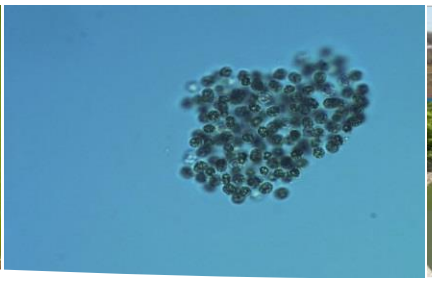
U.S. Environmental Projection Agency, Office of Research and Development

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM




Introduction to Microcystins

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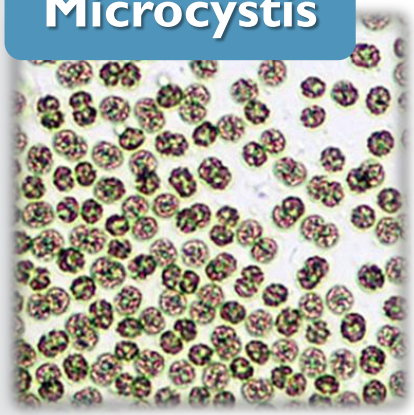
2016 OCTO Water Laboratory Analyst Workshop
May 2016

Harmful Algal Blooms (HABs)

- HABs exact a cost of approximately $2.2B annually in the US, including costs associated with restricted use of recreational waters, declining waterfront real estate value, spending on recovery of biodiversity, and drinking water treatment.
- Wide variety of taxa can produce blooms
- Typically detrimental to the aquatic system and can be harmful to humans and land animals (contact and consumption)
- Blooms are dependent on numerous factors, including nutrient loading, temperature, water flow and weather patterns

Microcystin Toxin Producers

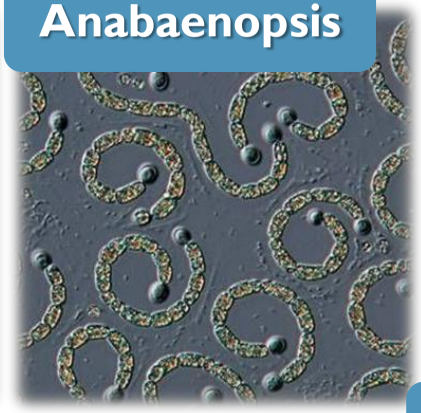
Microcystis



Oscillatoria

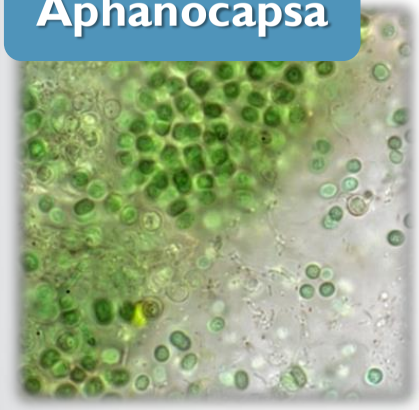
Nostoc

Anabaenopsis

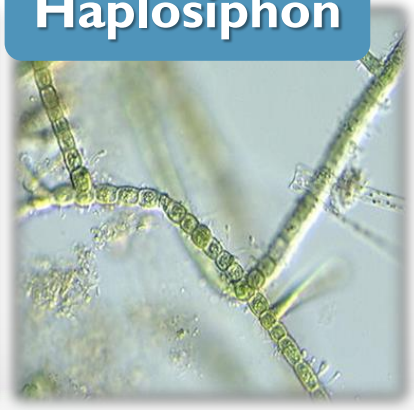


Synechococcus

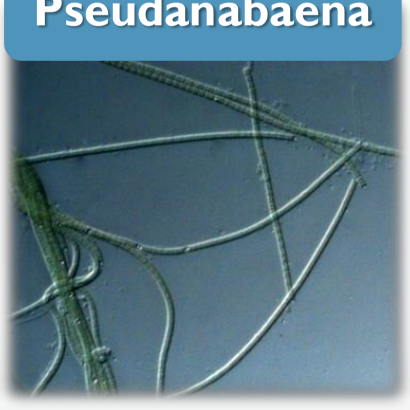
Aphanocapsa



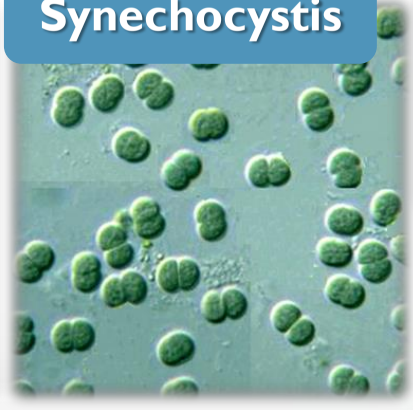
Haplosiphon



Pseudanabaena



Synechocystis



Possible Health Effects

- Drinking/Swallowing HABs-Contaminated Water
  - Severe diarrhea and vomiting
  - Liver toxicity (abnormal liver function, abdominal pain)
  - Kidney toxicity
  - Neurotoxicity (weakness, salivation, tingly fingers, numbness, dizziness)
  - Difficulty breathing
  - Death

- Skin Contact with HABs-Contaminated Water
  - Rashes
  - Hives
  - Skin blisters

Ohio In 2013 and 2014

Celina (population 10,400)

Summer 2013: > 100 µg/L total microcystins and nodularin in treatment plant influent

Carroll Township (population 2,000)

September 4, 2013 = 1.4 ug/L

September 5, 2013 = 3.6 ug/L

Switched to emergency connection with Ottawa County

Began flushing distribution system

Ohio EPA's first "Do Not Drink" advisory issued due to microcystin

On Advisory 48 Hours

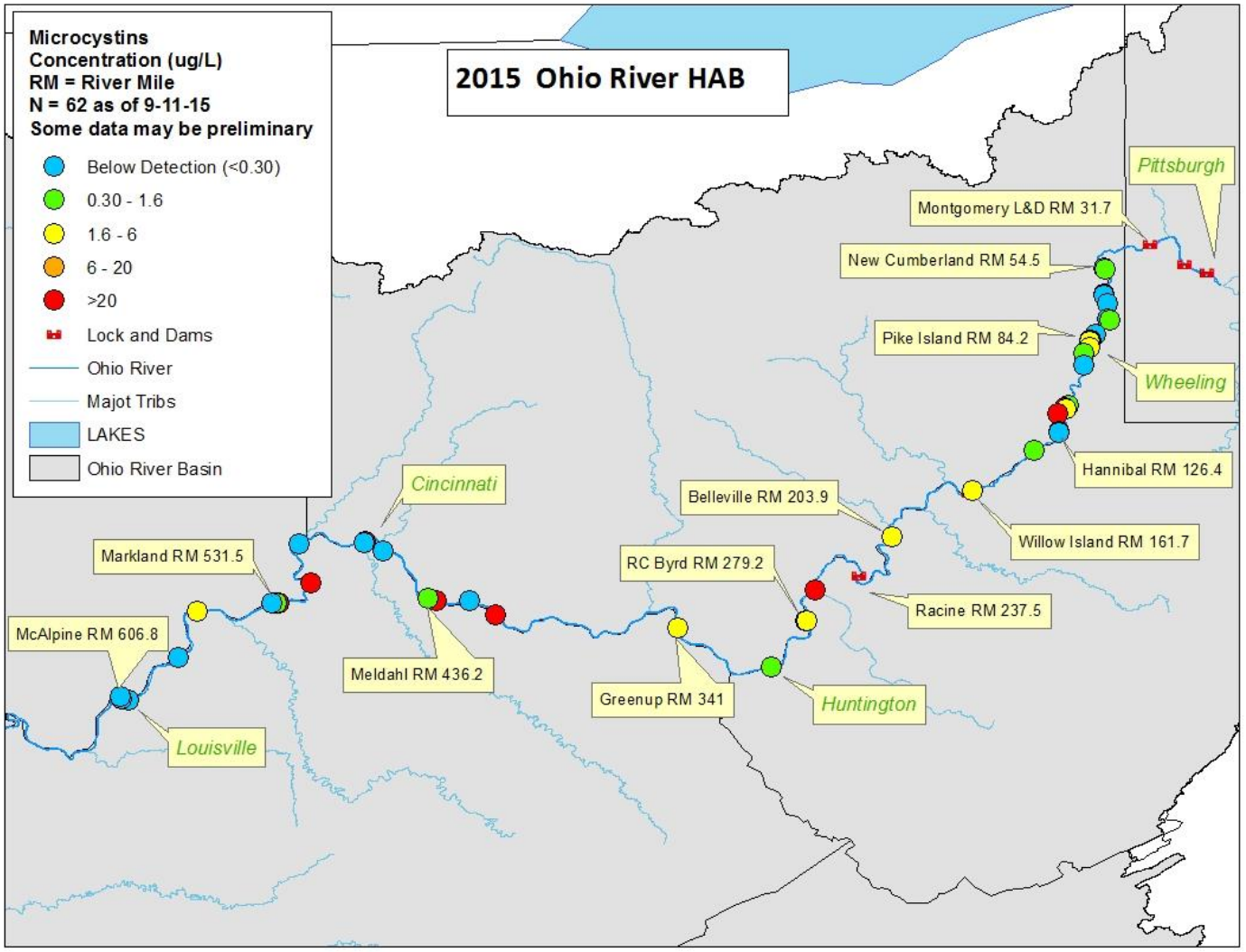
Toledo (population ~500,000)

September 2013: Detectable, but < 1 µg/L toxin in finished water

August 2014: >1 µg/L total microcystins and nodularin in finished water,

Ohio EPA "Do Not Drink" advisory

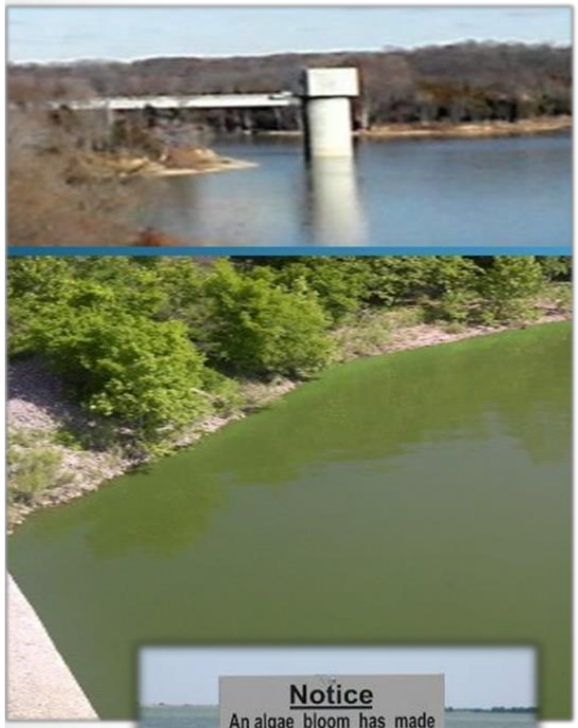
2015 Ohio River Bloom



Source: Ohio River Sanitation Commission

- **Approximate Dates:**
  August 19th through October 29th, 2015
- **Main Contributor:**
  Microcystis aerugenosa
- ***3x10^7 cells/ml***
  1x10^5 typically referenced as level for water impairment
- **Numerous recreational water advisories**

Current Regulations/Guidance



- 21 states have recreational water guidelines for harmful algae blooms
- Three states (MN, OH, and OR) have implemented standards or guidelines that apply to cyanotoxins in drinking water
- EPA's Office of Water has released its Health Advisory Level for microcystin-LR and cylindrospermopsin
- EPA's informational webpage http://www2.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-cyanohabs

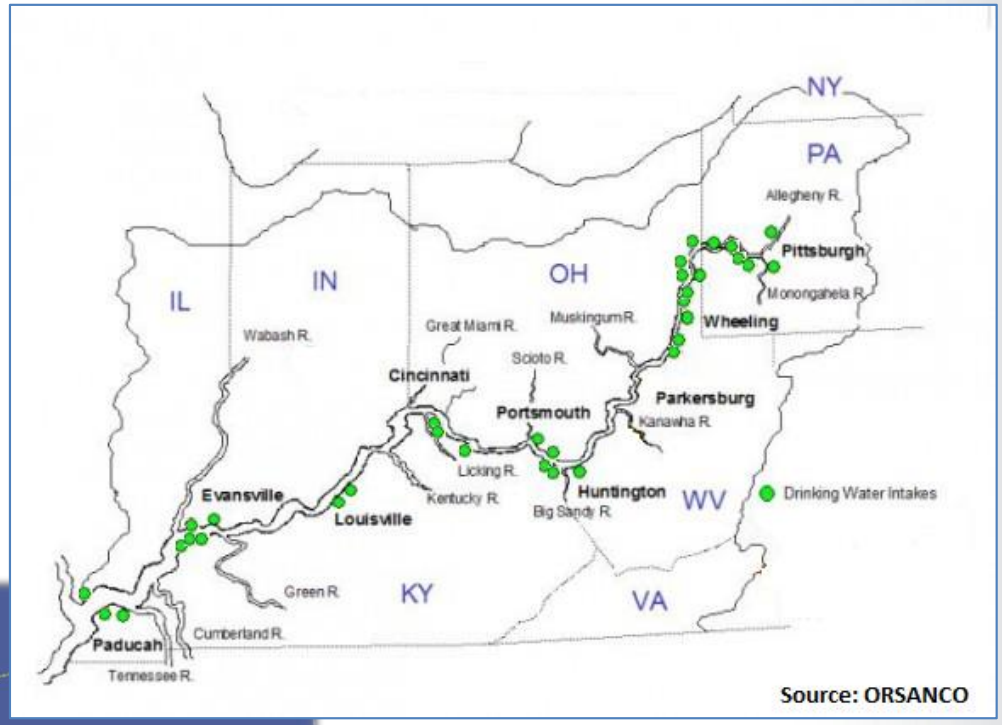
Current Regulations/Guidance

- State Health Advisory Levels
  - OH
    - "Do not Drink" Advisories"
      - 0.3 µg/L Tot-MYC Child < 6 yr & Sensitive Pop
      - 1.6 ug/L Tot-MYC Child > 6yr & Adults
    - "Do Not Use" Advisory
      - 20 ug/L
  - MN 0.04 µg/L as MYC-LR
  - OR 1.0 µg/L as MYC-LR
- Health Advisory Level for microcystin-LR
  Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins
  - 0.3 µg/L 10-Day Infants/Young Children
  - 1.6 µg/L 10-Day Adults
- The Harmful Algal Bloom and Hypoxia Research and Control Amendments Act of 2014
  - Delegates primary responsibility to NOAA in advancing the scientific understanding and ability to detect, monitor, assess, and predict HAB and hypoxia events in marine and freshwater
- Safe Drinking Water Act
  - Contaminant Candidate List 4 (draft) includes cyanotoxins
    - Microcystin-LR
  - Unregulated Contaminant Monitoring Rule (2018-2020)
    - US EPA Method 544

Lake Erie and the Ohio River are Major Drinking Water Sources

29 Ohio and 2 Michigan communities intake water directly from Lake Erie (Western and Central Basins)

Subsequent communities purchase either treated or untreated water from these primary DWTPs and may subject the water to further treatment



The Ohio River is a major source of drinking water along its entire reach

Source Water Impacts on Drinking Water

Problems facing the drinking water industry:

- Excessive nitrogen and phosphorous levels can cause harmful algal blooms
  - Agriculture (non-point source) is often the largest contributor of nitrogen load into waterways
- Forecasting is difficult because algal/cyanobacteria strains bloom under different conditions at different times
- Additionally, an algal bloom may not necessarily produce toxins

Algal blooms put pressure on drinking water facilities, requiring:

- Immediate operational changes (i.e. PAC addition) can be costly and effectiveness not well understood
- Possible shut-off of services
- Costly facility upgrades

Source Water Impacts on Drinking Water

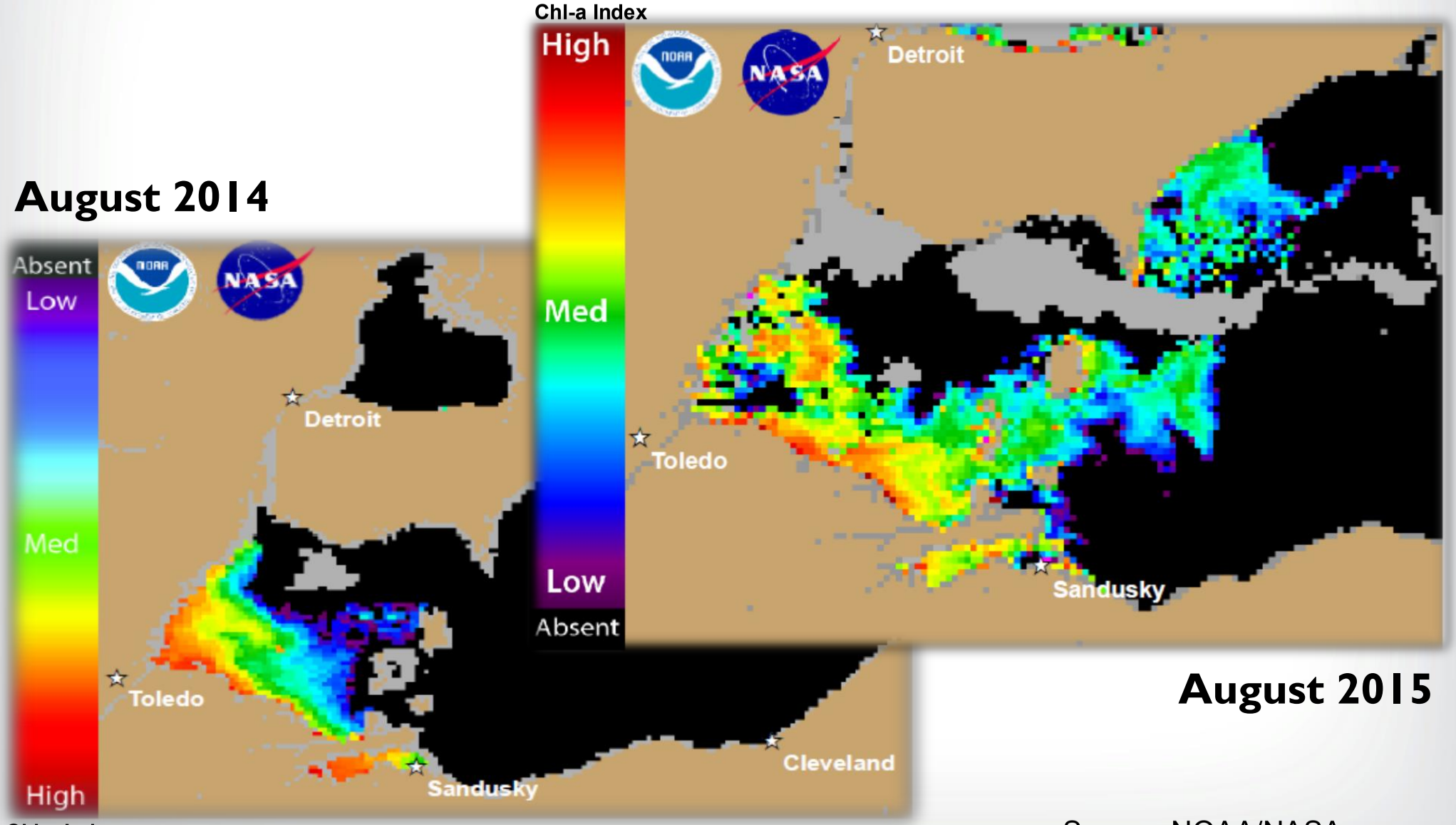
Possible Indicators of HABs:

- An increase in the presence of taste and odor due to MIB and geosmin, both of which can be produced concurrently with MYCs
- Increase in pH, especially during sunlight hours
- Increase in turbidity
- Decrease in filter run times
- Increase in chlorine demand or decrease in chlorine residual
- Increase in disinfection by-products

Possible Surrogate Measures of HABs:

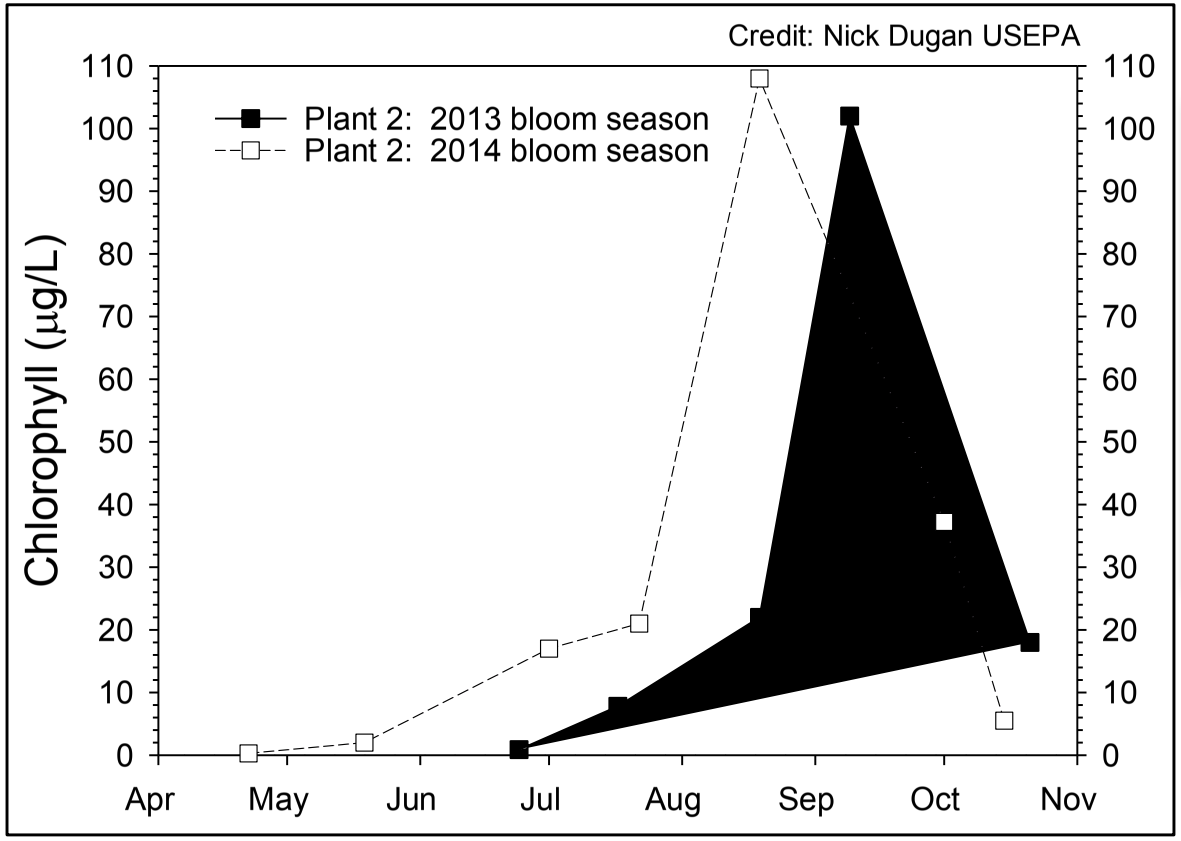
- Chlorophyll-a
- Phytocyanin
- MIB and Geosmin

Lake Erie Blooms 2014 vs. 2015 - Toxic vs. Non-Toxic Producing Blooms



Source: NOAA/NASA

Seasonal Bloom Dynamics



- Peak of a bloom season does not necessarily occur at the same time in a given year
- There may be more than one major bloom event

Microcystin Toxin Variants

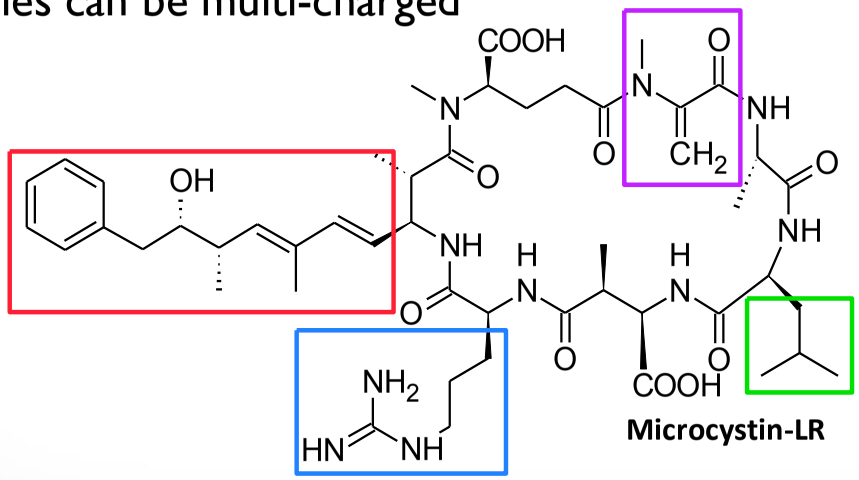
- MYCs are heptapeptides
- Varying strains produce different toxins at different rates and quantities
- Exist in multiple variants

  94+ known microcystin variants

  Significant differences in hydrophobicity

  and pKa

  Species can be multi-charged



Microcystin-LR

All MYCs include the ADDA (3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyl-4(E), 6(E)-decadienoic acid, red) and methyldehydroalanine (MDHA, purple) modified amino acids. Leucine (green) and arginine (blue) residues are sites of structural diversity, referred to as positions X and Z, respectively.

Variants differ in potency

Estimated cytotoxic IC_{50} values

MC variants name	IC_{50} (µg/mL)
[D-Asp^3, *Z*-Dhb^7] MC-LR	0.053
[D-Asp^3, *Z*-Dhb^7] MC-HtyR	0.120
[D-Asp^3, *E*-Dhb^7] MC-LR	0.133
[D-Asp^3, Dha^7] MC-LR	0.217
[D-Asp^3] MC-LR	0.217
[Dha^7] MC-LR	0.217
[D-Asp^3, *E*-Dhb^7] MC-HtyR	0.327
[D-Asp^3] MC-HtyR	0.347
[Dha^7] MC-YR	0.418
MC-LR	0.800
MC-YR	1.48
[D-Asp^3, Dha^7] MC-RR	4.11
[D-Asp^3, *E*-Dhb^7] MC-RR	4.95
[Dha^7] MC-RR	5.33
[D-Asp^3] MC-RR	>10
MC-RR	>10

Shimizu, Kumiko, et al. *Toxins* 6.1 (2013): 168-179.

Methods for Analysis

Non-Targeted Analysis

- Enzyme Linked Immunosorbent Assay (ELISA)
- Protein Phosphatase Inhibition Assay (PPIA)
- Liquid Chromatography - Photodiode Array Detection (LC-PDA)
- Derivatization
  - Gas Chromatograph
  - LC
- Microelectrodes
- Spatial analysis
  - Satellite Imaging/Aerial reconnaissance

Targeted Analysis

- LC - Mass Spectrometry Detection (LC-MS/MS)
- Thin Layer Chromatography (TLC)
- Liquid Chromatography Photodiode Array Detection (LC-PDA) *

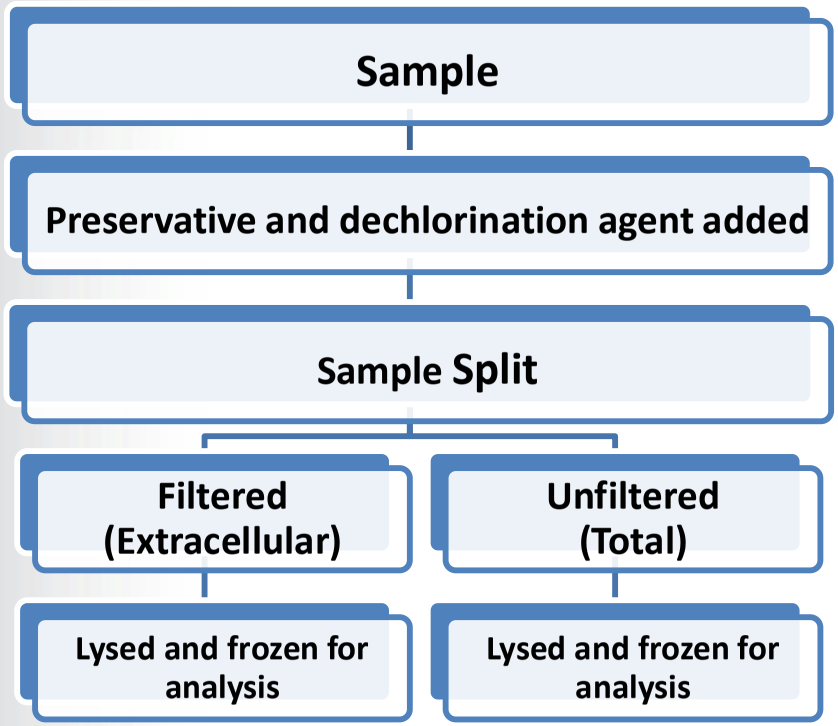
Measures the total microcystin concentration

- Broad-based method
- No differentiation between variants

Measures each individual microcystin concentration

- Total determined by sum of each
- Limited to reference standard availability

Sample Handling and Analytical Methods - Example



ELISA

- Broad-based method
  (measures total toxin concentration - All variants)
- Easy and relatively inexpensive

LC-MS/MS

- US EPA Method 544 measures 6 microcystin variants and Nodularin
- Several LC-MS/MS methods have been published by various venders
- Standards from various venders are currently available for approximately13 total variants (as of Nov 2015)

Other Analysis

- Chlorophyll A
- Phycocyanin
- Water Quality Parameters (i.e. Nutrients, Metals)

Sample Considerations

- Total vs. Dissolved
  - Filter
- In-Situ vs. ex-situ measures
  - Storage and preservation
- Total measurement time
  - Transportation
  - Sample preparation
  - Sample throughput
  - Reporting time
- Cost
- Level of expertise
- Availability of Instrumentation
- Sample Preparation

None < Filtering < Cell Lysing < Concentration < Derivatization

- Level of Sensitivity
- Selectivity
- Level of QA/QC

State of Ohio Action Plan for HABs

Ohio EPA Published a set of guidelines and rules

- Ohio Revised Code 3745.50 - Bill passed July 2015
  - Delegated and authorized Ohio EPA to serve as the coordinator of harmful algae management and response and implement an action plan

Relevant publications

- "Public Water System Harmful Algal Bloom Response." July 2015
- "Generalized Cyanotoxin Treatment Optimization Recommendations." March 2016

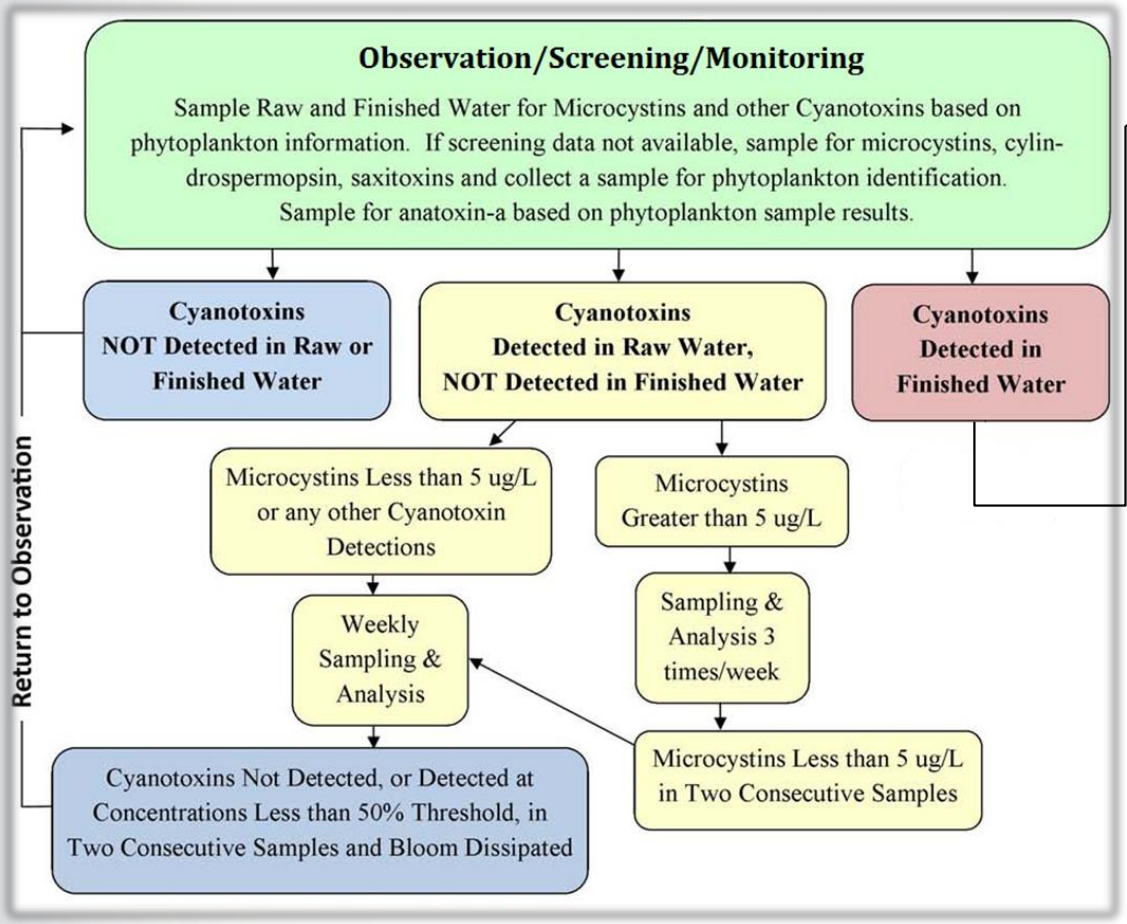
Ohio EPA – Recommendations for Monitoring for HABs in Source Waters

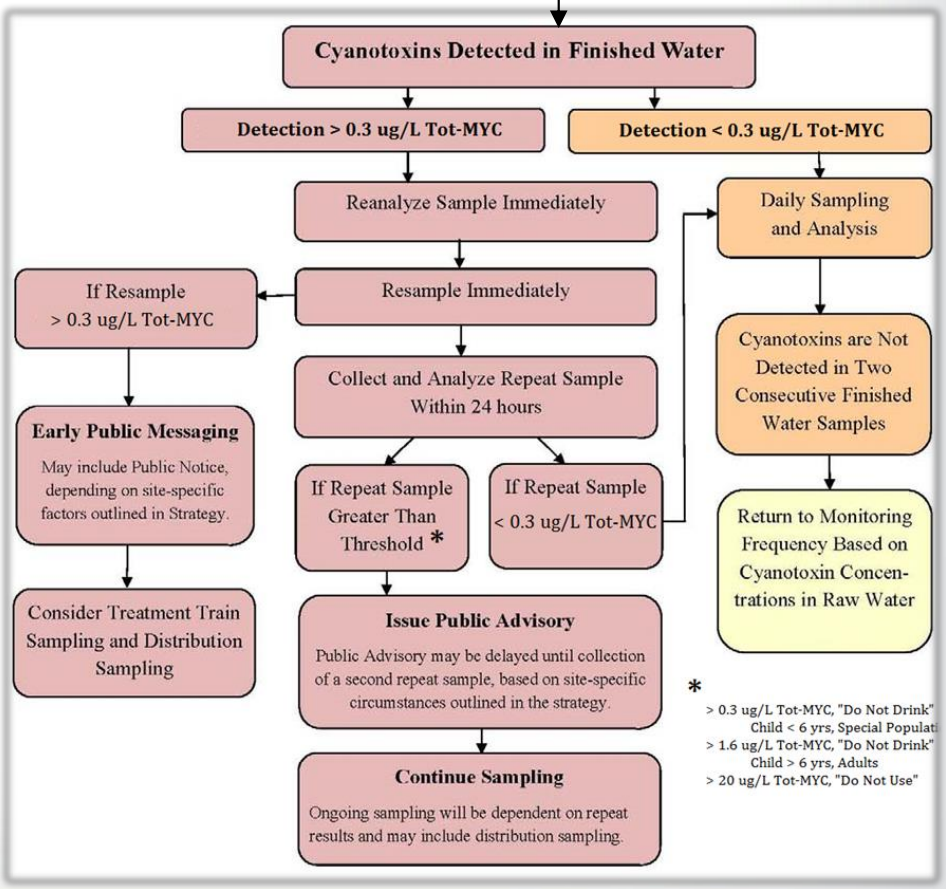
- Monitoring
  - Cyanobacteria cell count (or phycocyanin equivalents*) > 100,000 cells/mL
  - Biovolume > 10 mm^3 /L
  - Chlorophyll-a > 50 µg/L
  - Scum or surface accumulation is present and/or significant concentration of cells are visible throughout the water column
  - Presence of cyanotoxins, as indicated by test kit or laboratory analyses
  - Satellite imaging indicating a spike in Chl-a concentration
  - Operator observations (i.e. deviation from normal operation
- Methodology
  - ELISA Microcystins-ADDA method (total microcystins)
  - USEPA Method 544 is not recommended, not internally validated

Source: Ohio EPA "Public Water System Harmful Algal Bloom Response." July 2015

Ohio EPA – HAB Monitoring and Drinking Water Action Plan

Public Water Systems HAB Response Action Plan





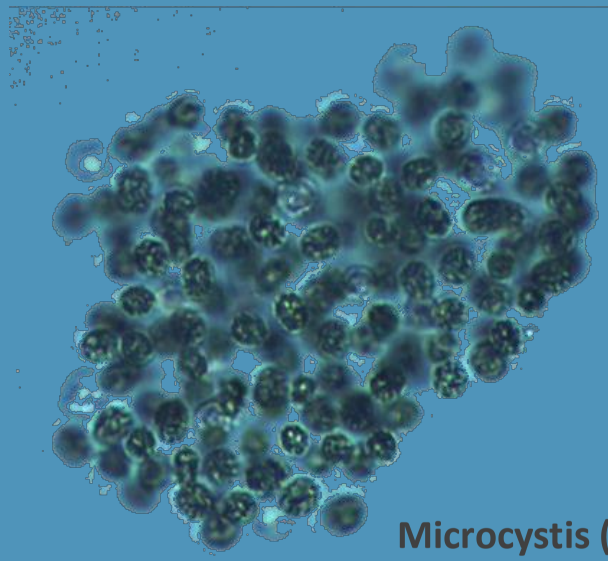
Source: Ohio EPA "Public Water System Harmful Algal Bloom Response." July 2015

Dissolved or Particulate?

Toxin within the cell and those that are dissolved require different sample processing and treatment

Particulates (toxin in cell)

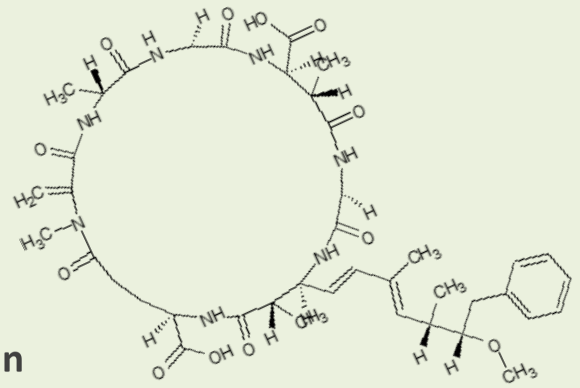
- Solids removal processes effective
- Do not want to lyse cell or toxin will be released



Microcystis (cells)

Dissolved (toxin released from cell)

- Solids removal processes ineffective
- Typical disinfectants or dosages may not be effective (e.g., permanganate, chlorine)
- More effective treatments are expensive and plants typically do not have them in place (e.g., GAC)



Microcystin Toxin

Ohio EPA – Recommendations for Conventional Treatment

Recommendations for intact cell removal when algal toxins are found primarily INTERCELLULAR

- Reduce or eliminate pre-oxidant addition prior to filtration
  - Avoid lysing of cells and release of toxin
- Optimize coagulation for particulate removal.
- Increase the sludge removal frequency.
- Increase the filter backwash frequency, do not recycle backwash.
- Increase PAC feed, use appropriate PAC
  - Wood-based for MYCs
- When possible, a slight reduction in pH is recommended
  - MYC degradation is greater when pH < 8.
- Increase free chlorine and contact time in clearwell to assist in toxin destruction
  - Increase chlorine residual 0.5-1.0 ppm, max residual of 4.0 ppm (total chlorine)

Source: Ohio EPA "Generalized Cyanotoxin Treatment Optimization Recommendations." March 2016

Ohio EPA – Recommendations for Conventional Treatment

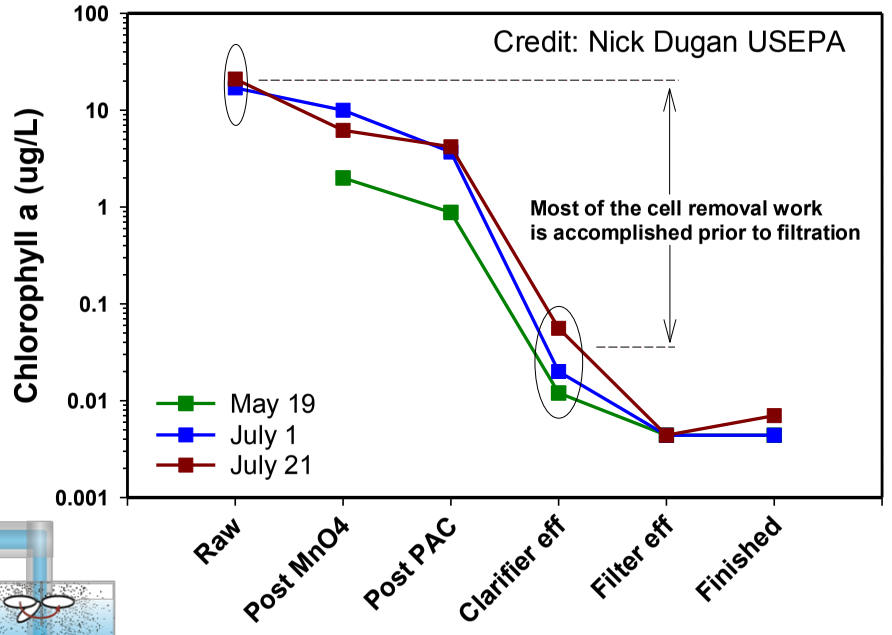
Recommendations when algal toxins are found primarily EXTRACELLULAR

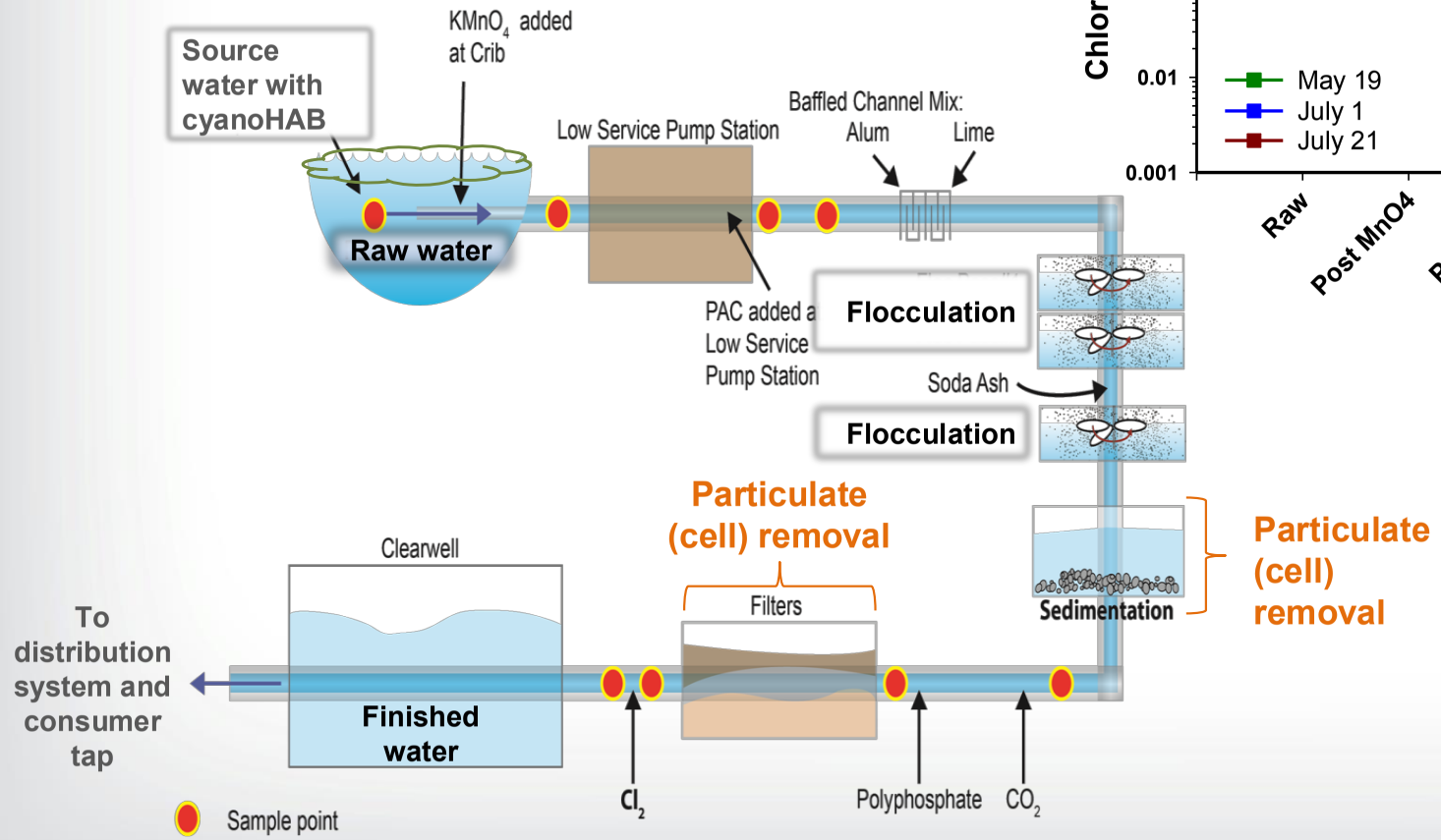
- Utilize pre-oxidation with chlorine or permanganate and maximize contact time for destruction of cyanotoxins
- Consider pre-filtration oxidant switch to chlorine and/or permanganate
  - Chlorine dioxide and chloramines are ineffective for toxin mitigation

Source: Ohio EPA "Generalized Cyanotoxin Treatment Optimization Recommendations." March 2016

Biomass is Effectively Removed Post-Clarification in Conventional Treatment

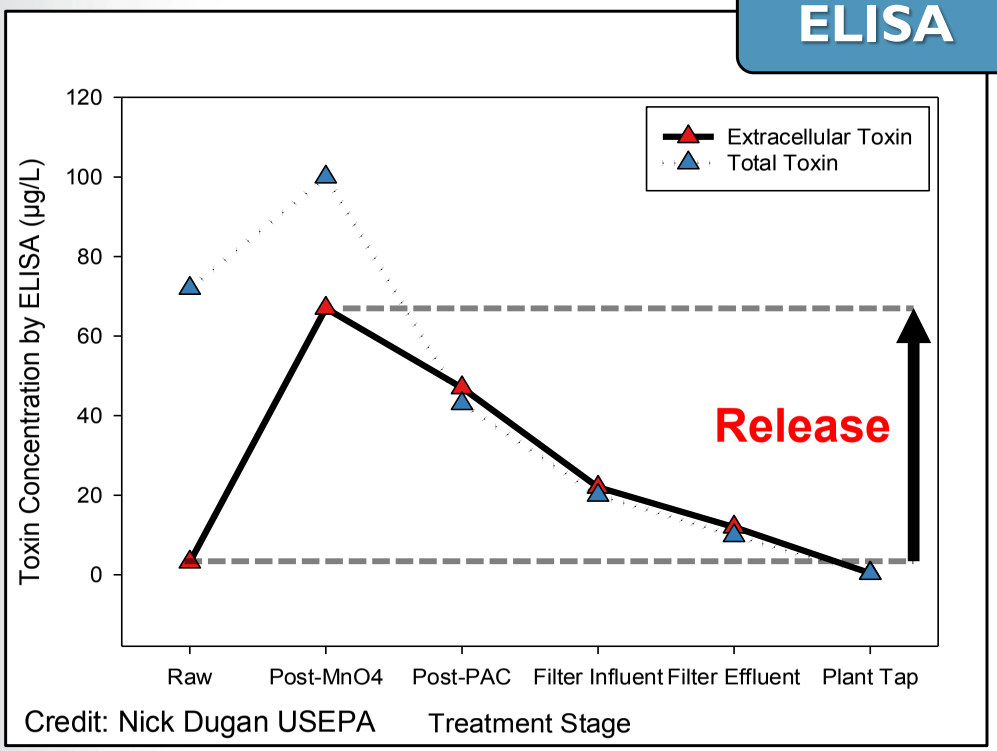
Most cell removal was accomplished prior to filtration





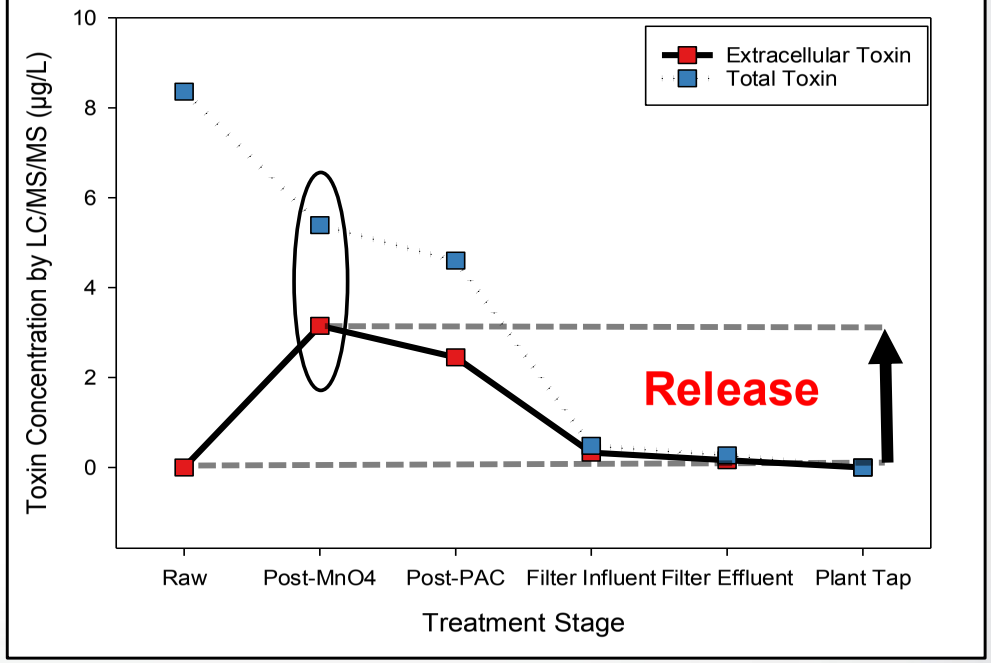
Toxin Propagation through DWTP - ELISA vs LC-MS/MS

ELISA



Intracellular toxin is release following MnO_4 addition

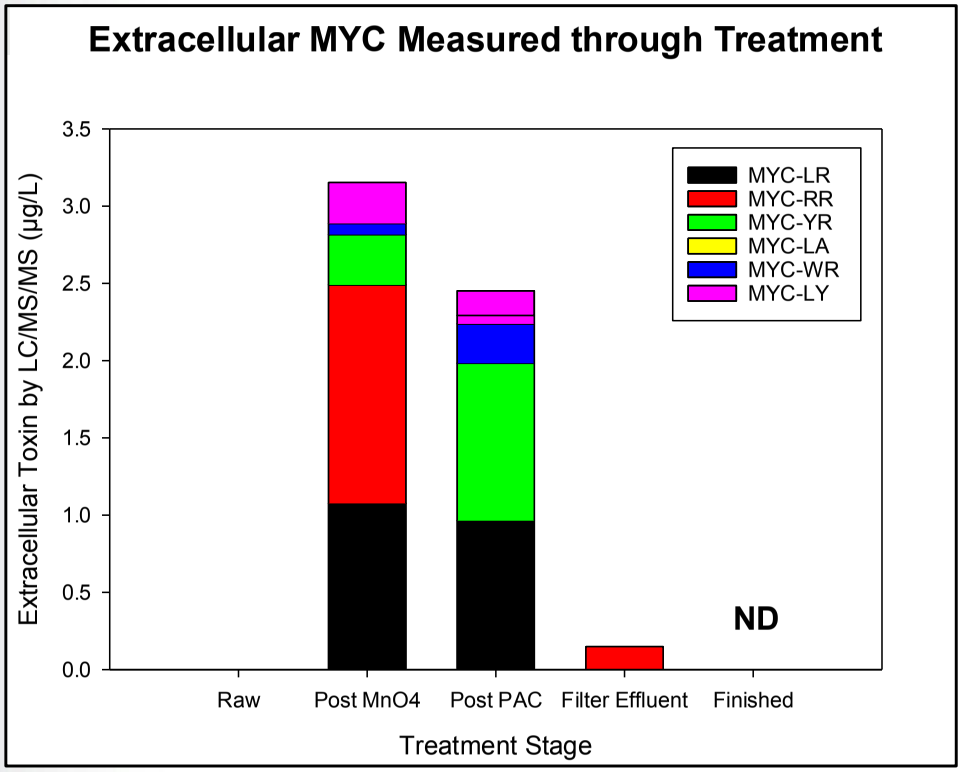
LC-MS/MS

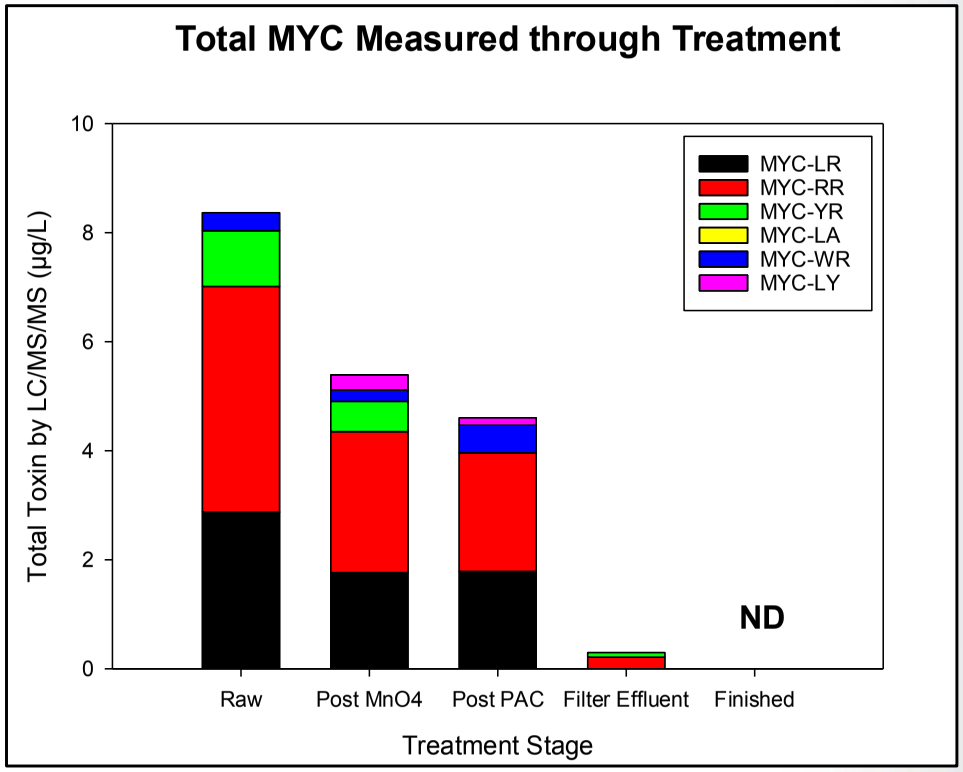


Unlike ELISA, total MYC measured by LC-MS/MS decreases after MnO_4 addition

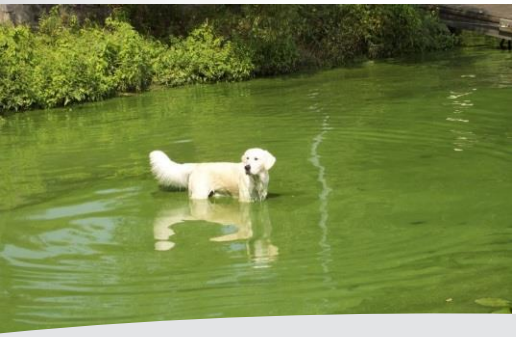
However, only 7 MYC variants were measured

Toxin Speciation and Propagation - LC/MS/MS





- Significant diversity in toxin variants
- Both extracellular and total MYC concentrations decrease through the treatment train; no MYC observed in finished water



Thank You

EPA's informational webpage

http://www2.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-cyanohabs