,88] The *Planktothrix* sp. filaments have also demonstrated to be highly resilient to shear stress and turbulent conditions,[89] and thus, are less likely to be broken up compared to other colonies of cyanobacteria like *Microcystis* sp.[90] The large size of these filaments (short atmospheric lifetimes),[91] coupled with the lack of MCs found in coarse particles, indicate that extracellular MCs are the dominant contributor to MCs in airborne exposure (both $PM_{2.5}$ (fine) and $PM_{10–2.5}$ (coarse)). Future studies will further explore the relationship between extracellular MCs in water and their aerosol concentrations.

Ambient Aerosolized Bacterial Genetic Material.

Aerosolized genetic material from bacteria, as determined by portions of 16S rDNA, was episodically observed at the beach and the porch site (Figure 5) between 8/5/2019 and 8/9/2019. Two types of aerosol samples were collected simultaneously: particles collected with a Bobcat sampler and size-resolved fractions collected with an Andersen impactor. On three of the 4 days, 16S rDNA was observed at levels above background with one sampler but not the other (Figure 5A). These concentrations ranged from 8.2×10^3 to 1.9×10^5 gc/m^3, summed across all size fractions (Figure 5B). 16S rDNA was found in size fractions <3.3 μm and not in larger ones (Figure 5C). On 1 day, 16S rDNA was not detected with either sampler. The gene that represents the total cyanobacteria was not detected in any aerosol samples. Previous studies have reported a range of 10^4–10^6 bacterial or bacteria-like particles/m^3 in outdoor air.[92–98] Considering that one bacterium usually possesses two to four 16S rDNA,[99] the actual total bacteria concentration in this study fell at the lower end of the reported range. Low levels likely contributed to seemingly inconsistent results. Concentrations of airborne bacteria depend on many factors, such as location, weather, season, and even air quality.[94–96,99] During the sampling campaign, the weather was usually clear and sunny with good air quality, conditions that have been associated with relatively low bacterial concentrations in air. Regarding particle size, Huffman et al.[100] reported a peak at around 2 μm for fluorescent biological aerosol, consistent with our finding of bacterial genetic material in particles <3.3 μm. However, Xie et al.[96] found bacteria in all size ranges. These differences can be attributed to different sampling and quantification methods, and regional variations can also contribute to these differences. The low concentrations of 16S rDNA in air and the absence of cyanobacteria DNA indicate that most MCs observed in the aerosol phase were likely extracellular and not contained within intact cells.

Overall, this study shows that MCs are aerosolized from GLSM, with detected concentrations between 1 and 156 pg/m^3 across different aerosol samplers. The aerosolized MC concentrations found in this study are comparable to concentrations reported in other ambient studies,[42–45,67] ranging from 0.090 to 2890 pg/m^3. While a high degree of variability in the aerosolized MC concentrations has been reported across various published works, the variability in airborne MCs found in this study can be attributed to meteorology and different LSA generation mechanisms. There were three likely pathways for aerosolization: natural wind-driven wave breaking, spillway, and aeration systems (Figure 1G), which led to different concentrations of airborne MCs. Despite the increased concentration of aerosolized MCs during the period of higher wind speeds found in the cascade impactors at the beach and porch, the concentrations were still lower than those in the $PM_{2.5}$ samples collected near the spillway on the western edge of the lake and the aeration systems on Prairie Creek. Size-resolved data showed that the MC-containing particles were primarily in $PM_{2.5}$, indicating the potential risk from inhalation.[41,101] Airborne MCs emitted are likely primarily extracellular based on size-resolved data and the patterns observed for airborne bacterial genetic material. The aerosol samples collected in this study were in proximity to the lake shoreline, with the furthest samples with detectable MCs collected 30 m, indicating the transport of MCs. The distance from the shoreline can potentially affect the concentration of aerosolized MCs. Sampling directly over the water, Murby and Haney[102] reported concentrations (up to 384 pg/m^3) of aerosolized MCs that were generally much higher than other published works. While the transport of microcystins from freshwater systems has not been well characterized, May et al.[39] found evidence of LSA in ambient particles collected >25 km from the shoreline. Given the sizes of most MC-containing particles found in the study, the toxins are likely transported downwind similar to LSA generated from other freshwater systems.[39,103] Therefore, further quantitative studies are needed to evaluate the long-range transport of aerosolized MCs. Future work is needed to explore additional congeners and peptides as well as intracellular versus extracellular toxins with respect to aerosolization. Overall, this study underscores that MCs present in $PM_{2.5}$ are most abundant in the accumulation mode with the longest atmospheric lifetimes. Therefore, further ambient measurements that account for additional factors that impact MCs and other aerosolized pollutants such as transport, distance from the shoreline, lofting into clouds,[40] and additional meteorological conditions are needed to comprehensively assess the environmental and public implications of aerosolized MCs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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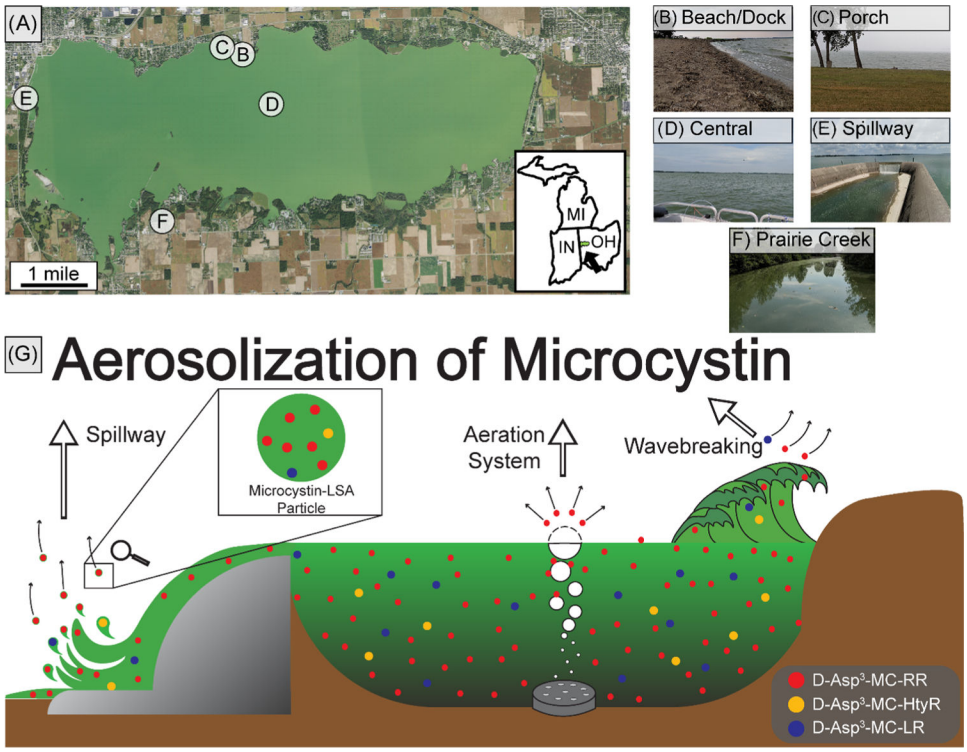

Figure 1.

(A) Satellite image of GLSM from the 2019 Ohio National Agriculture Imagery Program retrieved from the NOAA Office of Coastal Management. Inset is a diagram of Ohio, Michigan, and Indiana with an arrowing indicating the approximate location of GLSM. Pictures showing the approximate locations water and aerosol samples were collected near the (B) WSU-LC: beach/dock (40.5441°N, −84.5090°W), (C) WSU-LC: porch (40.5457°N, −84.5122°W), (D) the central location of GLSM (40.5295°N, −84.5026°W), (E) spillway (40.5345°N, −84.5740°W), and (F) Prairie Creek (40.5041°N, −84.5185°W). (G) Diagram illustrating the multiple mechanisms that can aerosolize MCs observed at GLSM. Image credits in (B–F) Dr. Andrew P. Ault.

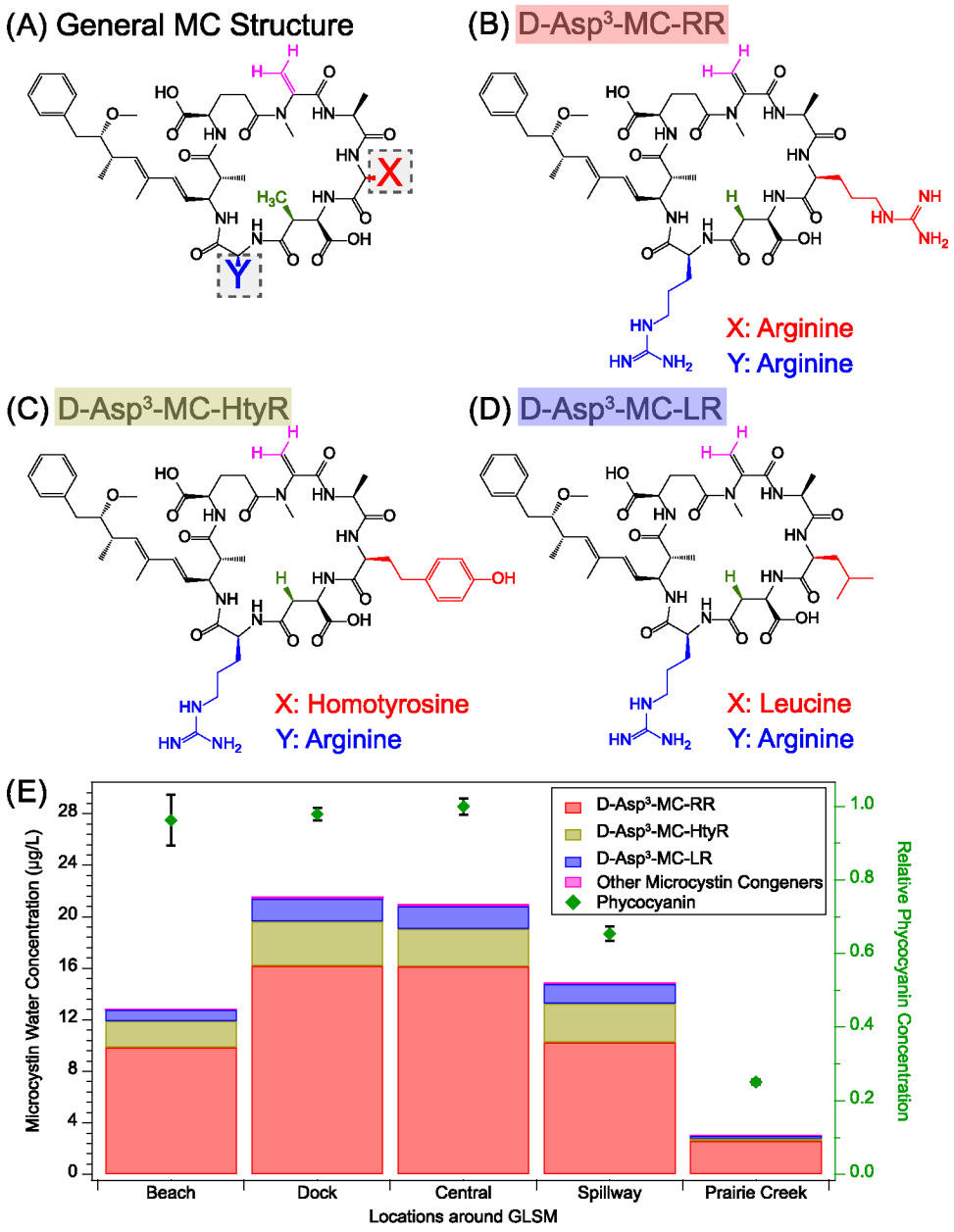

Figure 2.

(A) General cyclic structure of MC. (B–D) Structures of the three most concentrated MC congeners detected in water samples collected from GLSM. The Mdha is denoted in pink due to congeners being demethylated in that position, whereas the D-Asp^3-Dhb^7-MC-RR standard used for quantitative analysis is not. (E) Concentrations of the different MCs detected in water samples collected from GLSM and the relative phycocyanin levels. *MC standards are not commercially available; concentrations were determined using standards that most closely resembled the detected congeners.

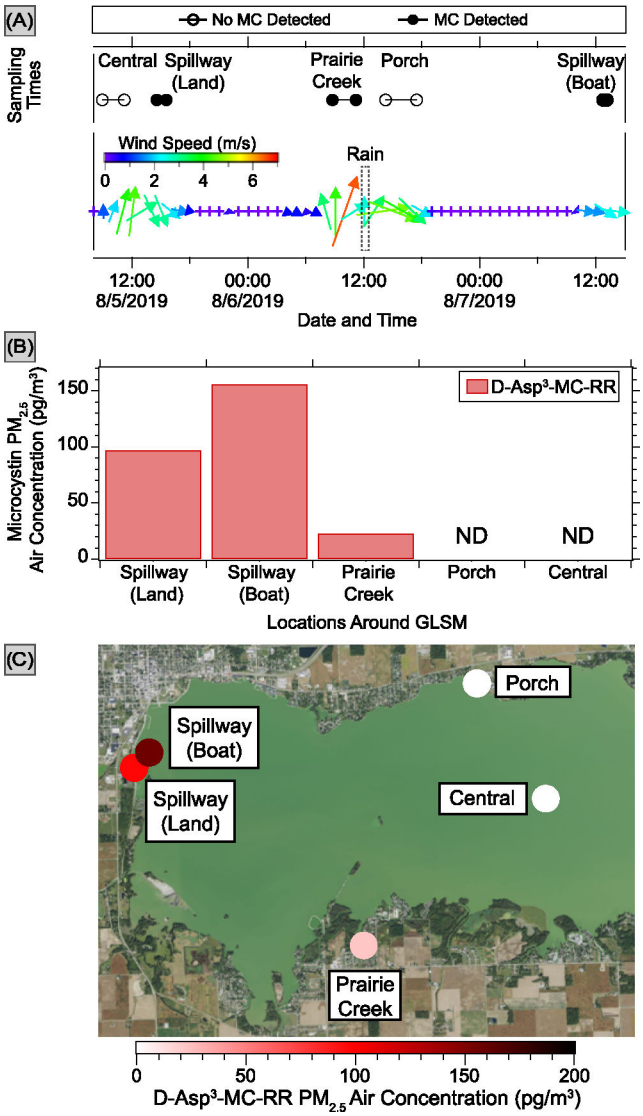

Figure 3.

(A) Sampling times for $PM_{2.5}$ samples with the corresponding wind speed and direction. The angles of the arrows point in the direction the wind was traveling toward. (B) The air concentrations of the MC congeners (D-Asp^3-MC-RR) were detected in $PM_{2.5}$ samples. Both the Porch and Central samples did not have detectable levels of MCs. ND = Not Detected. (C) Spatial variation of the detected MC around GLSM as colored markers overlaid on a satellite image from the 2019 Ohio National Agriculture Imagery Program retrieved from the NOAA Office of Coastal Management.

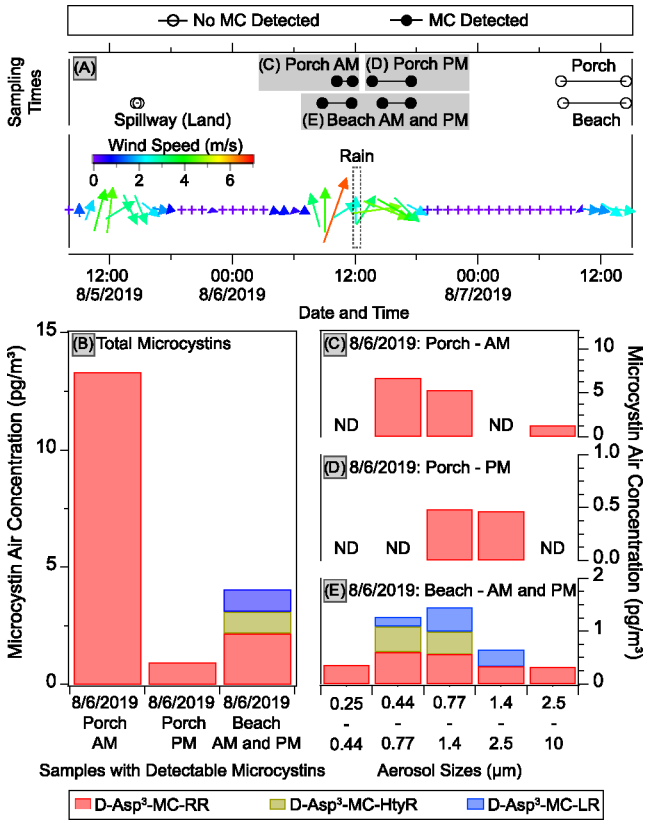

Figure 4.

(A) Wind speed and direction during cascade impactor sampling periods with the arrows pointing to the direction the wind was traveling toward. The + sign represents calm conditions (e.g., 0 m/s wind speed). (B) Total concentration of MCs detected on the TSI cascade impactor. (C–E) Size-resolved concentrations of aerosolized MCs sampled. ND = Not Detected. Size-resolved aerosol samples were briefly collected at the spillway, but insufficient sampling due to battery limitations led to an ND result.

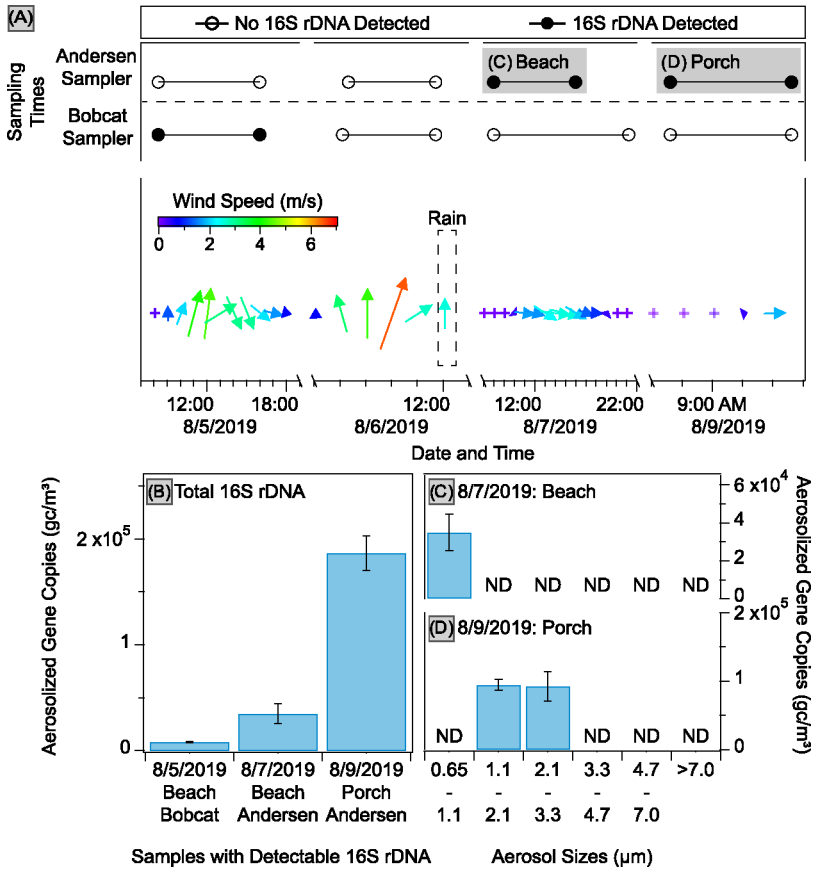

Figure 5.

(A) Sampling times for Andersen impactor and the Bobcat sampler with the average hourly wind measurements. Each arrow points to the direction the wind is traveling toward. Each tick represents 1 h. (B) Total 16S rDNA detected in aerosol samples collected. The size-resolved concentration of aerosolized 16S rDNA genes (B) 8/7/2019 at the beach and (C) 8/9/2019, porch (30 m inland). Error bars represent standard deviation of technical triplicates. ND = “Not Detected”.

Table 1.

Summary of Aerosol Samplers: Targeted Analytes, Sampling Locations, Sampling Time, and Sampling Volumes

sampler	collection material	analysis	particle size range	location	date	start time	end time	air volume sampled (m^3)
URG Corp $PM_{2.5}$ with cyclone	glass fiber filter	microcystins	<2.5 μm	central	8/5/2019	8:57	11:13	0.40
				spillway (land)	8/5/2019	14:35	15:35	0.18
				Prairie Creek	8/6/2019	8:46	11:12	0.44
				porch	8/6/2019	14:26	17:29	0.58
				spillway (boat)	8/7/2019	12:46	13:10	0.07
TSI high flow cascade impactor model 130	quartz fiber filter	microcystins	stage 1: 2.5–10 μm	spillway[a]	8/5/2019	14:35	14:43	1.5
						14:44	14:51	
				porch AM	8/6/2019	10:13	11:45	9.2
			stage 2: 1.4–2.5 μm	porch PM	8/6/2019	13:40	17:29	22.9
			stage 3: 0.77–1.4 μm	beach	8/6/2019	8:48	11:40	37.3
			stage 4: 0.44–0.77 μm	(AM and PM)[b]		14:16	17:29	
			stage 5: 0.25–0.44 μm	porch	8/7/2019	14:16	17:29	38.2
				beach	8/7/2019	8:06	14:28	36.8
				Celina Water Plant: aeration room	8/7/2019	16:08	20:57	28.9
Andersen cascade impactor	polycarbonate filter	16S rDNA	stage 1: 7.0+ μm	beach	8/5/2019	8:20	16:00	13
			stage 2: 4.7–7.0 μm	beach	8/6/2019	8:20	11:43	6
			stage 3: 3.3–4.7 μm					
			stage 4: 2.1–3.3 μm	beach	8/7/2019	8:00	21:12	22
			stage 5: 1.1–2.1 μm					
			stage 6: 0.65–1.1 μm	porch	8/9/2019	7:37	11:37	7
Bobcat air sampler	electret filter	16S rDNA	<10 μm	beach	8/5/2019	8:20	16:00	92
				beach	8/6/2019	8:20	11:43	41
				beach	8/7/2019	8:00	21:12	158
				porch	8/9/2019	7:37	11:37	48

[a] Sampler was halted for 1 min due to electrical issues.

[b] The sampler was halted prior to the storm (with filters secured within) and sealed; sampling resumed after the storm.