# Preliminary Report on Air Sampling of Particle-Associated Microcystins and BMAA Pilot Study in Lee County, Florida: Fall 2018 – Winter 2019


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Project Description. Extensive *Microcystis* blooms occurred in the tidal Caloosahatchee River and in adjoining Cape Coral canals in southwest Florida in late summer/early fall 2018. The Florida Department of Environmental Protection conducted monthly sampling in the area during the bloom, and reported the presence of *Microcystis aeruginosa* and microcystin concentrations as high as 46 µg $L^{-1}$ in their September 17, 2018 sampling event (https://floridadep.gov/dear/algal-bloom/content/algal-bloom-sampling-results). Exposure to concentrations >20 µg $L^{-1}$ in recreational waters are considered to be "high risk" for acute health effects by the World Health Organization (https://www.epa.gov/nutrient-policy-data/guidelines-and-recommendations). *Microcystis* (and most cyanobacteria) are also known to produce β-N-methylamino-L-alanine (BMAA), which is thought to be a factor in some neurodegenerative disorders (e.g., ALS).

Knowing that microcystins and BMAA were present in the waters affected by the cyanobacteria bloom, people were concerned that they were being exposed to these compounds via inhalation of aerosols or particles (i.e., "algal dust"). Discussions with Lori Backer (CDC) and a thorough review of the literature revealed that evidence for an airborne vector for microcystin and BMAA was sparse and often inconclusive (e.g., Codd et al. 1999; Cox et al. 2009; Backer et al. 2010; Chernov et al. 2017). To that end, we initiated a pilot study to determine 1) if microcystins and BMAA were present in airborne aerosols or particles; and 2) the sizes of particles these compounds were associated with. The latter is particularly important as the smaller the particle, the deeper in the lungs the particle can get and the more likely any associated toxins (or other potentially bioactive compounds) can get into the bloodstream (i.e., if particles can enter the alveoli). As people are still being exposed to *Microcystis* in some areas of Cape Coral and along the Caloosahatchee River, there is an urgent need to determine if airborne particles (or aerosols) are an exposure vector that needs to be addressed and accounted for when assessing risks to human health in the region.

Project Implementation. We used Anderson Impactor air samplers for this pilot study (Figure 1). These samplers offer many advantages to other methods, and provide additional data to allow us to test our primary question: is inhalation a viable vector for microcystin and BMAA exposure in humans? Firstly, the samplers size-fractionate the air-borne particles to allow us to assess if/and how many particles can enter the respiratory tract, and to what degree (i.e., head to bronchi to alveoli). Secondly, the vacuum pumps that suck the air into the samplers are calibrated, which allowed us to calculate the volume of air samplers and the concentration of the particles (and microcystins and BMAA) in the air.

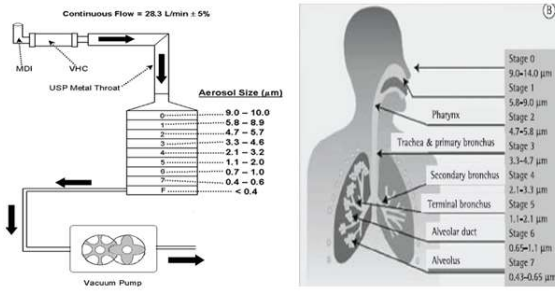


Figure 1. Schematic representation of the impactor sampler and the Section of the respiratory tract represented by each stage. Image provided by PharmaTutor.

Two deployments were conducted for this study. The first involved the deployment of two air samplers. One sampler was deployed next to an ongoing *Microcystis* bloom, and another at a control site upwind of the bloom) for 20 days (September 21 – October 11, 2018). The "bloom site" was a residential property on a dead end canal in Cape Coral (26.59861 N; 81.93139 W; Figures 2 & 3). A *Microcystis* bloom was evident at the site at the initiation of sampling (but past the peak in biomass earlier in the month; Figure 4), but waned over the course of the sampling period (Figure 5). The "control site" was the FGCU Vester Field Station in Bonita Springs, FL (26.33055 N; 81.8375 W; Figure 6). No cyanobacteria blooms were evident in the vicinity of the field station during the course of the sampling period.

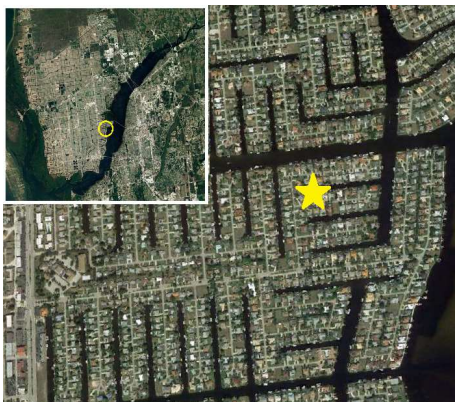

Figure 2. Location of Cape Coral "bloom site" at a residential property on a dead end canal.

We also collected water samples before and after each deployment at each site (50 mL Falcon® centrifuge tubes; four total each sampling trip) to determine toxin concentrations in the water during and after deployment. The filters (one for each stage in each sampler) and water samples were tested for microcystins and BMAA using Enzo Life Sciences ELISA microcystin test kits (http://www.enzolifesciences .com/ALX-850-319/ microcystins-adda-specific-elisa-kit/) and ABRAXIS ELISA BMAA test kits (https://www.abraxiskits.com/ uploads/products/docfiles/387_BMAA% 20R010213.pdf).

Wind data were also collected from the Page Field Airport in Fort Myers from weather.gov (station KFMY) to determine the predominant wind directions and speeds during sampler deployment.

Figure 3. Deployment of air sampler at Cape Coral residence. (Adam Catasus, graduate student, FGCU).

Figure 4. *Microcystis* bloom present at the Cape Coral study site at the beginning of the air sampler deployment on September 21, 2018.

Figure 5. The *Microcystis* bloom waned over the course of the sampling period at the Cape Coral study site (photograph taken on October 11, 2018).

The second deployment took place from December 21, 2018 through January 18, 2019 (28 days) at the FGCU Buckingham campus (Figure 7). This area was chosen because 1) it is a secure location (part of FGCU); and 2) it is at least 2 km away from any "large" bodies of water (i.e., those that show up on Google Earth). The purpose of this second deployment was to test new filters (Whatman filter paper 41) to reduce potential signal noise in the analysis. We also obtained two more air samplers, so four samplers in total were placed at the Buckingham property on a picnic table at an outside pavilion (Figure 8). The use of four samplers provided more material to examine for microcystins and BMAA (and possibly qPCR to detect and quantify *Microcystis*), as well as the ability to test replicates (and reproducibility).

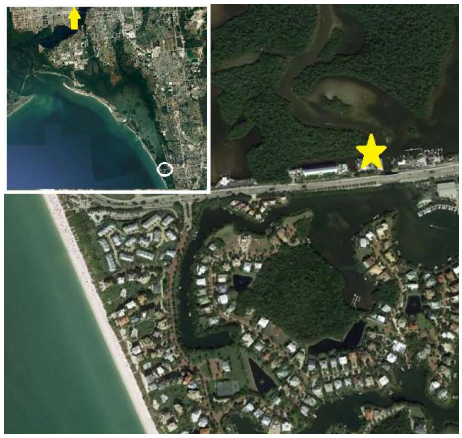

Figure 6. Location of the Vester Field Station "control site" in Bonita Springs, Florida.


Figure 7. Location of the Buckingham campus. The red scale bar = 2 km. The white circle shows the location of the Buckingham campus. The yellow star shows the location of the pavilion the air samplers were set up under.

Figure 8. A view of the area, samplers, and pavilion at the Buckingham campus.

Findings. First deployment. The ELISA test kits meet all QA/QC standards needed for this study. Calibration curves worked well, positive controls were within range, and negative controls were non-detect. The microcystin standard provided by the manufacturer produced a concentration value of 0.6 (µg $L^{-1}$), within the acceptable range of 0.75 ± 0.185 µg $L^{-1}$. The blank produced a (false) microcystin value of 0.05 µg $L^{-1}$, below the established limit of detection for the analysis (0.15 µg $L^{-1}$).

Analysis of the filter samples from the control and bloom sites indicated that microcystins and BMAA were detected at both sites, and concentrations were not statistically different between the two sites (Figure 7). Both compounds were present in all size fractions tested at both locations (Table 1 & Figure 8), and were not consistently higher in any size fraction between the sites. Also note that the compounds were detected on the filters with the smallest pore sizes (< 1.0 µm), which

indicates these compounds can reach the alveoli, and therefore have access to the respiratory pathway into the bloodstream.

Microcystins and BMAA were found in the water samples at both locations (Table 2), albeit at lower concentrations at the control site versus the bloom site, particularly in regard to microcystin. Microcystin levels were 5,627 µg $L^{-1}$ at the Cape Coral site at the initiation of air sampling on September 21, 2018 (approximately 200x higher than the 20 µg $L^{-1}$ "high risk" threshold for recreational waters established by the World Health Organization (Table 2). As the bloom waned over the 20-day period, however, microcystin concentrations fell to 1.2 µg $L^{-1}$, and were through below this threshold value. While the Vester Field Station water sample collected at the initiation of sampling was not tested, the microcystin concentrations in water at the termination of the sampling were found to be very low (0.1 µg $L^{-1}$).

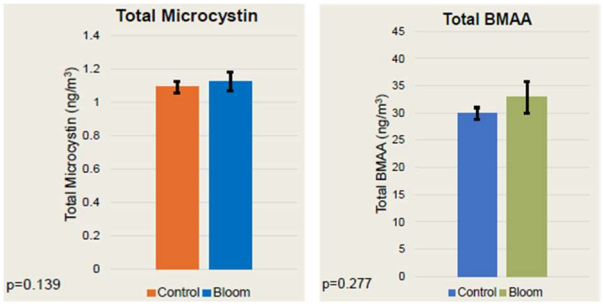


Figure 7. Comparison of total microcystin and BMAA concentrations at Vester (Control) and Cape Coral (Bloom).

Table 1. Microcystin concentrations measured in the air impactor stages from the "Bloom" site (Cape Coral) and "Control" site (Vester Field Station). Note that no filter was used in Stage 0 of the "Control" sampler.

| Cape Coral (Bloom) Sample (respiratory system equivalent) | microcystin concentration (ng/m³) | Vester Field Station (Control) Sample | microcystin concentration (ng/m³) |
|---|---|---|---|
| Stage 7 (head) | 0.1937 | Stage 7 (head) | 0.1656 |
| Stage 6 (head) | 0.2095 | Stage 6 (head) | 0.1631 |
| Stage 5 (pharynx) | 0.1560 | Stage 5 (pharynx) | 0.1663 |
| Stage 4 (trachea) | 0.1209 | Stage 4 (trachea) | 0.1639 |
| Stage 3 (secondary bronchi) | 0.1083 | Stage 3 (secondary bronchi) | 0.1300 |
| Stage 2 (terminal bronchi) | 0.1236 | Stage 2 (terminal bronchi) | 0.1349 |
| Stage 1 (alveoli) | 0.0635 | Stage 1 (alveoli) | 0.0531 |
| Stage 0 (alveoli) | 0.0572 | Stage 0 (alveoli) | not used |
| Stage F (alveoli) | 0.0826 | Stage F (alveoli) | 0.1112 |

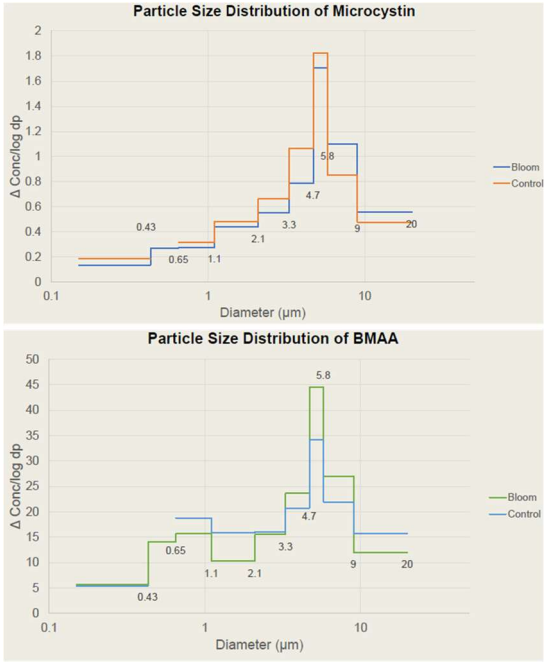


Figure 8. Difference in toxin concentration between Vester (Control) and Cape Coral (Bloom) size fractions (filter pore diameters in µm).

The home owner who graciously allowed us to set up the air sampler at their residence had saved the HVAC filters and an air mask that were used during the height of the cyanobacteria bloom in late August – early September, 2018. The microcystin concentrations were higher on these filters, but also represent a composite sample (i.e., equal to the sum of most of the filter stages (0 – 5) of the air samplers. While the different nature of sampling between the air samplers and HVAC filters (and dust mask) prevent direct comparison of the microcystin and BMAA concentrations, the fact that both compounds were detected indicates the compounds were associated with particles inside the residence.

Analysis of the Page Field airport wind data indicated that the predominant wind direction over the course of the study was East South East (ESE; 99.65°). Therefore, it is not likely that the detection of microcystins and BMAA at the control site (Vester) was due to wind transport from the bloom at Cape Coral.

Second deployment. The filters from two of the air samplers (one Yale unit; one FGCU unit) were analyzed for toxins. Microcystin extracts were run in triplicate; BMAA in duplicate (more extract volume was needed for the BMAA analysis). The standard curves for both analyses were >0.99, indicating that toxin quantification data are highly accurate. For both compounds, concentrations were far lower than seen in the fall; approximately 10 times lower for microcystin and 5 times lower for BMAA. Interestingly, however, toxins were still detected, and at all size fractions.

Table 2. Concentrations of microcystin and BMAA from water samples at the two study sites at the beginning and end of deployment.

| Sample | Microcystin (µg/l) | BMAA (µg/l) |
|---|---|---|
| Control Site | 0.09625 | 1241 |
| Bloom Site, during bloom | 5627 | NA |
| Bloom Site, after bloom | 1.207 | 1562 |

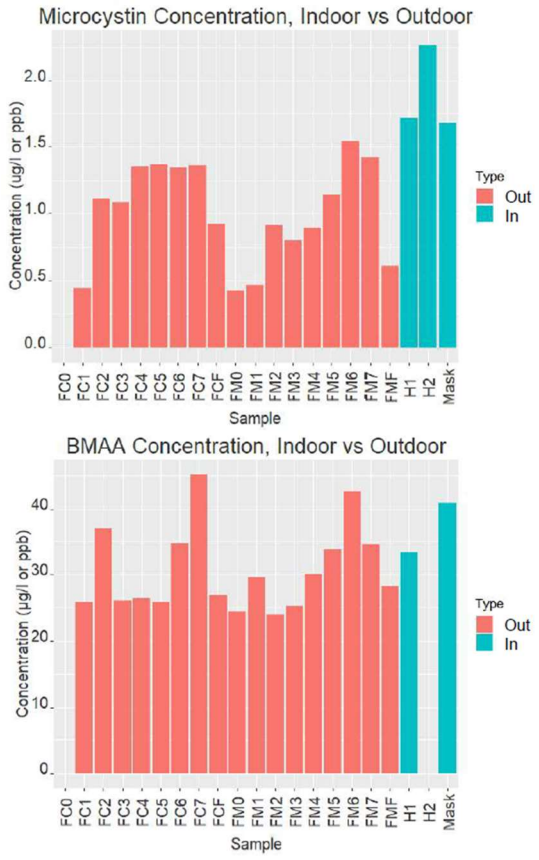


Figure 9. Microcystin and BMAA concentrations from the air samples (FC and FM; Out; red) and the HVAC (H1 and H2) and dust mask (Mask) filters from Cape Coral residence.

Interpretation of Results. Although these results are preliminary, the findings presented herein are significant. The most important finding was that microcystins and BMAA were found in association with particles that can travel through the respiratory tract to the alveoli (Figure 8 and Table 1), demonstrating that 1) microcystins and BMAA can be airborne; and 2) inhalation is a viable vector for microcystin and BMAA exposure in humans. Secondly, both compounds were

detected at both locations in the fall, albeit at low concentrations. The second deployment at Buckingham also detected both compounds at all size fractions, but at much lower concentrations (10x and 5x lower for microcystin and BMAA, respectively). These findings suggest that we measured "elevated" concentrations of microcystins and BMAA in the fall, and that lower background concentrations (i.e., present in the everyday environment) were documented in the winter (Buckingham) deployment. Although concentrations were "elevated" in the fall, microcystin concentrations were very low (<0.21 ng $m^{-3}$; not known if BMAA concentrations are considered low or not due to lack of health-related data). Additionally, the Vester Field Station (located south of Cape Coral) was upwind of Cape Coral during the course of the fall study, indicating that the Cape Coral cyanobacteria bloom was not likely the source of the compounds at filtered at Vester. Additionally, the fact that microcystin and BMAA levels were similar at both locations in the fall indicates there was no expected "dilution effect" if Cape Coral was a source of the particles (i.e., we would expect microcystin and BMAA concentrations to be higher near the source).

Our fall sampling effort occurred during the waning periods of this bloom in late September/early October, as depicted by the low microcystin concentrations measured at the end of the sampling (1.2 µg $L^{-1}$). As the *Microcystis* bloom was much larger in August and early September, it is likely that toxin-laden particles would have been higher during the peak of the bloom. In a study of freshwater lakes in California, however, Backer et al. (2010) reported that airborne microcystins were not correlated with *Microcystis* cell concentrations, indicating that the production of airborne microcystins appears to be more complicated than being a simple function of cell concentrations.

Conclusions. This pilot study demonstrated that microcystins and BMAA are associated with airborne particles capable of being inhaled deep into the lungs (the alveoli), thereby representing a possible vector of exposure in humans. This work expands upon other studies that have demonstrated that microcystins and BMAA can aerosolize and be inhaled (Backer et al. 2010; Cox et al. 2009) by providing more data on the size fractionation of the particles with which microcystins and BMAA were associated. By doing so, the argument for an inhalation-based vector of exposure is further strengthened, meriting further examination and assessment.

The other significant findings of this pilot study was that 1) microcystin and BMAA concentrations were similar in the fall at the "Bloom" and "Control" sites, suggesting that the source of these compounds may be more ubiquitous to the region (i.e., anywhere near water); and 2) the lower concentrations measured at Buckingham in the winter suggests that levels may be lower inland away from water bodies (and the potential cyanobacterial source of the compounds) And/or in the winter/dry season (when cyanobacteria biomass is lower). Further research is needed to assess whether these concentrations represent any chronic health risks, as has been reported in other studies; e.g., Zhang et al. (2015) reporting a 3% increase in non-alcoholic liver disease for every 10% increase in cyanobacteria bloom coverage in their study of freshwater ponds and lakes and nearby communities across the United States.

This study was a pilot study; future deployments will expand temporal and spatial coverage and improve statistical/analytical power. Inclusion of other research expertise (e.g., epidemiology) will also allow us to put results in context with human health exposure risk assessment.

References

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