Relationships between Volatile organic compounds and harmful algal blooms: Can VOCs be used as a time-sensitive and economical means for predicting bloom dynamics?

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Introduction
Freshwater harmful algal blooms (HABs) are an expected occurrence during the summer months in the eutrophic Upper Klamath River Watershed. Agency Lake (AL) and Upper Klamath Lake (UKL) experience transitioning blooms that are dominated by the cyanobacterial genus Anabaena. Aphanothece, Gloeotrichia, and Microcystis, HABs can negatively influence the ecology, potability, and recreation opportunities of these freshwater systems, and are themselves impacted by nutrient loading, temperature, pH, and a myriad of other environmental factors. The interactions of HABs and volatile organic compounds (VOCs) are scarcely understood. An individual cyanobacterial strain can produce a wide range of VOCs depending on the form of nutrients that are available; some VOCs can inhibit growth of competing phytoplankton (1). However, in situ studies on the impacts of VOCs in freshwater systems and how they are influenced by biotic and physicochemical properties are rare (2). The full spectrum of these VOCs may be unique to community composition or seasons, and could pose additional risk to humans health and crop production due to their contributions to ground ozone formation (3). To address this lack of knowledge, we carried out an exploratory study to determine how VOC production, environmental variables, and microbial community are interconnected in UKL and AL. We envision that a full analysis of these interactions could be facilitated by machine learning that will yield a quick and efficient monitoring practice that may have predictive capacity for HAB dynamics.

Methods
Samples were obtained at 1 site within UKL and AL, chosen based on their proximity to existing monitoring sites (Figure 1). Sampling was conducted May-December of 2018 and 2019. Abiotic properties were collected (chlorophyll, carbon, nitrogen, toxin concentrations, ozone, conductivity, pH, etc.) and are being processed either in house or through the Institute for Water & Watersheds’ Collaboratory on Oregon State University campus. DNA samples were obtained via filtration for community analysis by 16S rRNA sequencing using MiSeq at OSU’s Center for Genome Research and Biocomputing. To obtain VOC profiles (the full spectrum of VOCs in water samples) 100 mL of sample was bubbled to “strip” the VOCs from the water and feed the VOCs into a PTR-FID (see methods flow chart for details). The samples were run in triplicate with blanks of HPLC grade water as controls for the profiles. Unigene cultures were also gathered from each genus of interest and VOCs were being assessed in both stationary and exponential phases. To avoid samples and isolate data will be combined to use for unsupervised training of a convolutional neural network. Multivariant statistics and complex network theory are being used in parallel to elucidate connections between community, environmental variables, and VOCs.

Discussion and Future Work
VOCs profiles from the 2018 season cluster based on season rather than site (Figure 2), and specific VOCs show seasonality with a steady decreasing/increasing in correlation with each successive sampling (Figure 3). Enriched unialgal cultures show that specific VOCs accumulate to a higher extent in exponential compared to stationary phases of growth. This result supports our idea that bloom phase is an important aspect to our analysis. We are using machine learning, specifically a convolutional neural network, to predict bloom dynamics. To circumvent our problem of a small training set, the enrichments are being supplemented with the environmental data and a protein mined model from a similar arc of marine species and VOC profiles from the North Atlantic Aerosols and Marine Ecosystems Study (NAAMES) is being used as the model.

Further research and data processing is underway including 16S sequencing, processing of several environmental parameters, and incorporation of continuous monitoring data obtained from deployed buoys to account for bloom phase. Data processing of several environmental parameters, and incorporation of continuous monitoring data obtained from deployed buoys to account for bloom phase. Data processing of several environmental parameters, and incorporation of continuous monitoring data obtained from deployed buoys to account for bloom phase.

References

Notes:
- VOCs are being assessed in both stationary and exponential phases.
- To address this lack of knowledge, we carried out an exploratory study.
- DNA samples were obtained via filtration for community analysis by 16S rRNA sequencing.
- Samples were run in triplicate with blanks of HPLC grade water as controls.
- Enriched unialgal cultures show that specific VOCs accumulate to a higher extent in exponential compared to stationary phases of growth.
- We are using machine learning, specifically a convolutional neural network, to predict bloom dynamics.
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