Presence of Harmful Cyanobacteria in Local Water Bodies

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Abstract

Harmful algal blooms (HABs) have become a significant issue over the past few years, resulting in the contamination of drinking water and public health hazards. In order to study the presence of these harmful bacteria in Knox County, water samples were collected in two sampling rounds and filtered from twenty-six unique ponds, wetlands, and lakes on both public and private properties. ArcGIS software was used to create theoretical buffer zones around each site in order classify a site as either agricultural or non-agricultural. On site tests were conducted to determine DO, water temperature, pH, and conductivity. The on site tests revealed no significant differences between agricultural and non-agricultural sites. Water samples were collected at each site and filtered in the lab. Chemical tests were performed to determine microcystin (a hepatotoxin produced primarily by the cyanbacteria *Microcystis*) levels. The was no significant difference between agricultural and non-agricultural sites. The filters were prepared for qPCR (targeting PC-IGS gene) sequencing and 16S barcoding to determine if *M. aeruginosa* (another toxin producing cyanobacteria) was present in the samples. Further chemical tests were run to determine nutrient (phosphate, ammonia, and nitrate) levels for each site. Nutrient detection tests revealed no significant difference between agricultural and non-agricultural sites. Select samples were cultured and studied using phase contrast and light microscopy.

Introduction

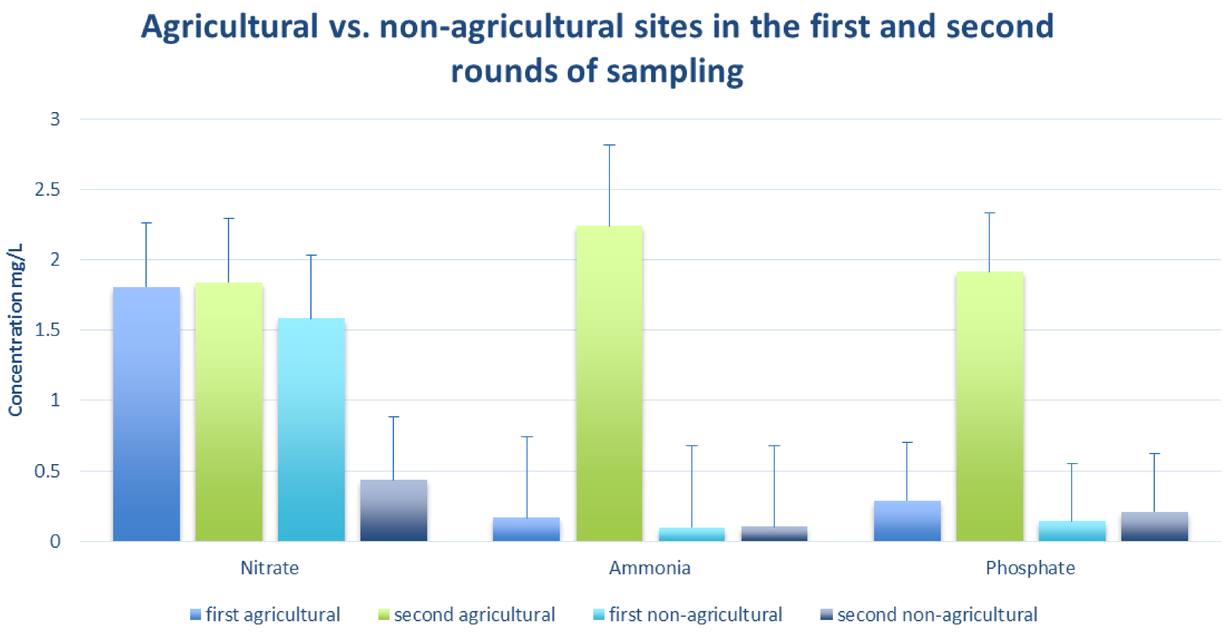
- *Microcystis* is toxin producing genus of cyanobacteria that contributes to the formation of HABs (Harmful Algal Blooms)[1]
  - From August 2-4 2014 the city of Toledo, OH was without consumable tap water due to elevated levels of microcystin in the Western Basin of Lake Erie caused by a large HAB[2,3]
  - Microcystin is a cyclic peptide which means it is stable at high temperatures and as a result the toxin cannot be boiled off[3]
- The presence of HABs in large bodies of water is well documented
  - However, the presence of these toxic cyanobacteria in small ponds, wetlands, lakes, etc. is mostly unstudied
  - Eutrophication is known to contribute to the presence of HABs
  - An influx of excess nutrient allows for the unrestrained growth of cyanobacteria resulting in HABs[4]
- Where are these excess nutrients coming from?
  - When fertilizer is applied to crops a portion of the nutrients are washed away by rain which then drains into either the groundwater or surface water bodies (like the sample sites)
- In order to study agricultural runoff and its effect on the presence of *Microcystis* in local water bodies several factors were analyzed
  - Buffer zones were created to classify sites as either agricultural or non-agricultural
  - Concentration of essential nutrients (nitrate, ammonia, phosphorus) were tested in the lab
  - On site general data (DO mg/L, DO% saturation, pH, conductivity, water temperature) were collected
  - Samples were sent to OSU for microcystin and genetic analysis

Methods

Collection of samples: Samples were collected at the surface with a Nasco Swing Sampler. The bottle (1000 mL) was rinsed with 1HCl:2H_2O acid-wash in between samplings to remove nutrients from previous sites. Water samples were placed in 24-oz Whirl-Pak® stand-up bags. Two bags were filled per site. Each site was sampled twice. This is an ongoing project. A third round of sampling is currently being conducted.
On site tests: A pH meter was used to collect data on pH and conductivity and was calibrated prior to each sampling session. A DO (dissolved oxygen) meter was used to collect data on DO mg/L, DO% saturation and water temperature and was calibrated at each site.
Filtration: Samples were filtered using sterile Microfil® V Filtration devices (Millipore) and membrane filters (Isopore TM membrane filter; 0.4 µm HTTP; Millipore). Filtration was conducted in quadruplicate, two filters were kept by the Kenyon lab and two were sent to OSU. The amount of water filtered from site to site varied due to the amount of material in the sample (generally 20-100 mL per filter). The filters were placed in 2-mL sterile centrifuge tubes and stored at -80°C.
Nutrient tests: Hach® water chemistry kits (nitrate (high and low), ammonia (high and low), and phosphate (high and low)) were used to determine the nutrient levels of filtered water samples.
Creation of buffer zones: GIS mapping was used to create 100 m and 1000 m buffer zones for each site. Sites were then classified as either agricultural or non-agricultural using estimated percentages of agricultural land use. Sites with an estimated agricultural land use of greater than 33% or sites with direct drainage from tiled crop fields is classified as agricultural. Sites with an estimated agricultural land use of less than 33% is classified as non-agricultural (excepting the Gambier Fishing Club which had direct drainage from a tiled crop.
Microcystin levels and qPCR: Using the sample water (20 mL) from each site Dr. Lee's lab at OSU was able to determine the concentration of microcystin at each site. Using the duplicate filters the lab was able to determine the concentration of *M. aeruginosa* (includes both toxin and non-toxin producing bacteria) at each site. The results from the first shipment were received; the second set of data is not yet complete.

Results

Average Essential Nutrient Levels



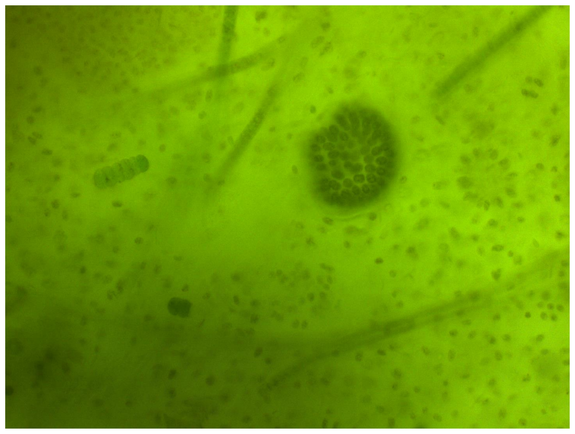
Agricultural vs. non-agricultural sites in the first and second rounds of sampling. This graph is representative of the average essential nutrient levels of each site based on its type (agricultural vs. non-agricultural) and time of sampling (first or second round). Nutrient levels for individual sites were obtained through the use of Hech® water chemistry kits on filtered water samples. The nutrient levels for each grouping were averaged and standard error bars for each nutrient calculated. T-tests performed in R-Studio revealed that there was no significant difference between agricultural and non-agricultural sites in relation to any of the nutrient levels. There was also no statistical difference between groups in regards to DO% saturation, pH, conductivity or water temperature.

Microcystin

Site	Type	Concentration of microcystin (µg/L)
Fishing Club (July)	agricultural	13.441
Bennet	agricultural	0.208
Co-op	agricultural	0.195
Fishing Club (June)	agricultural	0.174
Lee	agricultural	0.172
Foundation 2	non-agricultural	BDL
Rice	non-agricultural	BDL
Blackjack	non-agricultural	BDL
Sacks	agricultural	BDL
Green Cemetery	non-agricultural	BDL
John BFEC	non-agricultural	BDL
Lawhon	agricultural	BDL
Foundation 3	non-agricultural	BDL
Foundation 2	non-agricultural	BDL
Dharma 2	agricultural	BDL
Reynolds	agricultural	BDL
Dharma 1	agricultural	BDL
Wolfrun	non-agricultural	BDL

Microcystin concentration. This table is representative of the first set of microcystin concentration data received from OSU. Sites are classified as either agricultural or non-agricultural. A t-test performed in R-Studio revealed that there was no significant difference between agricultural and non-agricultural sites in relation to microcystin concentration. However, it should be noted that only agricultural sites had detectable levels of microcystin. All other sites were BDL (Below Detection Level).

Microscopy and Sites



The top image was obtained with light microscopy (400x). The dark dense cluster of cells in top center of the image is similar in structure to the kind of formations that *Microcystis* is known to arrange itself into. The sample depicted came from the Gambier Fishing Club in July. The bottom image was taken at the same time the sample in the top image was being collected. The bottom image depicts the peak bloom experienced at the site. The coloration and other characteristics indicate the presence of blue-greens. This sample also revealed elevated microcystin levels. Therefore it is probable that cluster of cells depicted in the top image are *Microcystis* cells.

Conclusion

- Based solely on a statistical analysis of the data it would appear that agricultural sites were not different from non-agricultural sites
  - However these data are still incomplete
  - A third round of sampling is currently being conducted
  - The qPCR and microcystin data is incomplete as well
- Whether or not a site had more than 33% estimated agricultural land use is not as relevant as whether or not a site had direct drainage from a tiled crop field
- The Bennet site has an effluent pipe that drains directly from a tiled crop field into the site
  - In the first and second round of sampling the Bennet pond had by far the highest nitrate concentration at 16.3 mg/L and 12.0 mg/L, approximately double the next highest reading 7.7 mg/L and 6.7 mg/L respectively
  - It had the second highest level of the toxin (0.208 µg/L)
- The Gambier Fishing Club was the most drastic site
  - The owners have had problems with algal blooms since the installation of tiles three years ago in the crop fields that drain directly into the pond
  - As illustrated in the photograph, the coloring is characteristic of blue-green algae (like *Microcystis*)
  - The mat emitted a strong odor when disturbed, similar to that of fertilizers and animal waste, characteristic of blue-green algae
  - In the second sampling, the site had very high levels of ammonia (30.0 mg/L) and phosphate (21.7 mg/L)
  - The most important data is the microcystin concentration (13.441 µg/L) from the second sampling, over fifty times greater than the next highest (Bennet 0.208 µg/L)
  - At this level of microcystin, a Public Health Advisory should be issued, meaning that direct contact with the water should avoided as much as possible[6]
- The results indicate that percent land use does not influence the presence of Microcystis but direct drainage from tiled fields may
  - In a future study it could prove useful to compare sites with direct drainage from tiled fields to sites without direct drainage

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