EPA Monthly Webinar Series:

Challenges and Treatment Solutions for Small Drinking Water and Wastewater Systems

Hosted by EPA's Office of Research and Development (ORD) and Office of Water (OW)

May 31, 2016, 2:00-3:00 PM EST (Optional Q&A session from 3:00-3:30)

TODAY'S TOPIC:

Responding to Harmful Algal Blooms, Optimization Guidelines, and Sampling for Utilities

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EPA

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Presentation 1:
U.S. EPA Office of Research and Development

Removal Capabilities of Common Treatment Processes and Facility Evaluation Strategies and Performance Improvement

Harmful algal blooms (HABs), which include blooms of cyanobacteria, pose particular challenges and questions for small drinking water systems. Two of the most important are: “how well equipped is my facility to handle cyanobacterial cells and the toxins that may be released?” and “how can I improve my facility’s performance within rigid financial constraints?” This presentation will review the removal capacities of common processes used in drinking water treatment, present a strategy for evaluating an existing treatment facility and, finally, discuss how to use this information to improve a facility’s performance.

Nicholas R. Dugan, P.E.

Nick is an environmental engineer with U.S. EPA’s Office of Research and Development/National Risk Management Research Laboratory in Cincinnati, Ohio, where he specializes in drinking water treatment. In addition to his work with cyanobacteria and cyanobacterial toxins, he has performed treatment studies to evaluate the control of cryptosporidium, nitrate, perchlorate, pesticides, and disinfection byproduct precursors.

Contact: dugan.nicholas@epa.gov

Presentation 2:
Ohio EPA Division of Drinking and Ground Waters

Source and Finished Water Monitoring Options and Their Limitations and Benefits

There are a variety of tools that can be utilized to monitor a water system's source and finished waters for HABs. Monitoring data can help a water system develop appropriate reservoir management strategies and optimize treatment for cyanotoxin removal. This presentation will cover source and finished water monitoring options and their limitations and benefits. It will also provide a few examples of how water systems in Ohio are using monitoring data to both focus reservoir management and optimize treatment following source and finished water cyanotoxin detections.

Heather Raymond

Heather has almost 20 years of experience in Ohio EPA's Division of Drinking and Ground Waters where she currently serves as the Harmful Algal Bloom Coordinator. She helped develop Ohio's Harmful Algal Bloom Monitoring and Reporting Rules, the State of Ohio Recreation and Public Water System HAB Response Strategies, and HAB-related public water system guidance documents. She also co-teaches a practical workshop on HABs at Ohio State University's Stone Laboratory. She has helped water systems effectively respond to HABs in both their raw and finished drinking water.

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

Small Systems Webinar Series

Responding to Harmful Algal Blooms: Treatment Optimization

Nicholas R. Dugan, PE

US Environmental Protection Agency, Water Supply & Water Resources Division

May 31, 2016

Organization of presentation

- Introduction and definitions
- Background
  - –Cyanobacteria
  - –Pigment analysis
  - –Toxin analysis

Fundamental to making good treatment decisions

- Treatment
  - –Focus on the conventional treatment process
  - –Focus on microcystins

Introduction

- What is a bloom?
  - Sudden increase in the rate of growth or accumulation of phototrophic micro-organisms
- What is a phototrophic micro-organism?
  - An organism that depends on light from the sun as its primary energy source
- Phototrophic organisms include:
  - Algae (contain a nucleus → eukaryotes)
  - Cyanobacteria (no nucleus → prokaryotes)

Introduction

- Why is a bloom potentially harmful?
  - Unpleasant appearance
  - Unpleasant tastes and/or odors
  - Negative impacts on aquatic food web
  - Some species of cyanobacteria produce toxins that can negatively affect various systems in the human body, including the liver and central nervous system
  - Loss of confidence in the quality of treated drinking water

Introduction

- If a bloom is caused by cyanobacteria, why is it called a "harmful algal bloom?"
  - Cyanobacteria were formerly classified as "blue-green algae"
  - Terms such as "cyanobacteria" and "blue-greens" are still used interchangeably
  - "Algal" has two syllables, "cyanobacteria" has seven → "harmful algal blooms" and "HABs" roll off the tongue more easily than "harmful cyanobacterial bloom"
- For this presentation, "harmful algal blooms" and "HABs" will be used

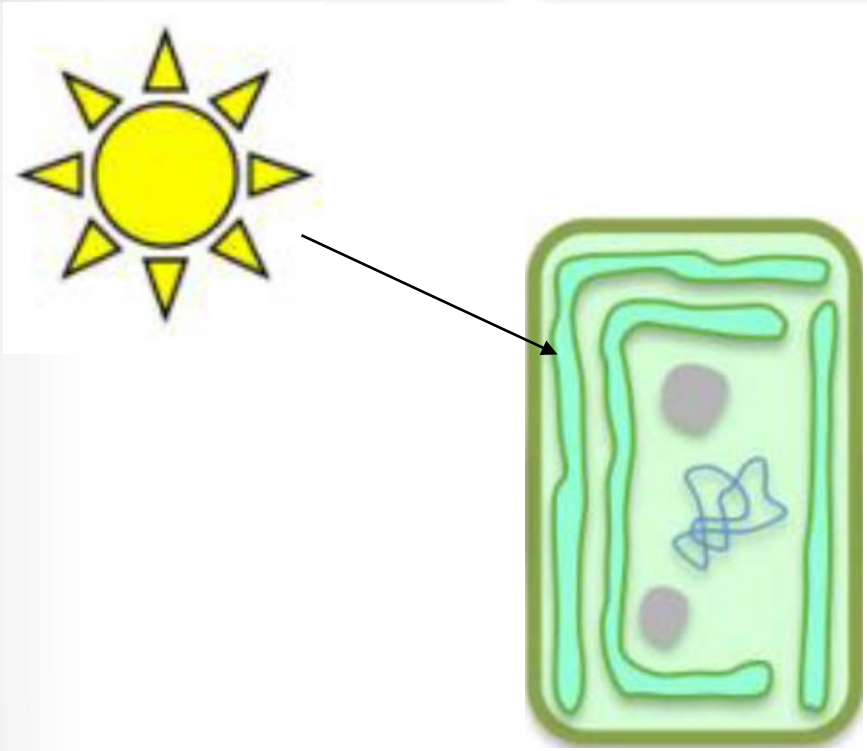
Introduction

- HABs are a relatively new concern for operators who are used to managing filter effluent turbidities, distribution system chlorine residuals, storage tank levels, lead & copper rule compliance, etc.
- Some HABs are accompanied by taste & odor (T&O) events but, historically T&O training has discussed 2-methylisoborneol (MIB) and geosmin, never HABs-related toxins

Introduction

- Toxins with mammalian health effects are a complicating factor:
  - The word "toxins" carries emotional freight
  - Operators are not used to sampling or contracting for analyses
  - Events that may have passed without wide attention in prior years take on new potential significance:
    - "My dog swam in the lake and now he's sick – is it safe to drink the water?"
    - "The water is green and I found dead birds on the shore – is it safe to drink the water?"

Background



All cyanobacteria contain pigments:

- Chlorophyll
- Phycocyanin

- Not all cyanobacteria produce toxins
- In toxin-producing cyanobacteria – timing of toxin production can vary
- When toxins produced, they are usually contained within the cell membrane
- Sometimes toxins released from cells into solution

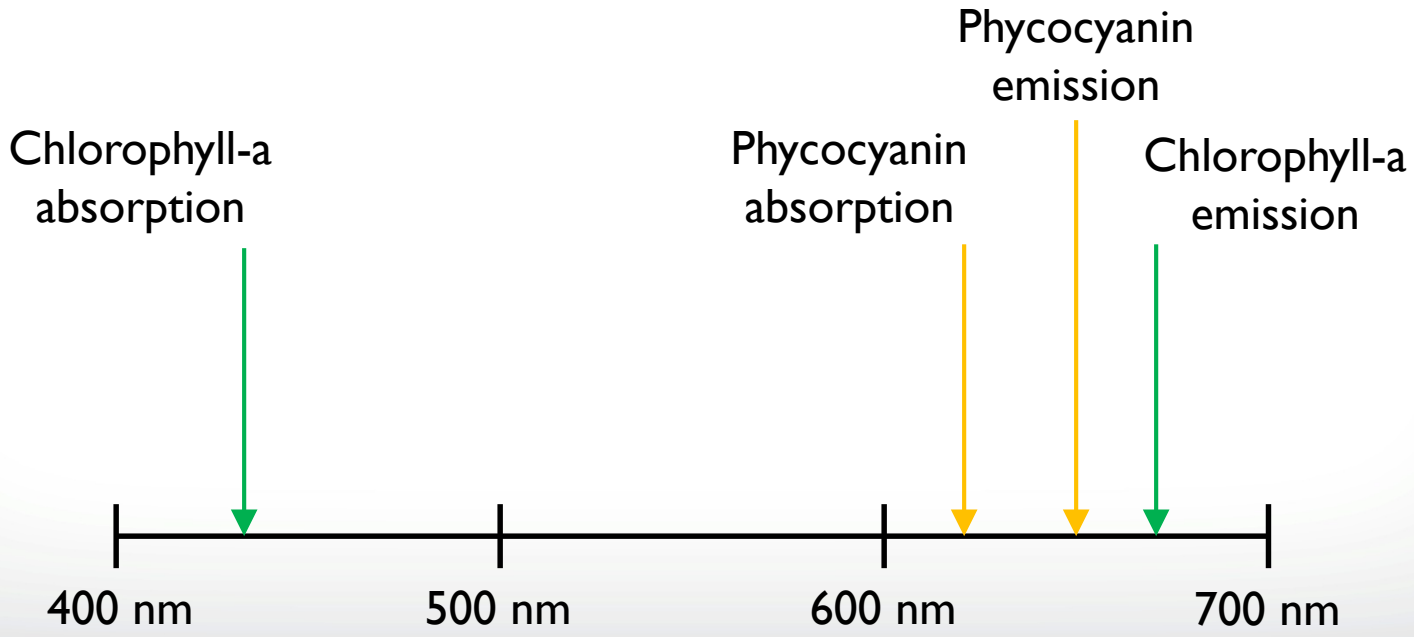
Figure adapted from: Kamennaya, N.A., et al; *Minerals* 2012:2:338-364

Background

- Chlorophyll-*a* and phycocyanin are pigments:
  - Large molecules
  - Essential components of the mechanism that harvests light and converts it to a form of energy that cyanobacteria can use for growth and maintenance
  - Both algae and cyanobacteria contain chlorophyll-*a*
  - Only cyanobacteria contain phycocyanin
  - This difference in pigment content is helpful in diagnosing the composition of a bloom

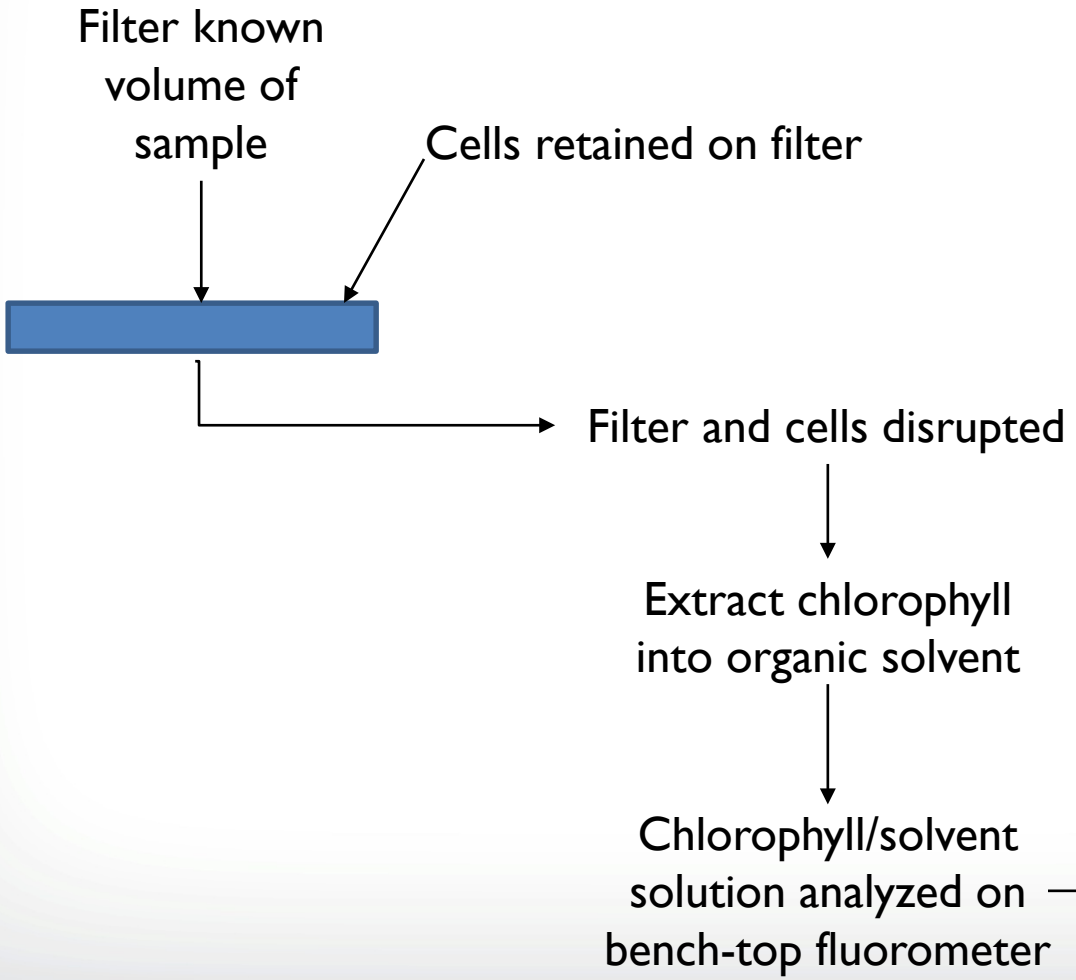
Background

Both chlorophyll-*a* and phycocyanin absorb light at shorter wavelengths and re-emit the light at longer wavelengths → fluorescence. Each pigment has a unique set of excitation and emission wavelengths (see figure below). Commercially available probes, monitors, hand-held and bench-top units use combinations of lamps, light emitting diodes (LEDs) and optical filters, to take advantage of these excitation and emission wavelength combinations to generate estimates of algal and/or cyanobacterial biomass in suspension, without additional sample processing → *in vivo* analysis (available for phycocyanin and chlorophyll-*a*).



Background

Extractive (*in vitro*) analysis for chlorophyll-*a*

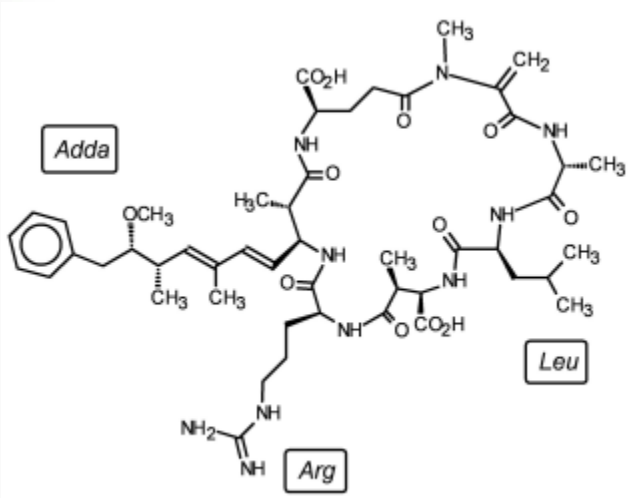


Background

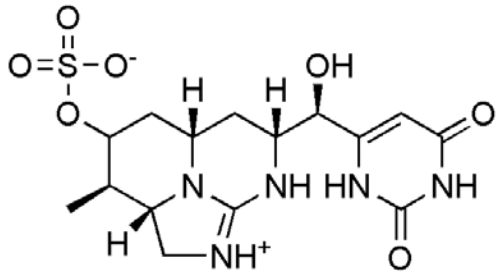
- Sensors, probes, hand-held and bench-top units (*in vivo* analysis):
  - Available for phycocyanin and chlorophyll-*a*
  - Advantages:
    - Rapid
    - Very little training required
    - High sampling frequency
  - Disadvantages:
    - No direct quantitation of pigments
- Extractive (*in vitro*) analysis:
  - Available for chlorophyll-*a* (EPA Method 445.0)
  - Advantages:
    - Sensitive
    - Large dynamic range
    - Analysis based on material retained on a filter → good proxy for suspended phototrophic biomass
  - Disadvantage:
    - Requires a higher level of training

Background

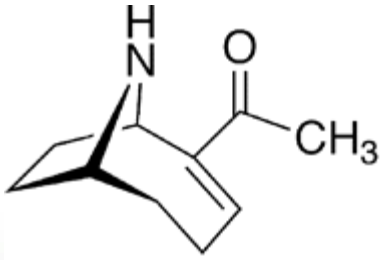
Microcystin → liver



Cylindrospermopsin → kidneys & liver

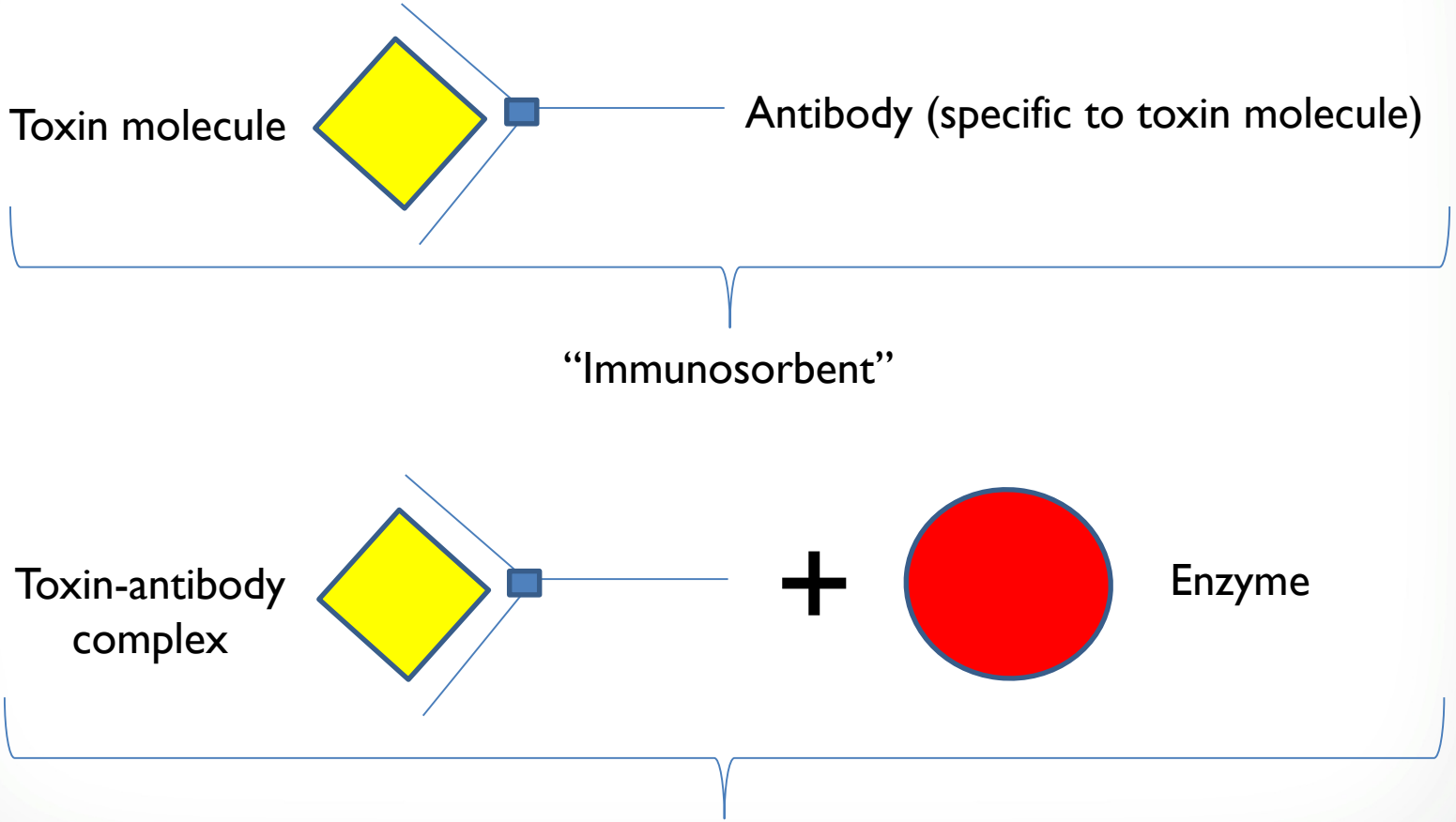


Anatoxin → central nervous system



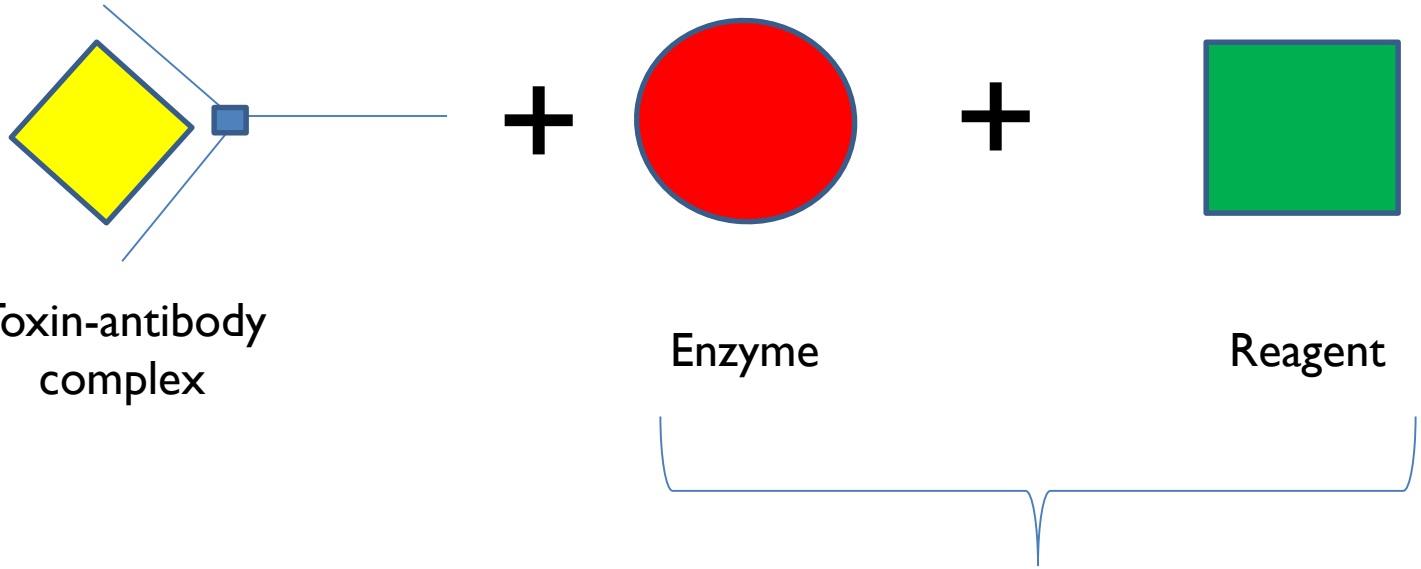
Background

Enzyme linked immunosorbent assay (ELISA)



Background

Enzyme linked immunosorbent assay (ELISA)



Toxin-antibody complex

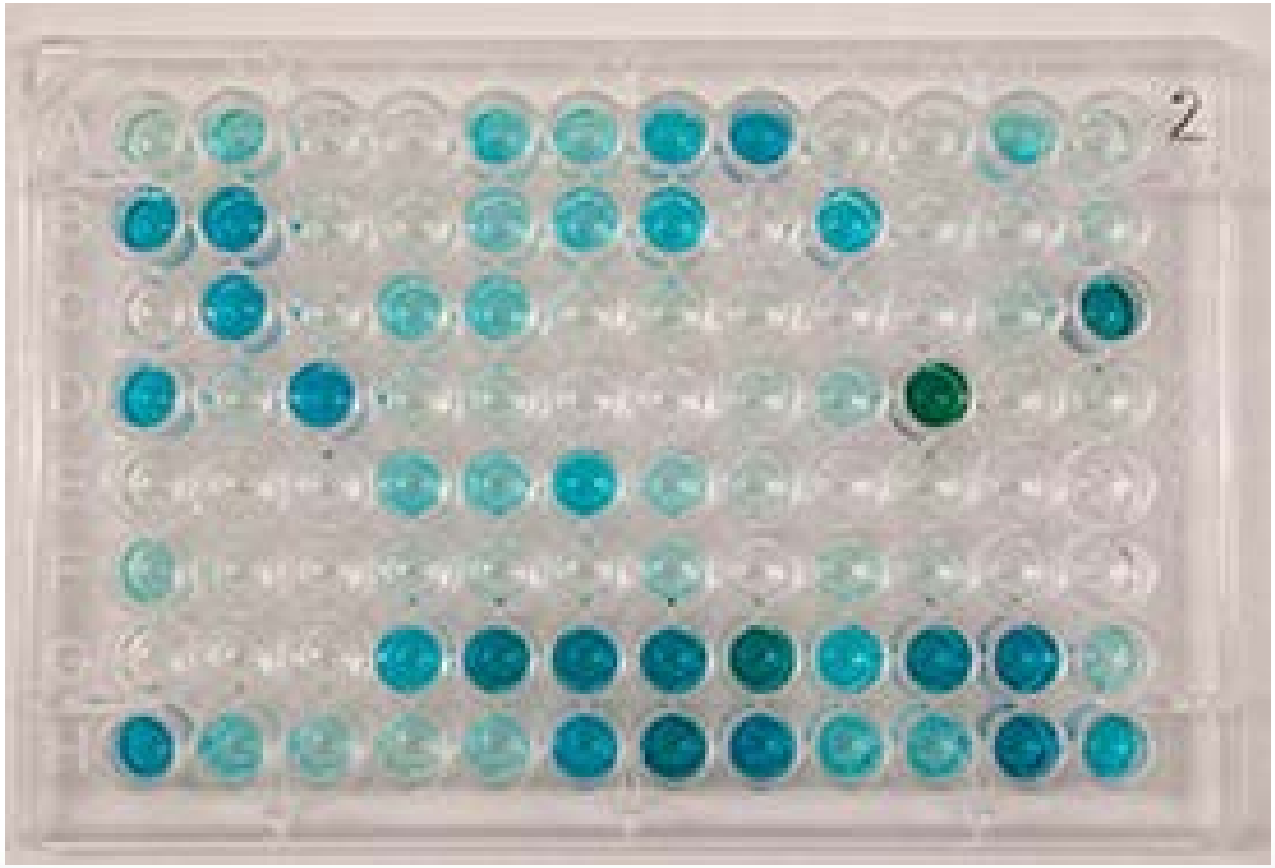
Enzyme

Reagent

Enzyme catalyzes reaction that results in color change proportional to toxin concentration

Background

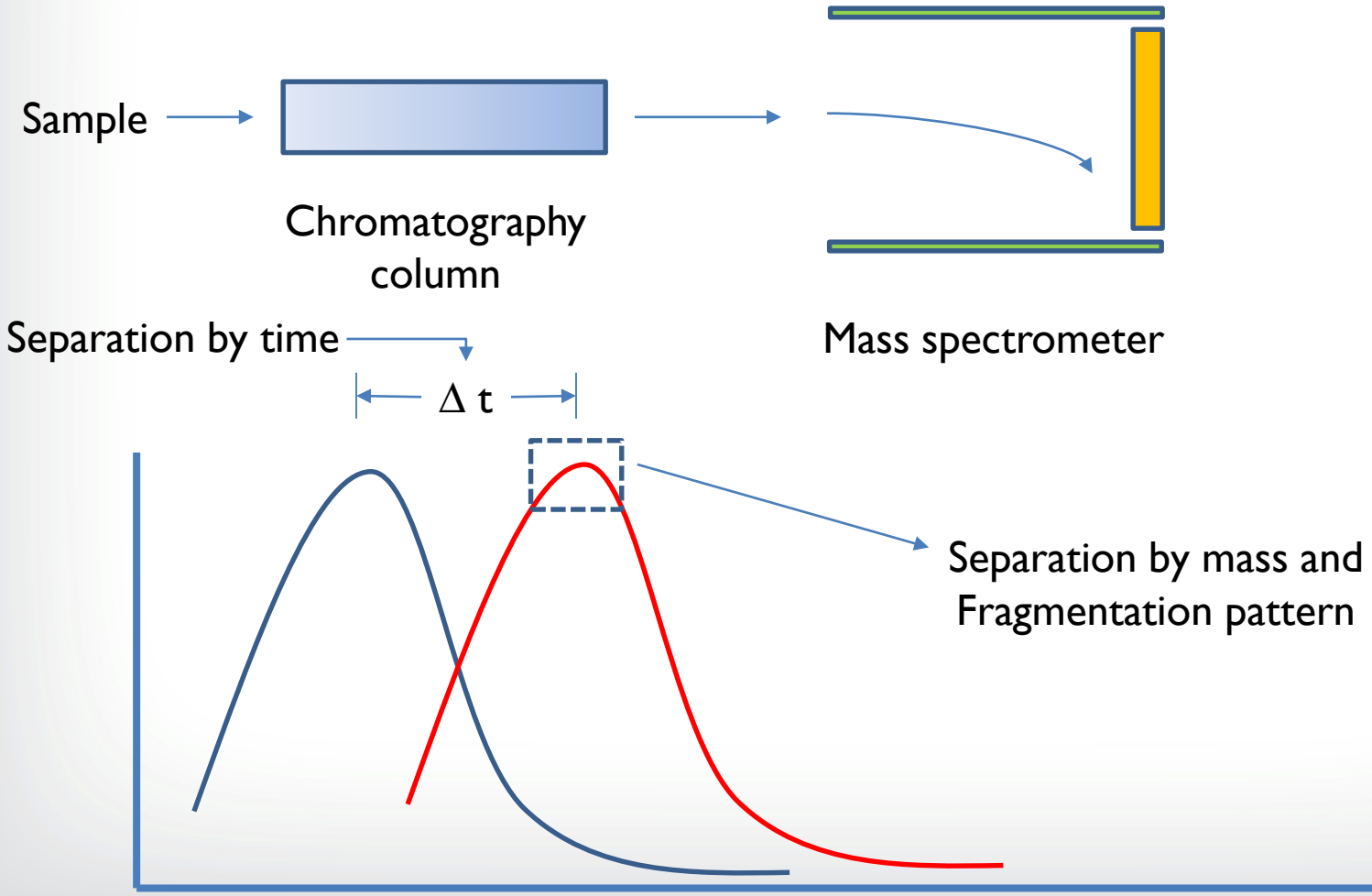
Enzyme linked immunosorbent assay (ELISA)



ELISA plate ready for reading

Background

Liquid chromatography with tandem mass spectrometer (LC/MS/MS)



Background

- ELISA assay:
  - Commercially available for total microcystins, cylindrospermopsin, anatoxin
  - Less expensive than LC/MS/MS
  - Not congener-specific
- LC/MS/MS analysis:
  - Available for cylindrospermopsin, anatoxin and subset of microcystin congeners
  - EPA Method 544 available for microcystin congeners in finished water
  - More expensive than ELISA

Background

- Both ELISA and LC/MS/MS can be applied to extracellular and total (extra & intracellular) toxins
  - Extracellular → filter sample & analyze filtrate
  - Total→ Disrupt cells (freeze/thaw, sonication or both) & analyze
- Understanding the relative amounts of extracellular and total toxins in your influent and how those are changing through the treatment plant are critical to making good decisions
- It's easier to treat water where the majority of toxins are still contained within the cell

Treatment – source/intake

Wyoming

7/29/15 Drinking water treatment plant intake: < 0.25 µg/L

7/29/15 Swimming beach: > 1.4 µg/L

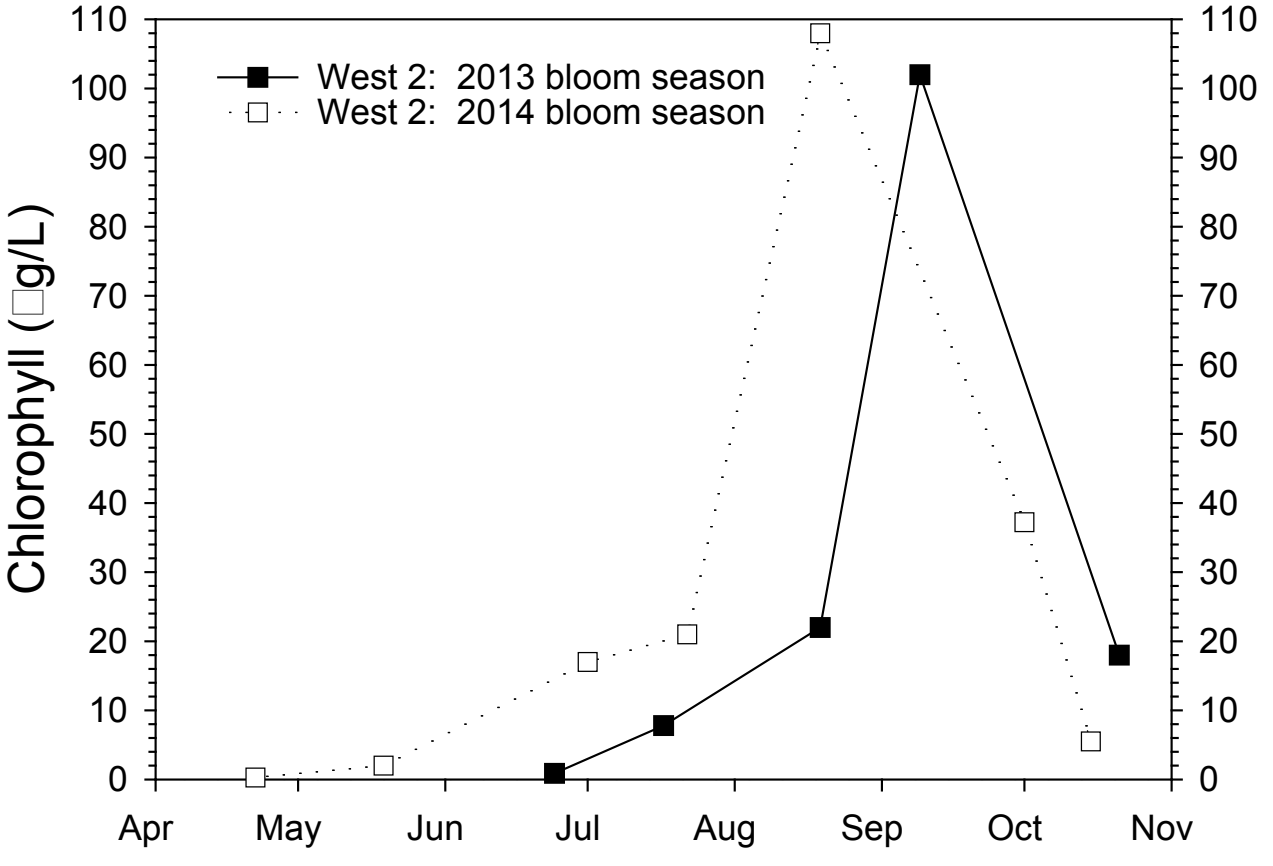
7/21/15 Boat ramp: > 290 µg/L

Treatment - source/intake

- A toxin detection in the source water, even a high one, does not necessarily mean that toxins will be detected at the intake
- A toxin non-detect in the source water does **NOT** necessarily mean that toxins will not be detected in the intake
- Need to maintain separate and regular source water and intake monitoring programs

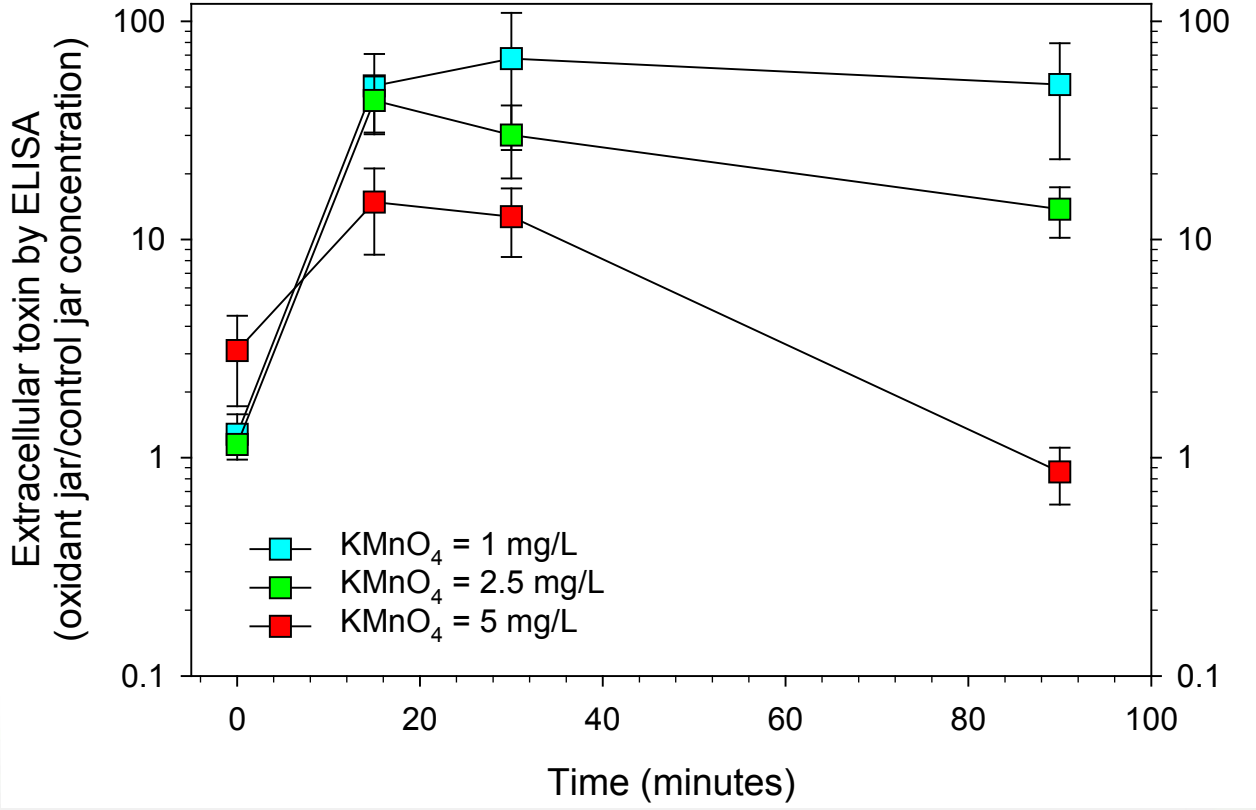
Treatment - influent

Lake Erie Western basin treatment plant influent *in vitro* chlorophyll-*a*
The exact timing of the bloom peak can change from year to year

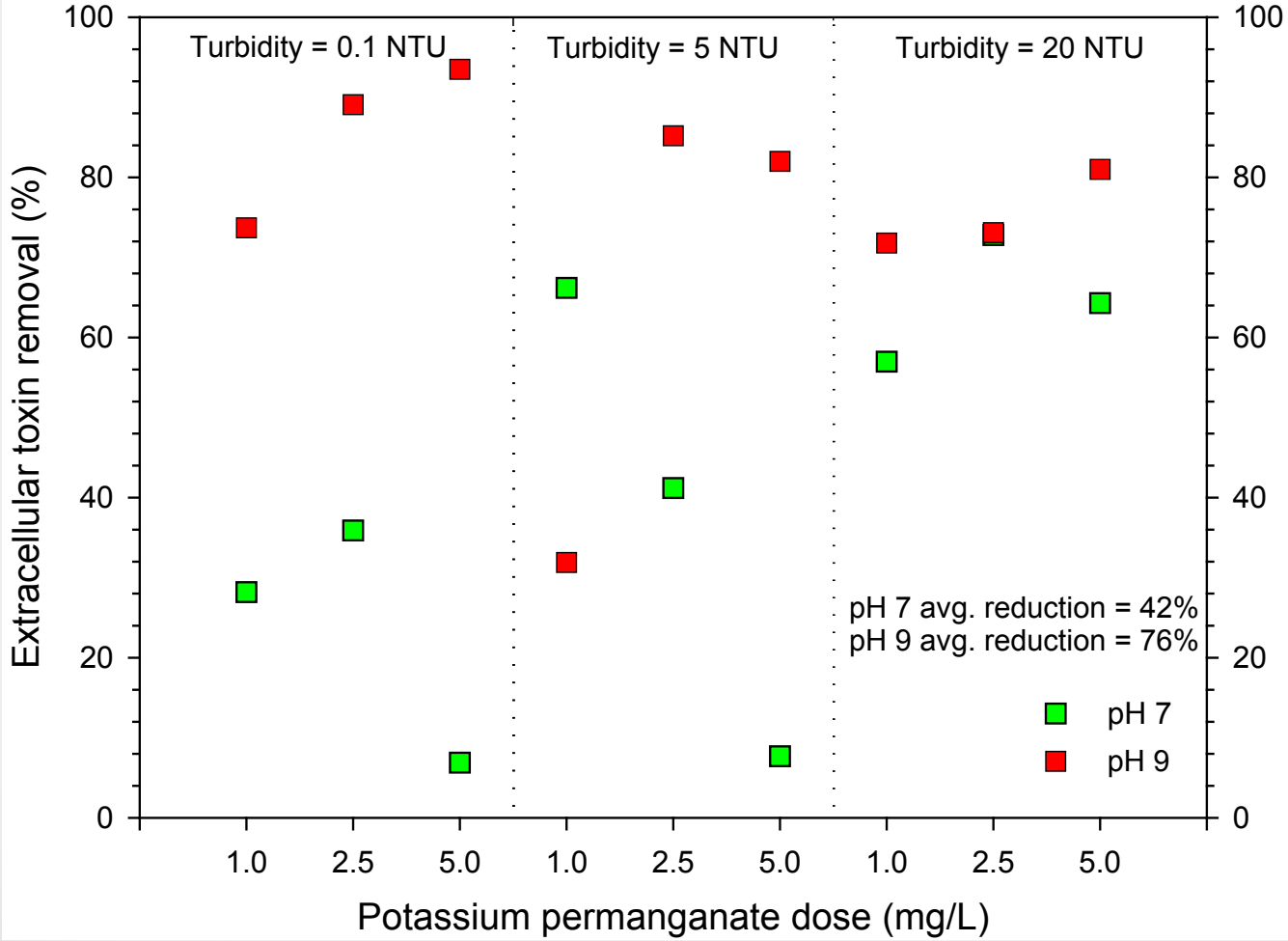


Treatment – permanganate addition

Potential for toxin release:
Bench-scale studies: Extracellular toxin concentrations (pH = 7, turbidity < 0.1 NTU)



Treatment - powdered activated carbon (PAC)



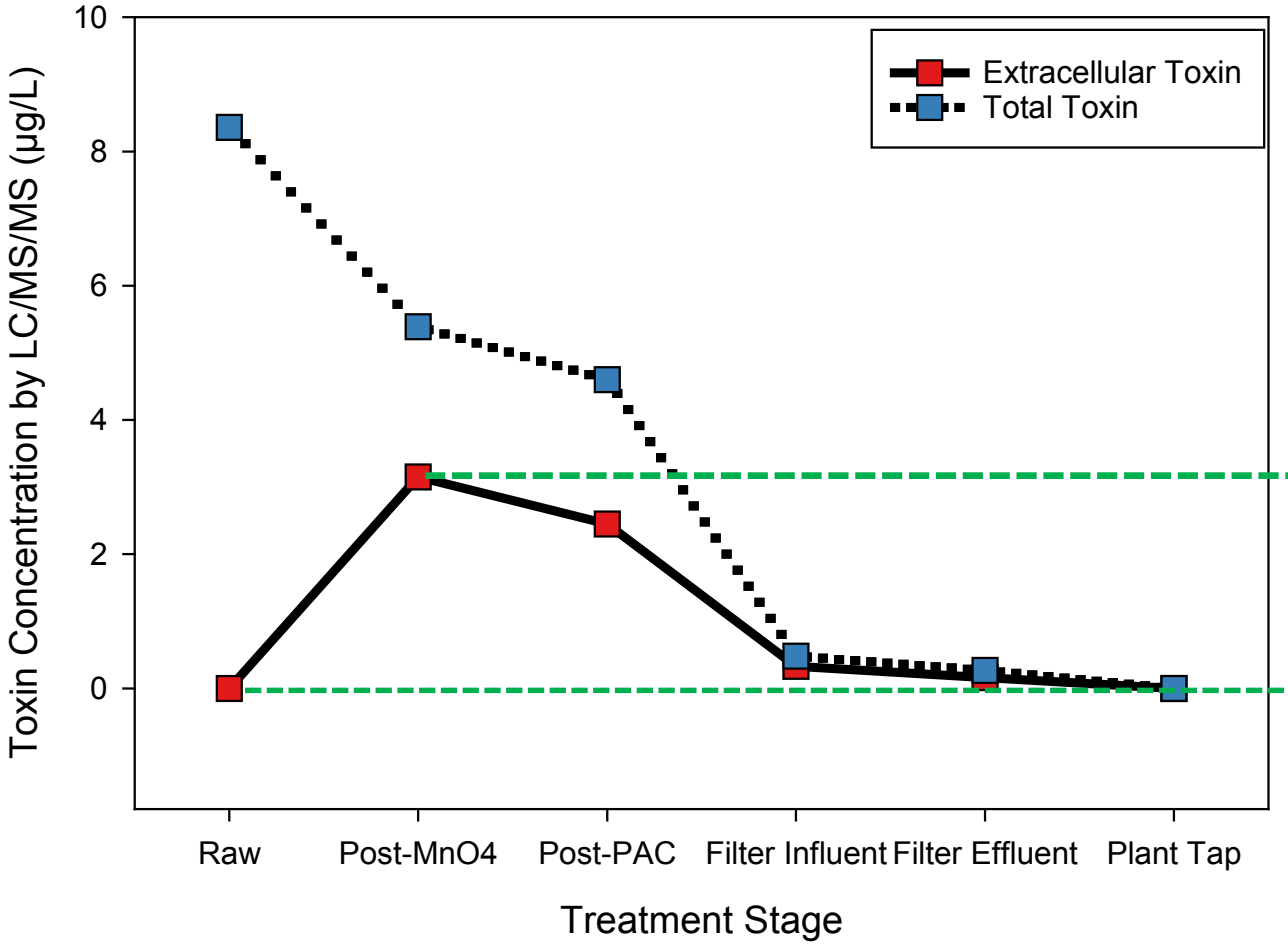
- At t = 0 min., $KMnO_4$ dosed at 1, 2.5 or 5 mg/L
- Toxin release observed
- At t = 30 min. PAC dosed at 10 mg/L
- 60 minute PAC contact time

Treatment – permanganate addition

- Several research groups have examined the phenomenon of toxin release upon permanganate addition:
  - Degree and timing of observed toxin release and accumulation varies between studies
- Awareness, vigilance and preparation recommended:
  - Monitor for extracellular and total toxins before and after the point of permanganate addition
  - Consider if and for how long permanganate feed can be interrupted – it helps if bloom dynamics in the source water are understood
  - Consider adding powdered activated carbon (PAC) or increasing dose if toxin release observed
    - Enough PAC on hand?
    - What is the maximum feasible PAC dose?
      - Capacity of feeding equipment
      - Sludge accumulation
      - PAC carryover to filters → head loss development, run times, effluent quality

Treatment – toxin propagation

Lake Erie western basin treatment plant - 2014 bloom season

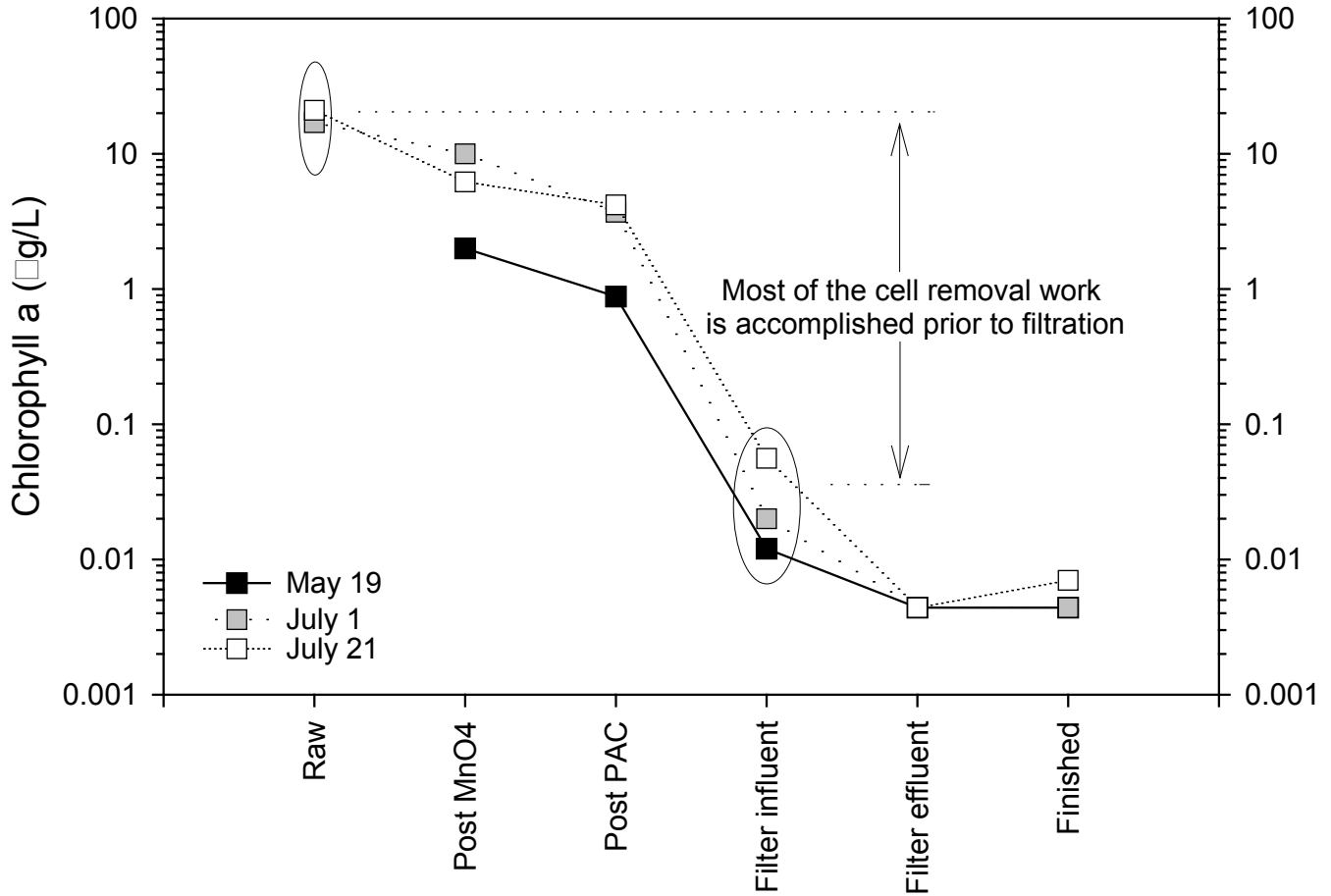


Intracellular toxin release following MnO_4 addition

Treatment – cell propagation

Lake Erie western basin treatment plant - 2014 bloom season

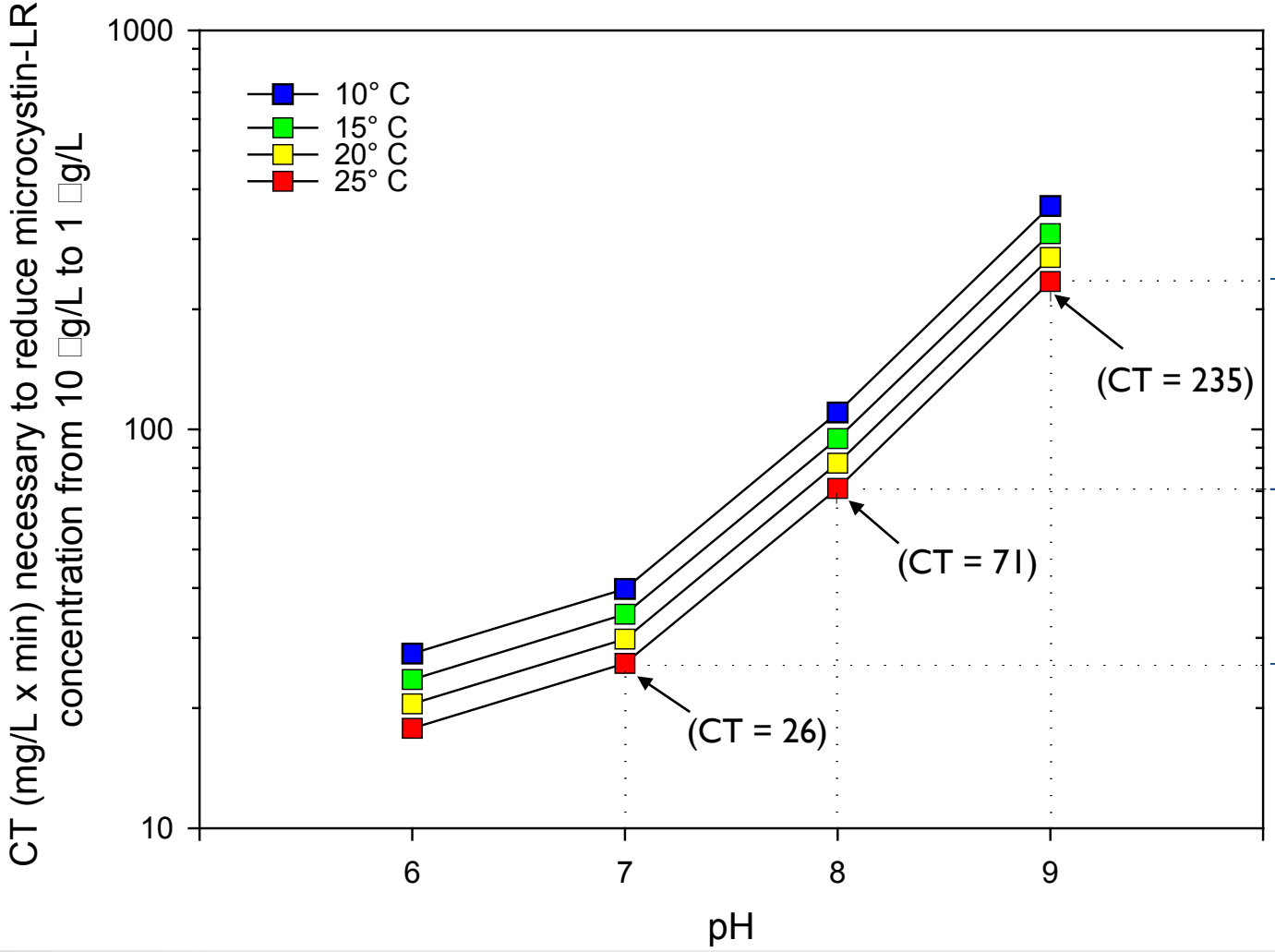
Minimizing settled and filter effluent turbidities correlates with best removals of phototrophic biomass



Treatment – cell propagation

- Effective coagulation, flocculation and sedimentation are critical → room for improvement?
- Blooms often associated with quiescent water conditions → significant fraction of the influent particulate load may be bloom material
- Be aware that specific gravity ~ 1, and may be less if cyanobacteria contain gas vacuoles to regulate buoyancy → potentially reduced settling efficiency
- Potential for improving process efficiency:
  - Consider changes in coagulant dose
  - Consider changes in coagulant type or combinations of coagulants and coagulant aids

Treatment - chlorination



CT for 3-log *Giardia* inactivation @ 1.0 mg/L Cl_2, t = 25° C:

- pH 7: 37
- pH 8: 54
- pH 9: 78

> 3X increase in CT

> 2X increase in CT

*Figure based on data from Acero et al, *Water Research*, 2005:39:1628-1638

Treatment - chlorination

- Data on previous slide generated using laboratory grade water
- Treatment plant effluent has a higher background chlorine demand → Site specific CTs to achieve a given amount of degradation may be higher
- Balance Stage 2 DBP Rule compliance with microcystin degradation → is there room to increase Cl_2 dose if necessary?
- Anticipate distribution system sampling approach in case of finished water toxin exceedance:
  - Toxins degrade with water age as long as Cl_2 residual is present
  - High toxin levels may be isolated depending on distribution system hydraulics → may impact public messaging

Strategy

- Understand available analytical tools
- Institute a monitoring program:
  - Source water
  - Intake
  - Through-plant
- Consider how plant operations may be optimized:
  - Coagulation/flocculation/sedimentation critical
  - PAC addition
  - Chlorine dosing

Strategy

- Contingency planning
  - Capable of dosing extra PAC?
  - Status of pre-oxidation (if applicable)?
  - Capable of dosing extra chlorine?
  - Public notification?

Disclaimer

U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for external publication. Any opinions expressed in this paper are those of the author(s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Acknowledgements & contact information

- Acknowledgements:
  - –Toby Sanan
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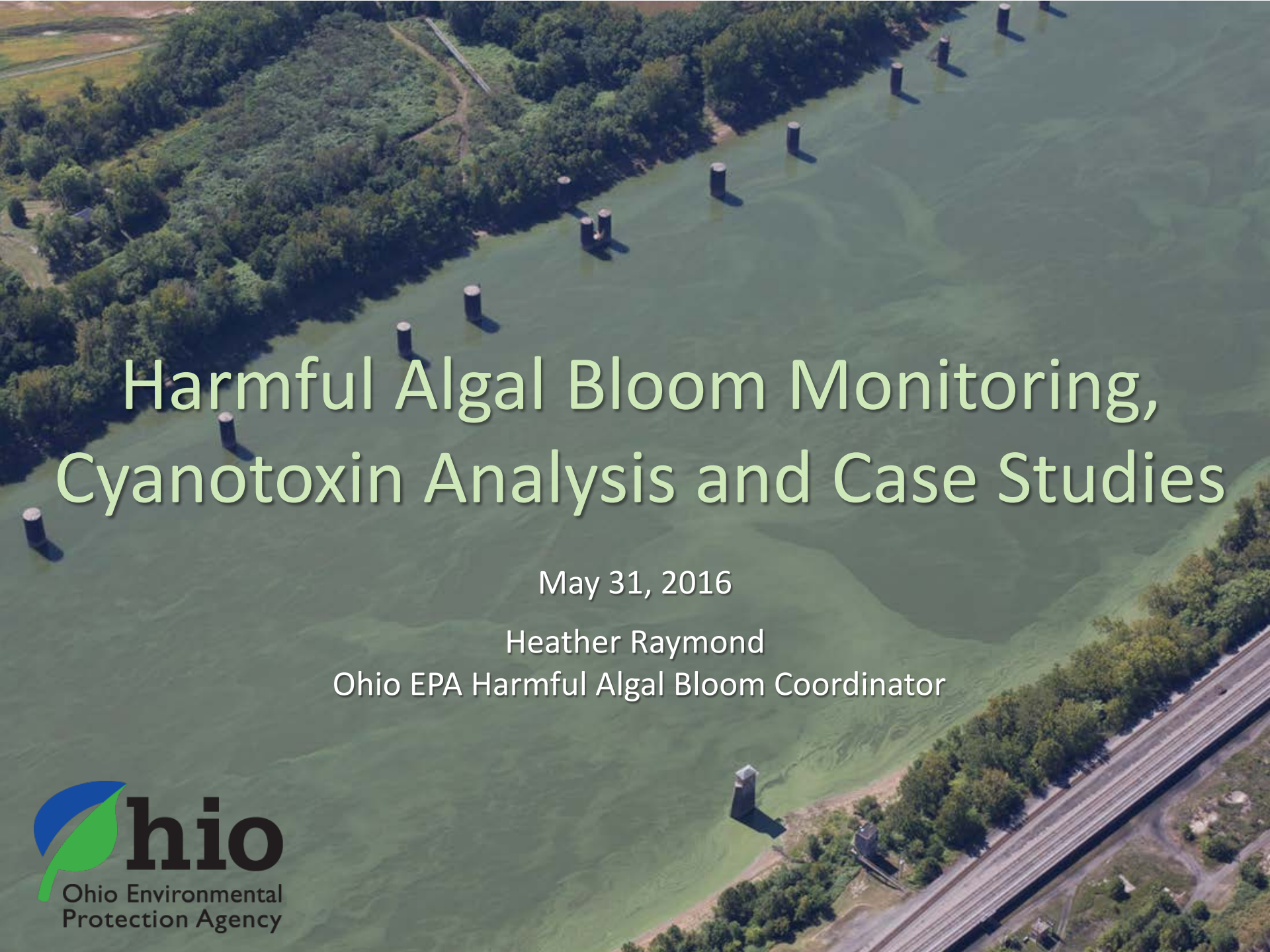


Harmful Algal Bloom Monitoring, Cyanotoxin Analysis and Case Studies

May 31, 2016

Heather Raymond
Ohio EPA Harmful Algal Bloom Coordinator

Ohio Environmental Protection Agency

Outline

- Ohio Harmful Algal Bloom (HAB) Response & Occurrence
- Source Water Monitoring
- Microcystins Analysis Methods
- Case Studies
  - Reservoir Management
  - Treatment Optimization

Ohio Environmental Protection Agency

Summary of Ohio HAB Response

2010: Ohio EPA began sampling for cyanotoxins at public water systems (PWSs)

- Finished water detection at inland PWS.

2011: OEPA/ODNR/ODH created Ohio HAB Response Strategy

2012: Separate Strategy documents developed for Recreation and PWS Response (updated annually)

2013: Finished water threshold exceedance at small PWS

- Drinking Water Advisory Issued.

2014: Finished water threshold exceedance at large PWS

- Drinking Water Advisory Issued.

2015: Revised Response Strategy to include U.S. EPA health advisories for microcystins and cylindrospermopsin

- Finished water microcystins detections at 5 PWSs
  - No Drinking Water Advisories Issued.
- Ohio Senate Bill 1 passed

2016: HAB Monitoring and Reporting Rules

- Effective June 1, 2016
- Updating response strategies, new guidance for PWSs

Ohio Environmental Protection Agency

Ohio HAB Rules Overview

HAB Rules: epa.ohio.gov/ddagw/rules.aspx

- PWS requirements - new rules in OAC Chapter 3745-90
  - Microcystins action levels in drinking water
  - Monitoring requirements: Microcystins & Cyanobacteria Screening
  - Treatment technique requirements
  - Public notification and Consumer Confidence Report (CCR) requirements
  - Recordkeeping requirements

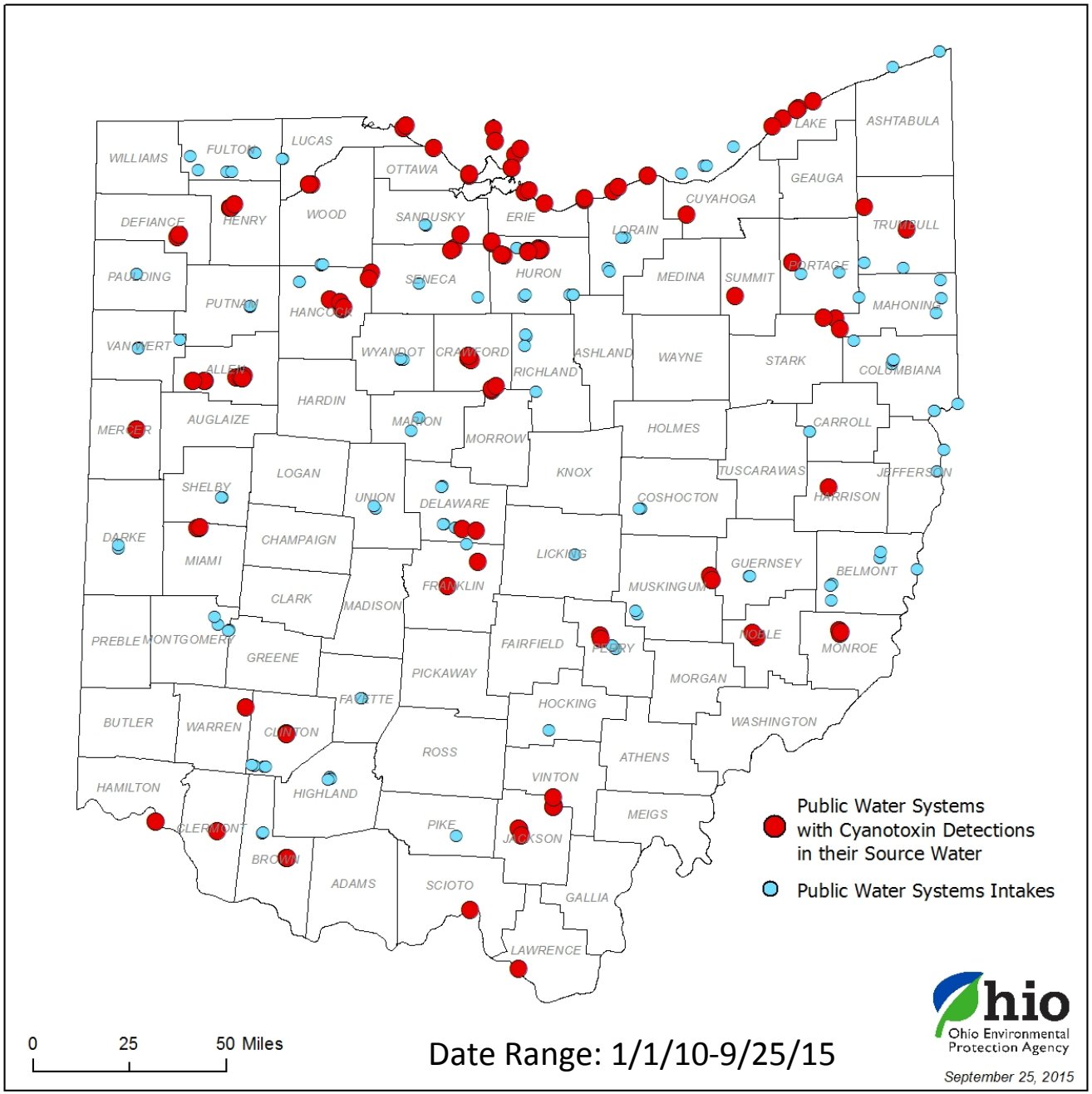
- Laboratory Certification requirements –

  New OAC rule 3745-90-04 and amended rules in Chapter 3745-89
  - Laboratory certification
  - Analytical techniques
  - Reporting deadlines

epa.ohio.gov/Portals/28/documents/labcert/TotalMicrocystins.pdf

Ohio Environmental Protection Agency

Cyanotoxin Detections at Ohio Public Water Systems



Sampled 60% of PWSs

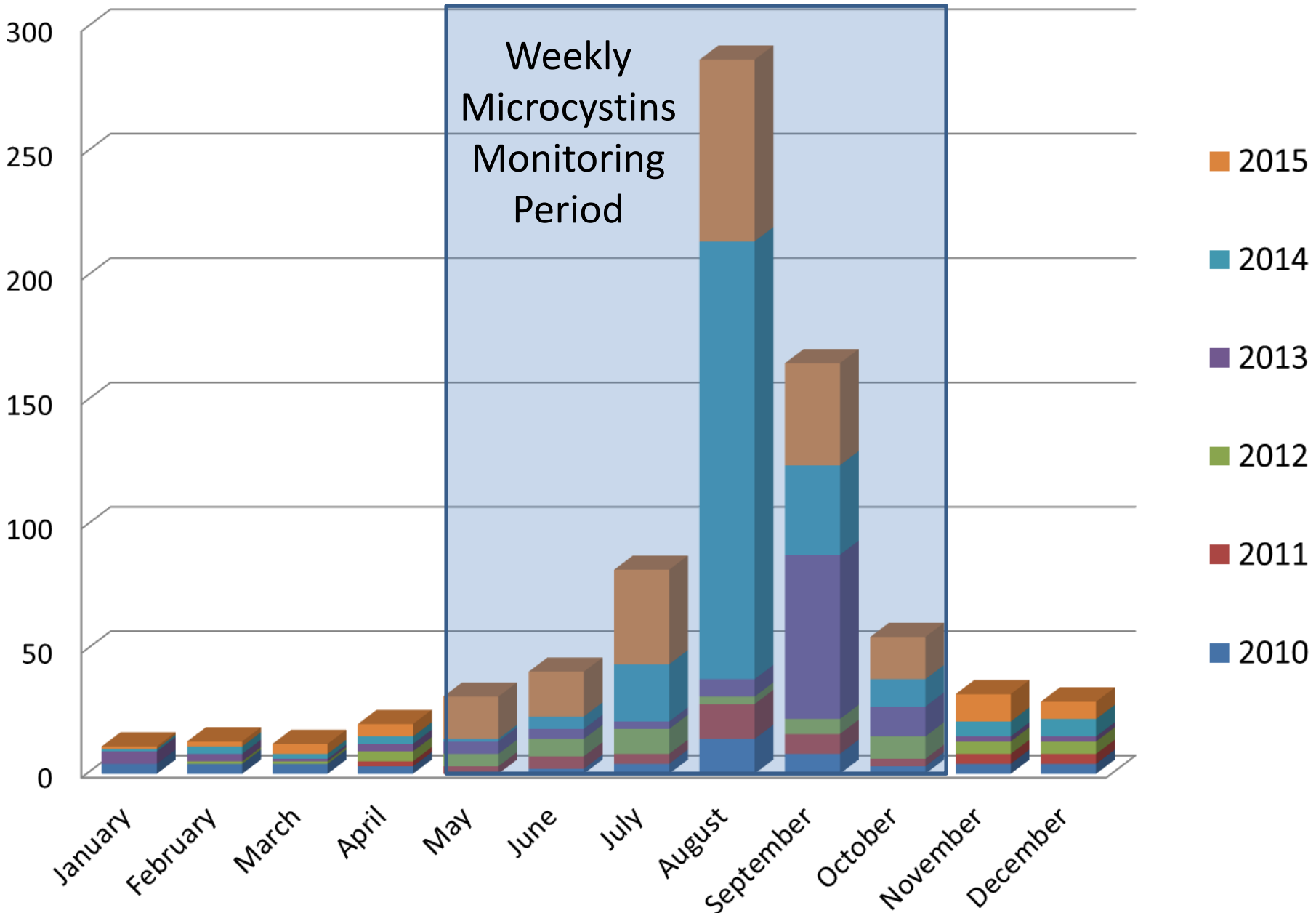
Microcystins detected in 75% of sampled PWSs

Saxitoxins detected in 35% of source water samples

Cylindrospermopsin detected in <1% of source water samples

Anatoxin-a not detected in source water samples

Frequency of Source Water Microcystins Detections > 1.6 ug/L in Ohio

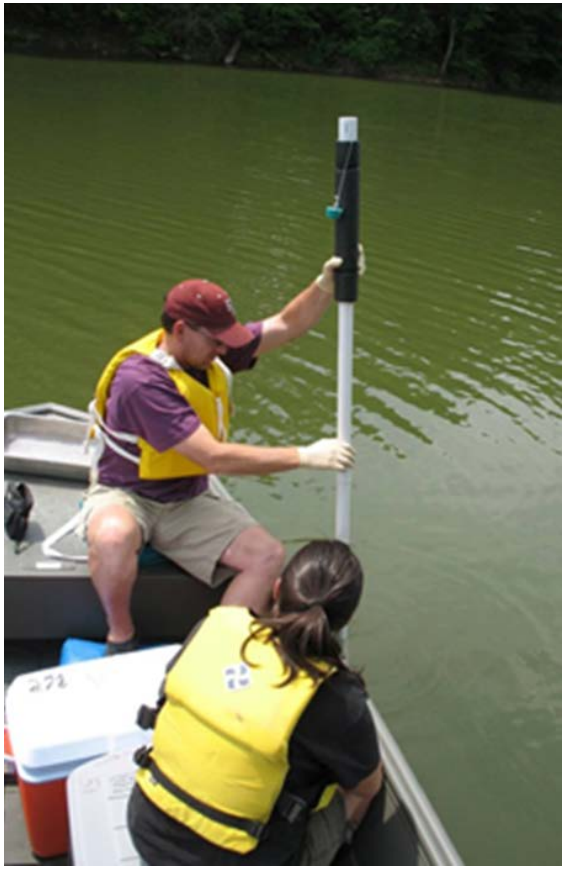


Grand Lake St. Marys Microcystin Concentrations at City of Celina Intake (Raw Water)

Data Source: Celina PWS

HAB Source Water Monitoring

- Phytoplankton Identification & Enumeration
- Nutrient Monitoring: Phosphorus & Nitrogen
- Other Water Quality Parameters: pH, DO, temperature, turbidity
- Accessory Pigments: **Phycocyanin** & Chlorophyll-a
  - Remote sensing
  - Datasondes
- **Molecular Methods (qPCR)**
- **Cyanotoxin Monitoring**

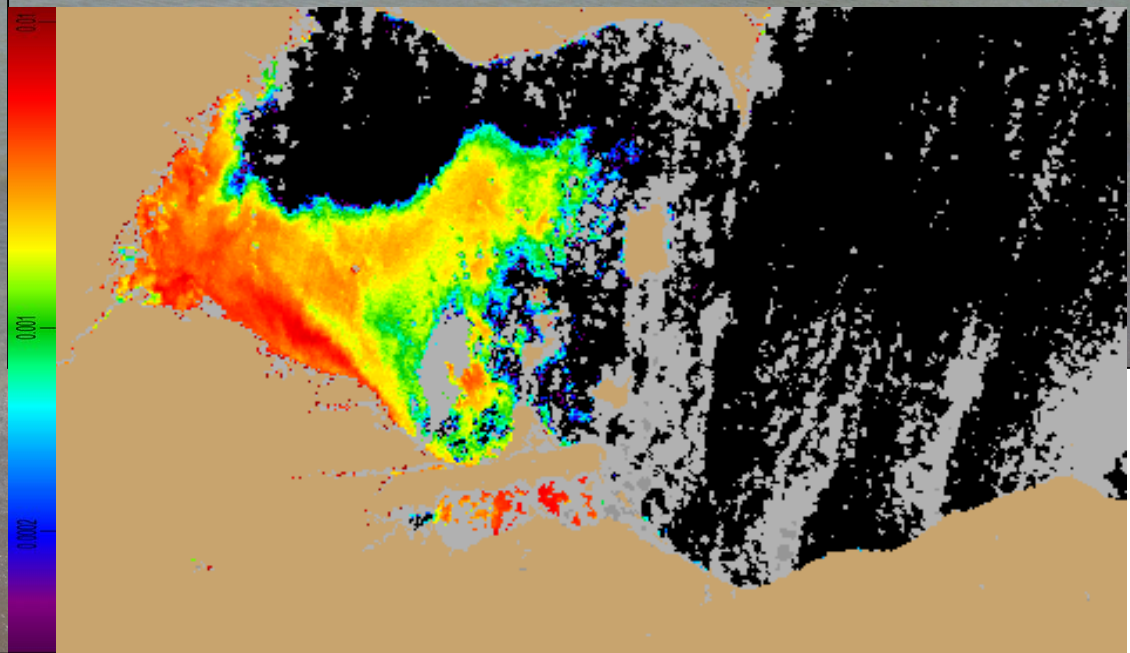


Ohio EPA provided grants to PWSs to support source water monitoring.

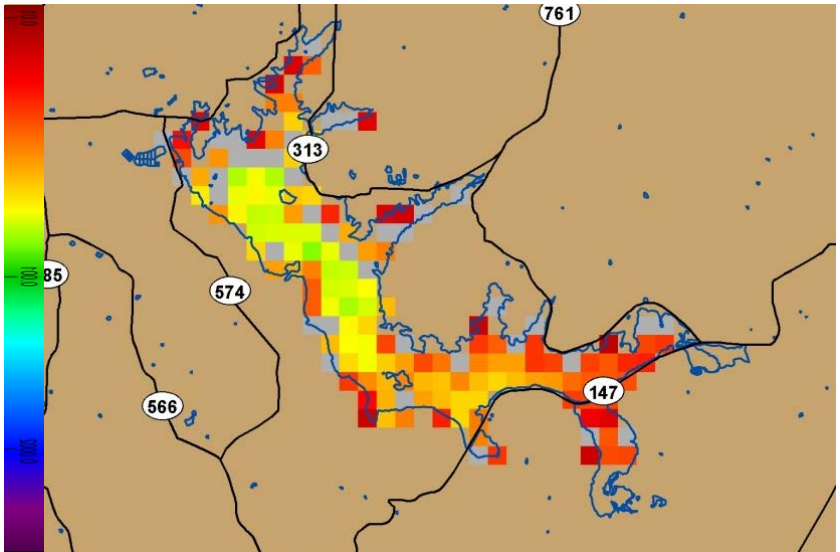
Ohio Environmental Protection Agency

Why is Source Water Monitoring Important? Detect "Non-Visible" Blooms

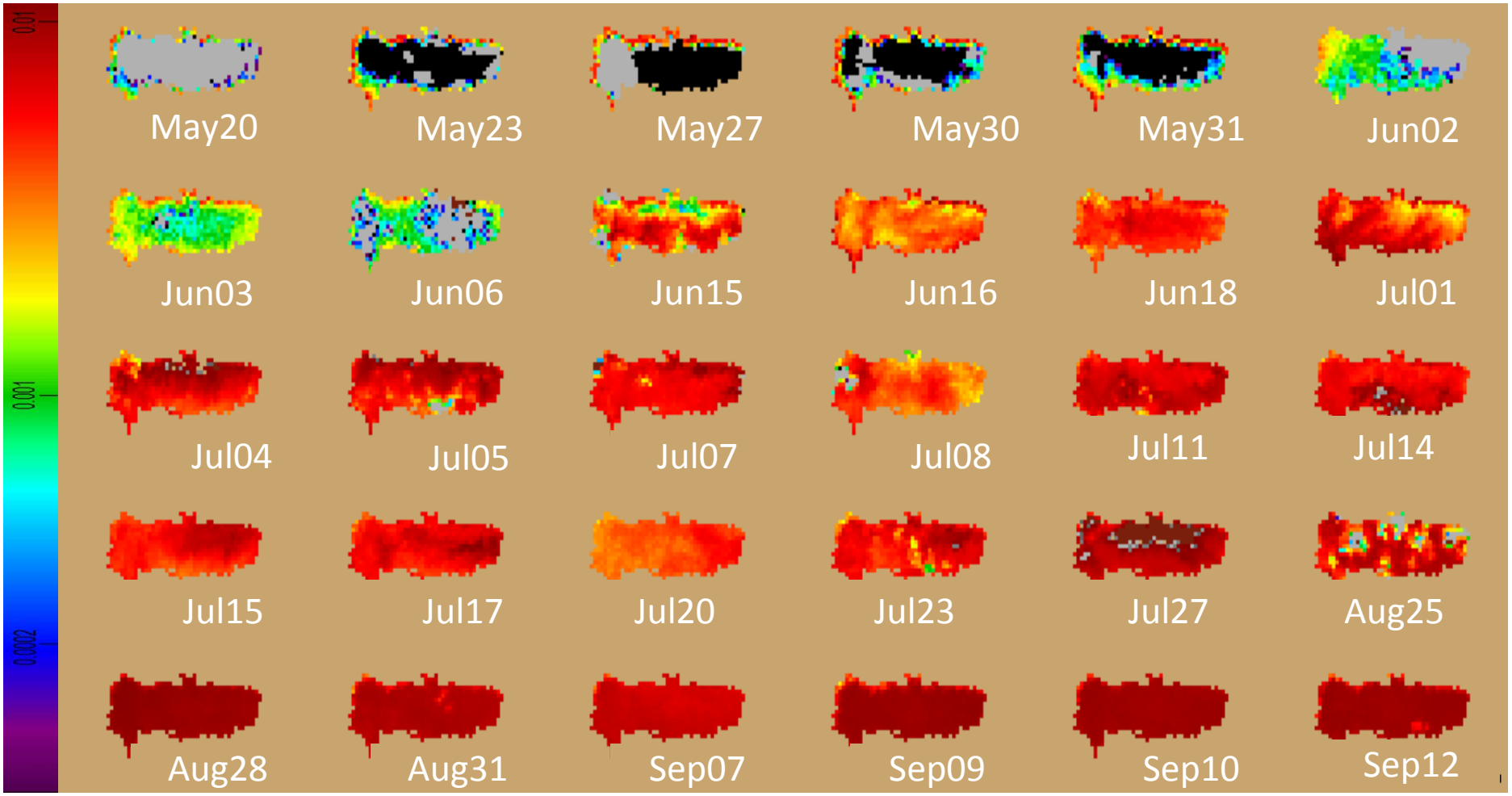
- **Microcystin concentrations at beach >100 ug/L**
- **Exceeded drinking water thresholds in raw water at all four area public water systems**



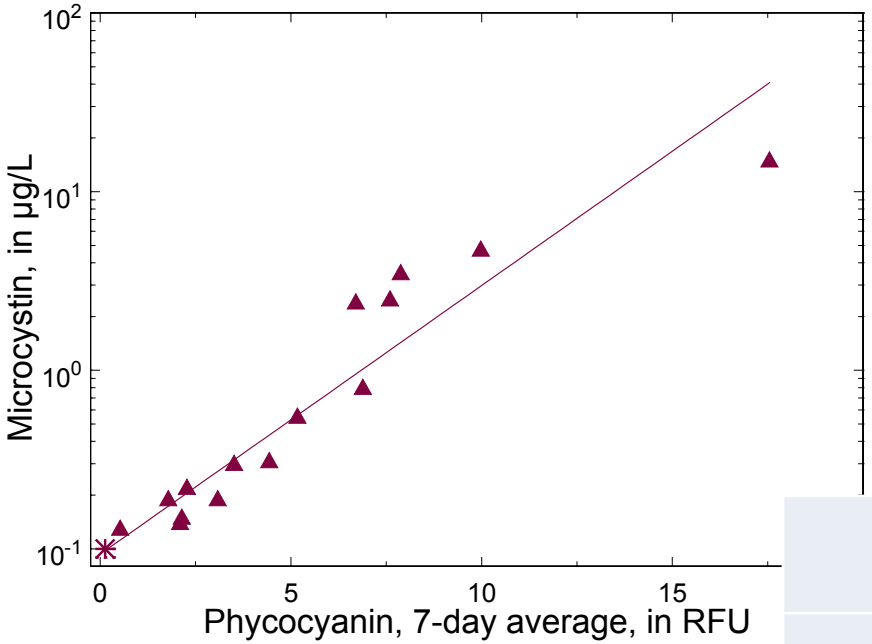
Application to Inland Lakes



Inland Lake Time Series



Use of Multi-Parameter Datasondes for Continuous Monitoring



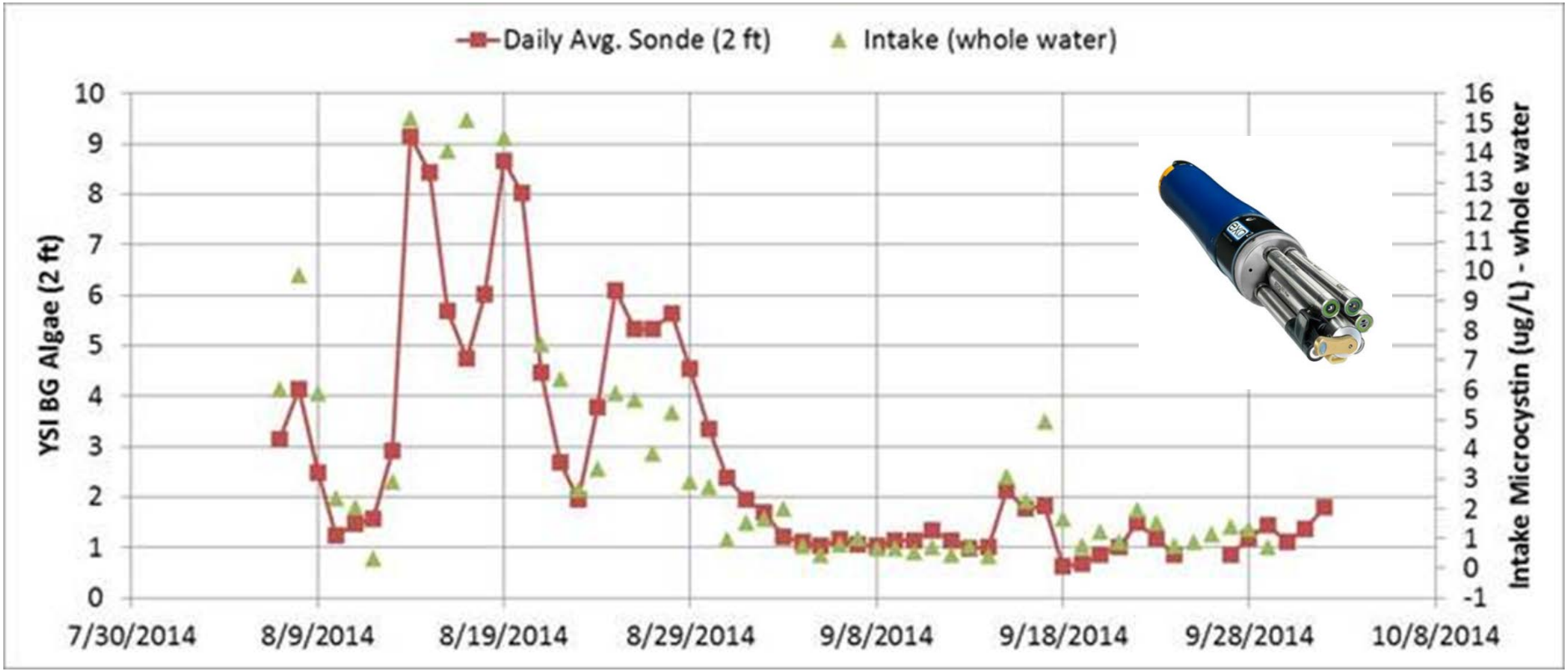
Data Courtesy:
Donna Francy, USGS

USGS Scientific Investigations Report 2015-5120

Spearman's correlation to microcystin concentrations	rho	p
Phycocyanin, 7-day average	0.98	<0.0001
Dissolved oxygen, 14-day average	0.88	<0.0001
pH, 7-day average	0.83	<0.0001
Temperature, instantaneous 10 a.m.	0.73	0.0031
Chlorophyll, 24-hour average	0.53	0.0358
Specific conductance, 3-day average	-0.20	0.4473

Phycocyanin Data Interpretation

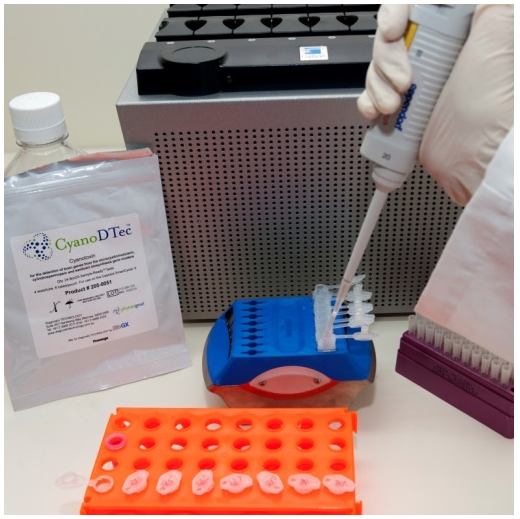
- Phycocyanin concentrations vary based on type of cyanobacteria present, turbidity of the water and other factors.
- Evaluate trends, not absolute values.
- Report data in Relative/Raw Fluorescence Units (RFUs) instead of cell counts.



-Graph provided to Ohio EPA by Ed Verhamme, Limnotech.

Cyanobacteria Screening: Molecular Methods (Multiplex qPCR)

- Quantitative polymerase chain reaction (qPCR) – identifies and quantifies the presence of genes unique to:
  - Cyanobacteria (16S rDNA, good correlation with cell counts)
  - Microcystin and Nodularin production (mcyE gene)
  - Cylindrospermopsin production (cyrA gene)
  - Saxitoxin production (sxtA gene)
  - Test completed within 2-3 hours (includes extraction)
  - Scalable
  - Cost-effective
  - Utilizes certified reference material
  - Specific: no gene, no toxin
- Ohio EPA method and certification in 2017
- Ohio EPA will use the data to trigger saxitoxins and cylindrospermopsin sampling and potentially reduce microcystins monitoring
- www.phytoxigene.com/products/



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Using Molecular Analysis to Direct Reservoir Management

- Saxitoxins detections in finished water from July 31, 2015 – September 21, 2015.
- Extracellular saxitoxins predominated all samples.
- 10 different potential saxitoxin producing genera found in multiple habitat zones (pelagic, benthic, periphyton, etc.) in multiple locations.
- qPCR results indicated benthic saxitoxin source, limited to shorelines. Data used to target algaecide application.

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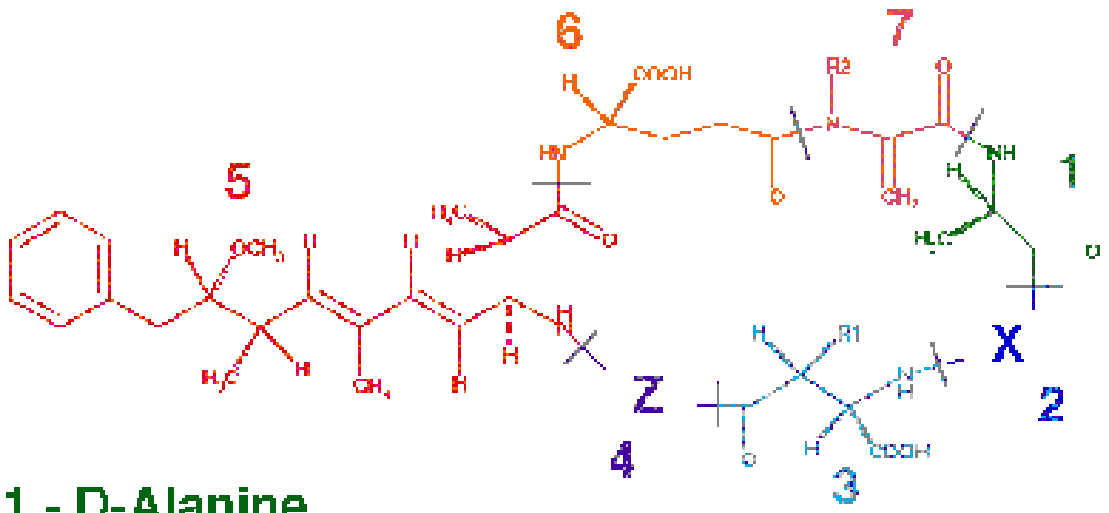
Microcystins Testing

No "Perfect" Analytical Method for Detecting TOTAL Microcystins



1 - D-Alanine
2 - Variable L-amino acid
3 - D-Methylaspartic acid
4 - Variable L-amino acid
5 - 3-amino-9-methoxy-2,6,8-trimethyl
-10-phenyldeca-4,6-dienoic acid (Adda)
6 - D-Glutamic acid
7 - N-Methyldehydroalanine

- Over 140 microcystin variants
- Standards not available for majority

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ELISA Microcystins-ADDA

Enzyme-Linked ImmunoSorbent Assay (ELISA) Microcystin-ADDA Method
(detection of antigen using an antibody)

- Measures total microcystins (all variants/congeners, based on ADDA)
- Highly selective/specific (for ADDA)
- Certified by U.S. EPA (ETV Program)
- Moderately sensitive (RL: 0.30ug/L)
- Suitable for raw & finished water (complex matrices)
- Quick (four hours), useful for operational adjustments
- Relatively inexpensive
- Does not require high end equipment or expertise to run (can be used in water system lab)
- Does not require pre-concentration solid phase extraction (SPE) step

- Does not provide concentrations of specific microcystin variants
- Is an indirect measure of the toxin

- **Ohio EPA Standard Method 701.0 & Lab Certification**
- **U.S. EPA Method Under Development**

Analytical Method Comparison & Microcystin Variant Evaluation

- 11 Sites: 4 up-ground reservoirs, 2 in-stream reservoirs, 2 Lake Erie locations, 2 canal-feeder lakes, and 1 river source
- 22 samples from 2014 selected to help evaluate spatial and temporal variability within source waters
- Variety of cyanobacteria genera represented
- Each sample analyzed using 5 separate analytical methods

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Liquid Chromatography (LC) – Ultraviolet (UV)

LC-UV

- Liquid Chromatography separates components
- Microcystins have UV absorption maxima at 238 nm
- Non-selective detector; co-eluting interferents prevent accurate identification of components and quantitation
- Less expensive than mass spectrometry
- Less sensitive than mass spectrometry (average LOQ ~ 0.3 µg/L)
- ISO 20179 Standard Method

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Liquid Chromatography(LC) –Tandem Mass Spectrometry (MS/MS)

- LC/MS/MS
  - Highly specific identification of components (based on standards)
  - MS can identify a component in the presence of co-eluting interferents but quantitation may be compromised
    - Presence of co-eluting interferents can act to suppress or enhance response resulting in analytical bias
    - Sensitive (LOQ ~ 0.02 µg/L)
  - "Weak" product ion abundance limits sensitivity. Requires pre-concentration with SPE to augment sensitivity (LOQs ≤ 0.02 µg/L)
    - Preconcentrates NOM too
  - U.S. EPA Method 544
    - Standard Method- includes QA/QC protocols and reduces variability in results between labs
    - Limited to 6 microcystin variants and finished water only
  - Expensive and requires highly skilled analysts

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LC-MS/MS MMPB Method

- MMPB (2-methyl-3(methoxy)-4-phenylbutyic acid) method analyzes the chemically cleaved Adda group common to all microcystin variants
- Measures total microcystins (all variants, based on ADDA)
- Quick (~2 hours, does not require freeze/thaw or sonication)
- Sensitive (0.05 ug/L)
- Suitable for raw water, some limitations with finished water
- Does not require standards for individual variants
- Utilizes 4 PB internal standard

- Does not provide data on individual variants
- Requires oxidation step
- Potential for detection of microcystins disinfection byproducts

Toxicon 104 (2015) 91-101 (Foss & Aubel): Using the MMPB technique to confirm microcystin concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS)

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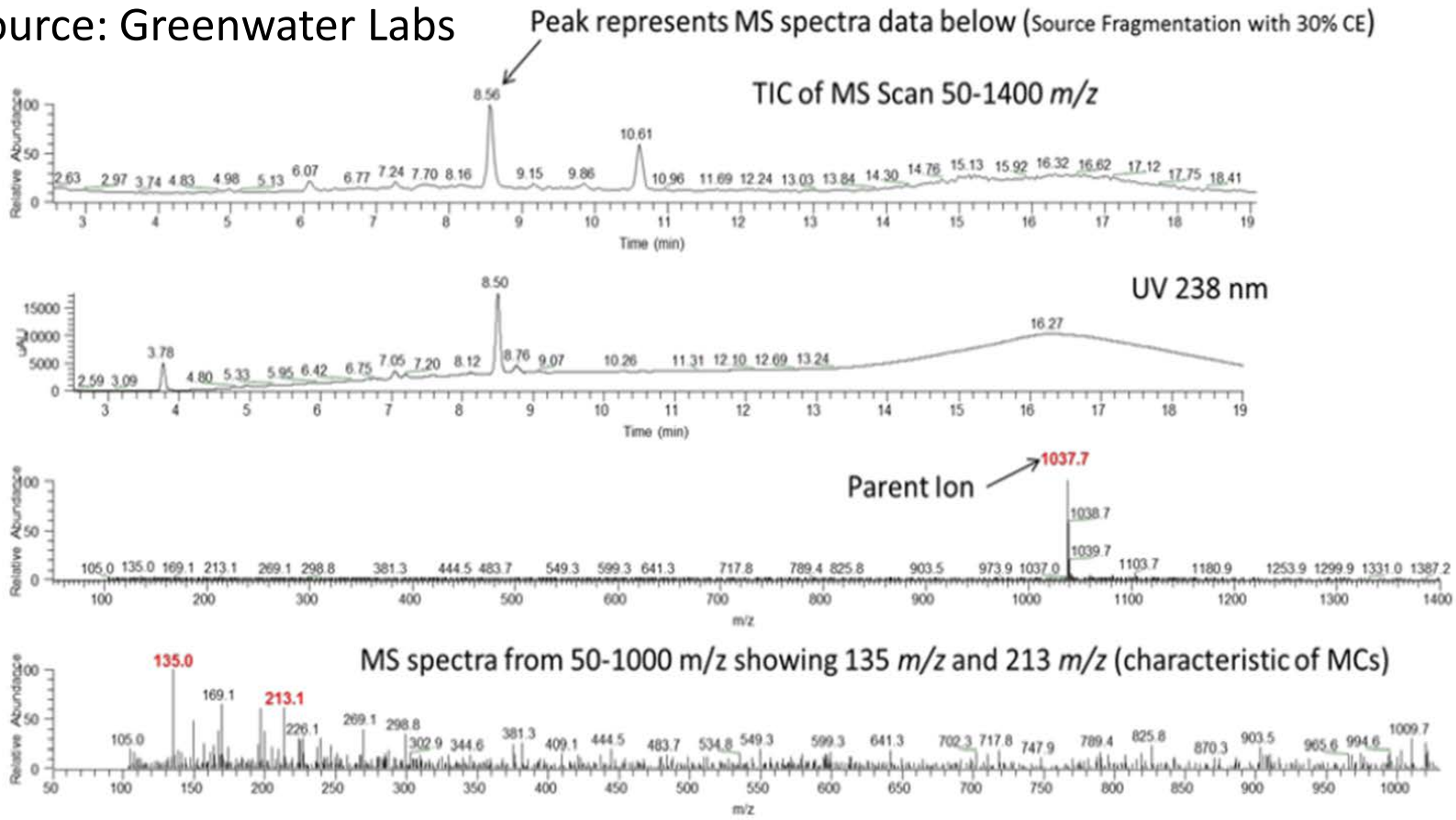
LC-UV/PDA & LC-MS Scan

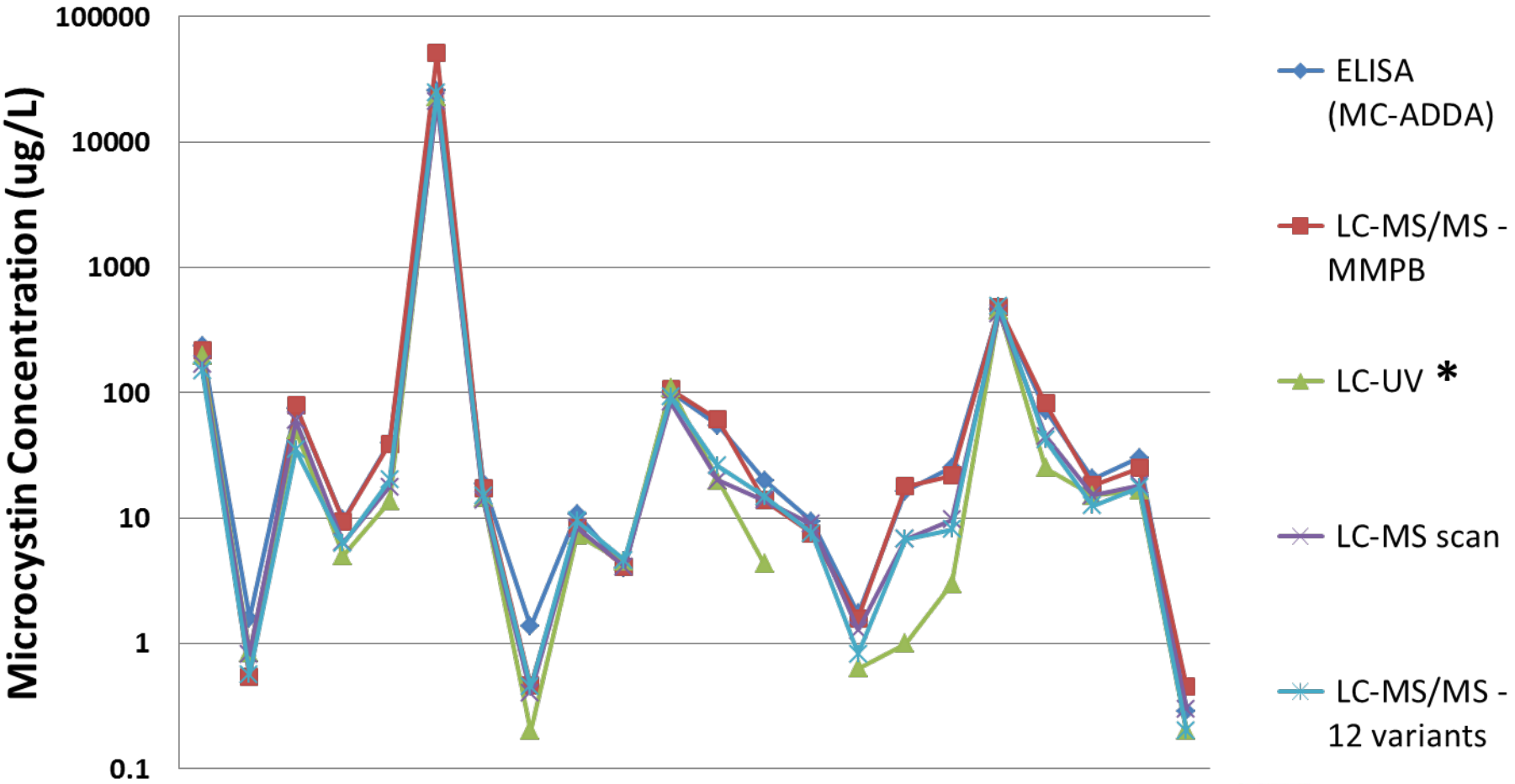
Uses two LC-based methods in tandem to independently confirm presence of microcystins

- Can detect microcystin variants without standards
- No standard methods, expensive, requires complex data-interpretation, time-consuming

Source: Greenwater Labs



Results of Method Comparison



* LC-UV data presented does not include false-positives that were eliminated from total (Based on lack of confirmation with LC-MS methods). Sample # 14 was non-detect using LC-UV.

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Ohio-EPA-10x-166165-E-Fork-Camp-Beach...
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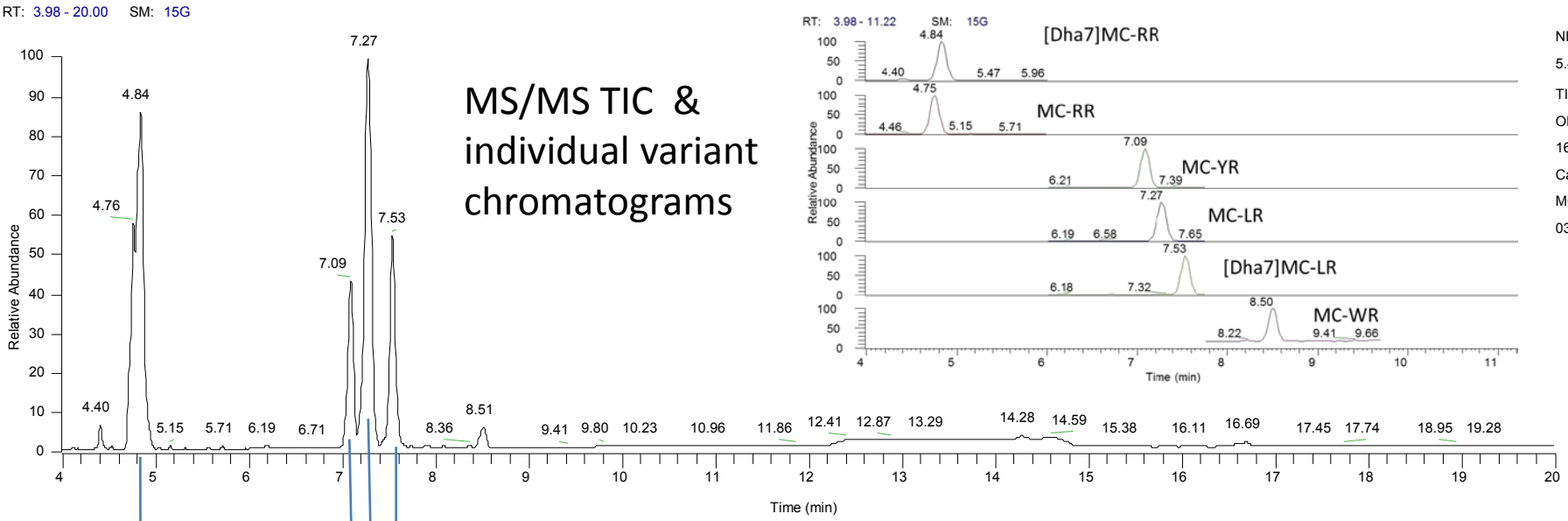
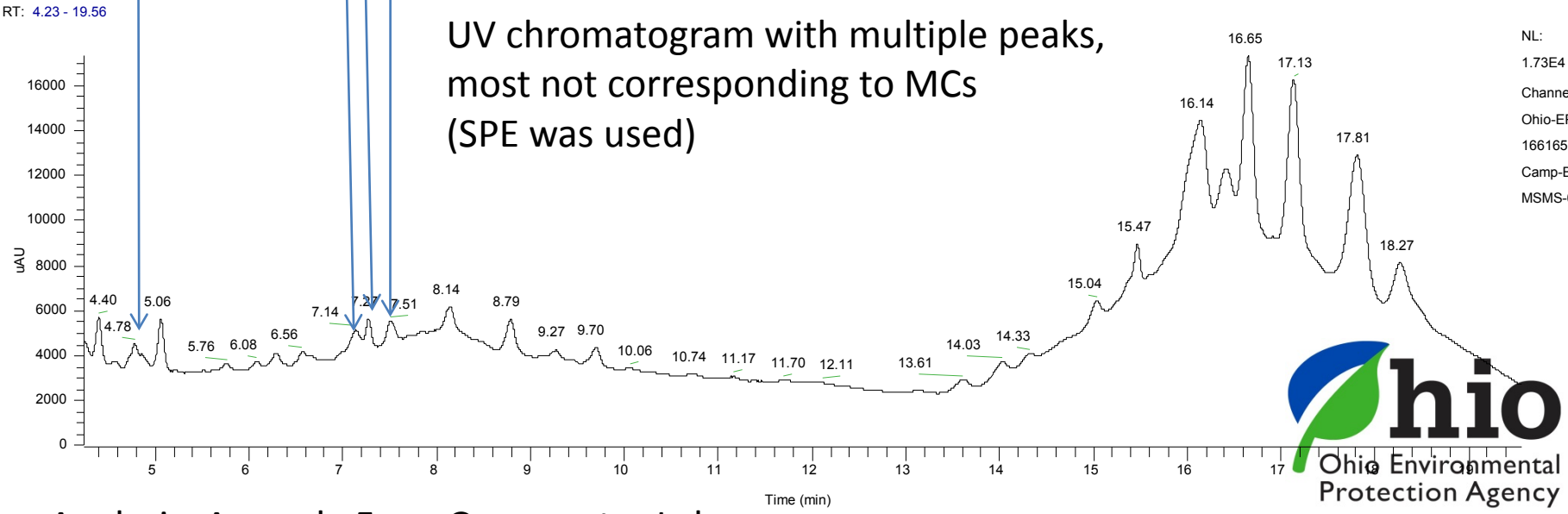
3/16/2015 4:38:28 PM



MS/MS TIC & individual variant chromatograms

NL:
5.49E2
TIC MS
Ohio-EPA-10x-166165-E-Fork-Camp-Beach-MC-MSMS-031615-2



UV chromatogram with multiple peaks, most not corresponding to MCs (SPE was used)

NL:
1.73E4
Channel A UV
Ohio-EPA-10x-166165-E-Fork-Camp-Beach-MC-MSMS-031615-2

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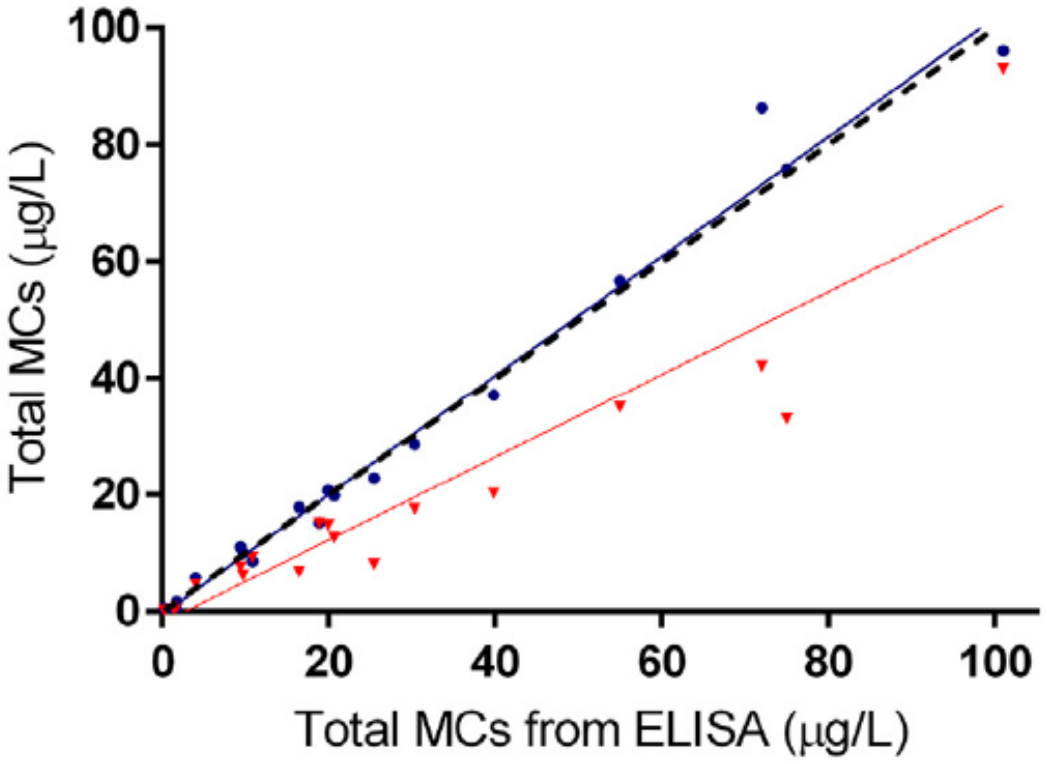
Analysis: Amanda Foss, Greenwater Labs

Results of LC-MS/MS MMPB and Individual Variant Analysis Compared to ELISA



MMPB

Y = 1.022*X - 0.4314

R^2=0.9830

Individual Variant Analysis

Y = 0.7095*X - 1.907

R^2=0.8724

ELISA

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Inland Lake Microcystin Variants (Planktothrix)			
MC-Variant	Site 1 6/16/14	Site 2 6/16/14	Site 2 9/2/14
[DAsp3] MC-RR	5.3	6.1	17.5
[Dha7] MC-LR	1.1	1.4	1.5
MC-YR	0.2-0.6	0.2-0.6	1.2
MC-RR		0.1-0.3	

Inland Lake Microcystin Variants (Mixed Bloom)				
MC-Variant	Site 1 6/18/14	Site 2 6/18/14	Site 2 7/9/14	Site 3 6/30/14
[Dha7] MC-RR	2.9	3-9	1.0	0.08
MC-RR	1.4	39	1.0	0.01-0.03
MC-YR	1.1	15	1.0	
MC-LR	4.0	67	2.4	0.55
[DAsp3] MC-LR	0.6	18	0.4	0.03
[Dha7] MC-LR	3.6		1.0	0.05
MC-WR	0.2-0.6		0.2-0.6	
MC-LA	0.2-0.6			
MC-LY	0.2-0.6	6	0.2-0.6	0.10

Key Findings

- 16 different MC-variants were detected
- MC-LR was only detected at 5 of 11 sites (45%)
- Most common variants were: MC-YR, [Dha7] MC-LR and [DAsp3] MC-RR
- LC-PDA methods prone to interference, potential for false positives and false negatives
- LC-MS/MS MMPB method confirmed ELISA results
- 91% of samples had MC-variants not detectable by U.S. EPA Method 544 (including dominant MC-variant in some samples)
- LC-MS/MS individual variant analysis under-reported total microcystins, based on MMPB and LC-UV/MS scan data

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ELISA MC-ADDA Matrix Interference Studies

Treatment Chemical	Microcystins – ADDA ELISA Assay Tolerance (< / =)
Sodium Carbonate (Soda Ash)	≤25 gpg
Sodium Hexametaphosphate	≤250 ppm
Sodium Silicofluoride	≤10 ppm
Aluminum Sulfate[1]	≤100 gpg (with pH adjustment within assay tolerance)
Calcium Oxide (Lime)[1]	≤2000 gpg (with pH adjustment to within assay tolerance)
Potassium Permanganate[2]	≤10 ppm (with quenching using 1 mg sodium thiosulfate per 1 ml sample)
Sodium Chlorite[2]	≤10 ppm (with quenching using 1 mg sodium thiosulfate per 1 ml sample)
Carbon[3]	≤2 ppm with filtering at time of sampling

[1]Natural pH of solution outside assay tolerance, tolerance levels determined after pH adjustment

[2] Oxidizers degrade microcystins, tolerance determined after quenching

[3] Tolerance level due to effect of carbon on toxin, not assay performance

Lisa Kamp, et. at, 2016. *The effects of water sample treatment, preparation, and storage prior to cyanotoxin analysis for cylindrospermopsin, microcystin and Saxitoxin.* Chemico-Biological Interactions.

ELISA MC-ADDA Matrix Interference Studies

Studies by U.S. EPA as part of ELISA MC-ADDA Method Development for UCMR 4:

- Storage Stability – Holding Times
- Sample Preservation and Container Studies
- Matrix Interference Studies
  - -Microcystins Variant Fortified Sample Studies (finished water, raw water, reagent water with chemical addition, etc.)
  - -Dilution Experiments (real world raw/finished water samples)
- U.S. EPA Method Validation & Interlab Validation

LC-MS/MS MMPB Method Evaluation:

- Potential concern regarding detection of microcystins disinfection byproducts
- ELISA MC-ADDA does not detect microcystins disinfection byproducts

Ohio Environmental Protection Agency

Analytical Methods Utilized by Ohio EPA

	Microcystins (µg/L)	Cylindro-spermopsin (µg/L)	Saxitoxins (µg/L)	Anatoxin-a (µg/L)
Surveillance sampling	ELISA (MC-ADDA)	ELISA	ELISA	LC-MS/MS
Repeat sampling in response to a finished water detection	ELISA (MC-ADDA)	LC-MS/MS	LC-MS/MS	LC-MS/MS

ELISA: Enzyme-Linked Immunosorbent Assay

LC-MS/MS: Liquid Chromatography followed by tandem Mass Spectrometry

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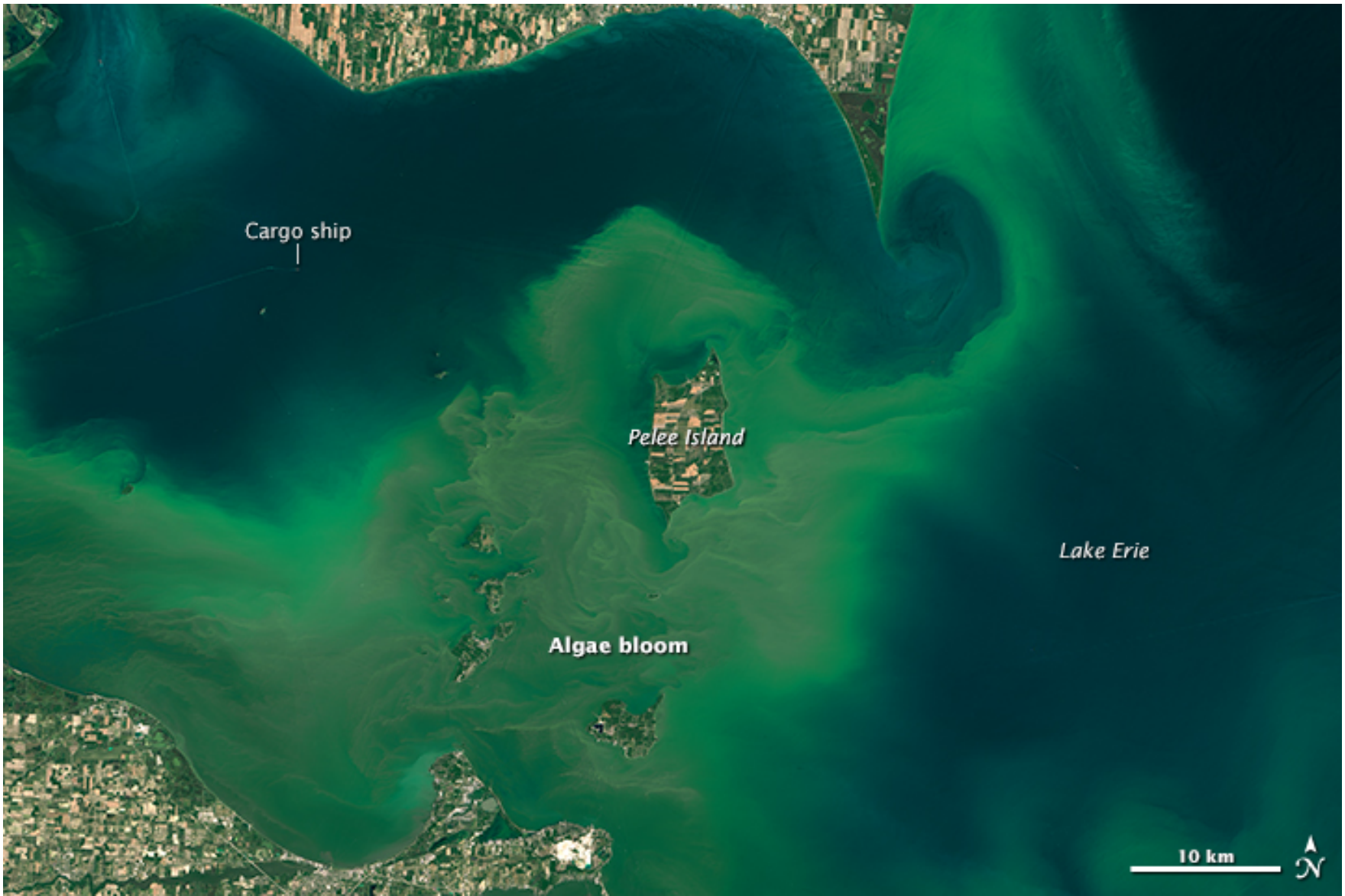
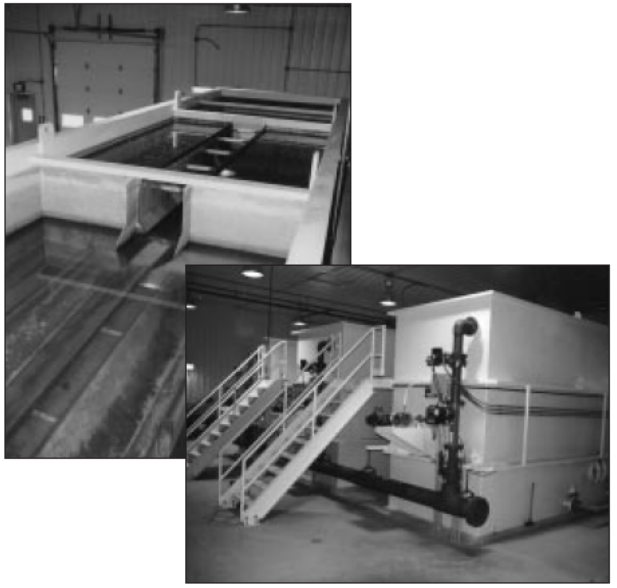
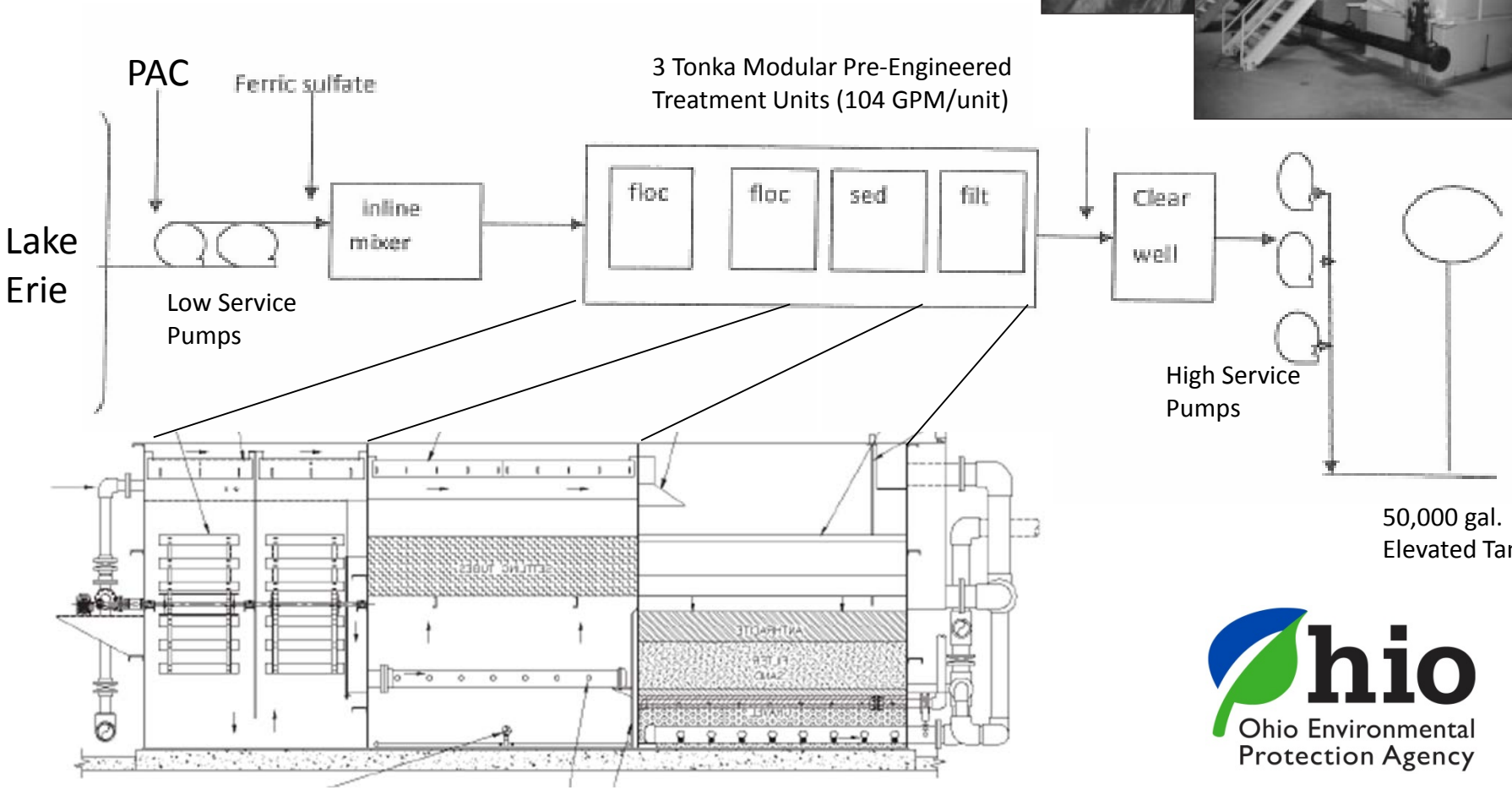
Treatment Optimization Case Study



Lake Erie Island PWS

- Conventional 0.3 MGD Surface Water Treatment Plant
- Plant detention time: 3 hours
- Wet well detection time: 35 minutes





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Treatment Optimization in Response to 1.8 ug/L Microcystins Finished Water Detection

- PWS worked with Ohio EPA to troubleshoot and optimize treatment
- U.S. EPA consulted for additional assistance
- Increased PAC dose to wet well (25 pounds/day)
- Added temporary PAC feed system to rapid mix (25 pounds/day)
- Additional PAC slurry to flocculators and some to top of tube settlers
- Changed PAC type
- Installed trash pump in wet well to promote mixing
- Removed sludge in sedimentation chambers nightly
- Decreased potassium permanganate pre-oxidant 50%
- Intake pre-chlorination off, small dose added prior to filters to address other treatment objectives
- Increased post chlorine, 1.6 to ~3mg/L (EP from 0.86 to 1.5 mg/L)
- Temporarily decreased pH to promote MC degradation, but affected other treatment objectives so discontinued
- All backwash to waste lagoon (no recycling)
- Slowed flow through plant

Post Event: purchased jar testing equipment, upgrading PAC feed systems

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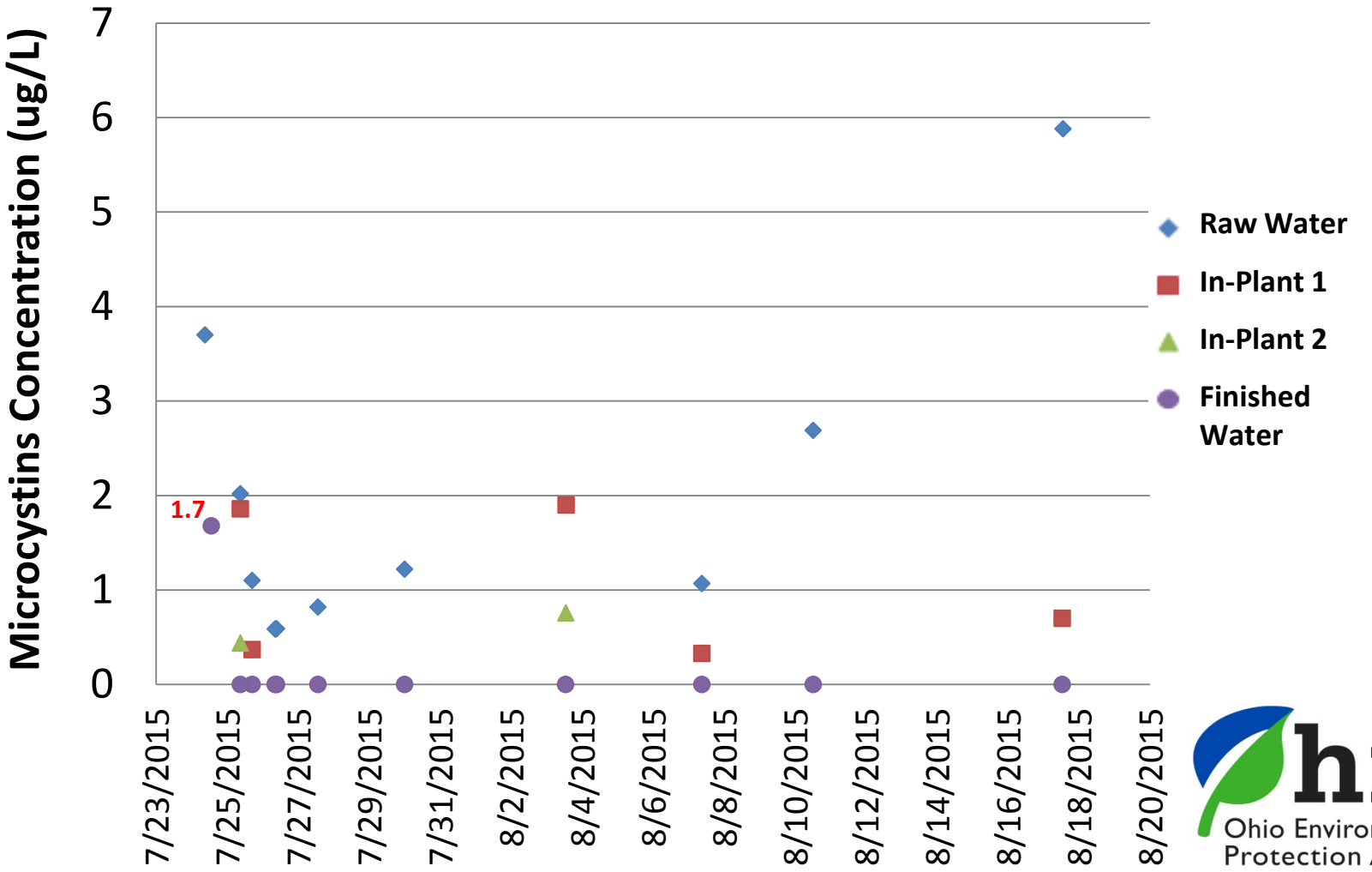
Microcystins Reduction in Treatment Train



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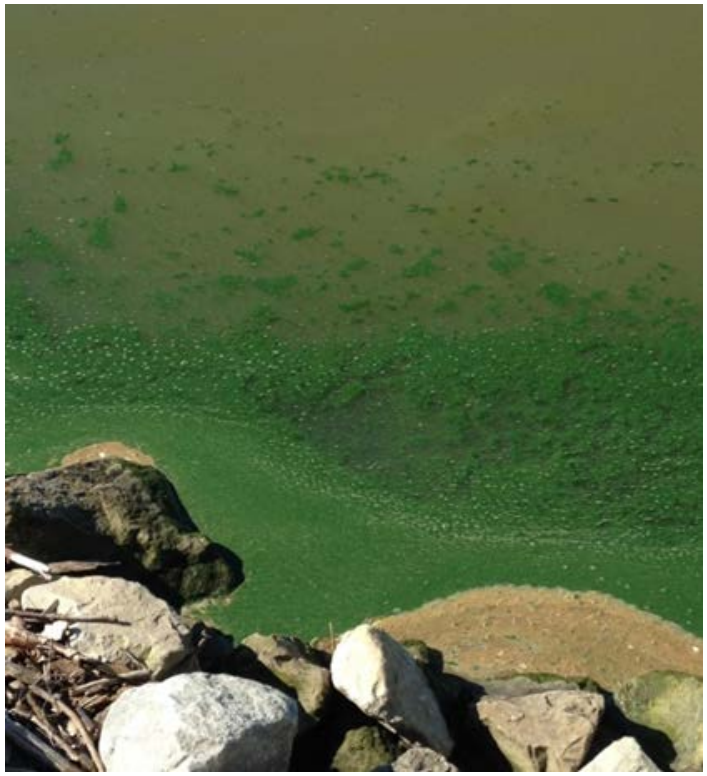
Reservoir Management Case Study

- 3 Reservoir System; 8 square miles, predominantly agricultural, watershed
- Used Ohio EPA Grants for ELISA testing equipment, microscope and data-sonde
- Historically, treated Lower Reservoir with copper-based algaecide every 2-3 weeks for total algae control

2014- Extensive *microcystis* bloom on Upper Reservoir. Just two days prior, reservoir appeared clear.

- Microcystins concentrations 15 ug/L. Posted recreational advisory.
- Dry weather left Upper Reservoir below capacity, enabling isolation.
- Two algaecide applications killed the m*icrocystis* in Upper Reservoir. HABs not detected on other reservoirs.
- Reservoirs checked daily until September, no further HABs detected.



June 2015 HAB on Upper Reservoir

August 2015 Isolated HAB

2015- HAB developed on Upper Reservoir much earlier, perhaps due to extremely wet June.

- Genera shift to *aphanizomenon* (dominant).
- Rain event caused some biomass to transfer to Memorial Reservoir.
- Treated Upper R. and spot treated Memorial R.
- Microcystins concentrations were >25 ug/L in Upper R. and in Memorial R. at the spillway, posted recreational advisories.
- Closed valve between Memorial and Lower Reservoirs to protect intake.
- Used monitoring data to focus algaecide application. Observed genera shift to *microcystis*.
- After algaecide application, took an additional week for cyanotoxins to dissipate. Continued applications until September.

Routine observation and monitoring focused algaecide application, timely reservoir isolation, and protected intake from cyanotoxins.

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Summary

- Some severe cyanotoxin-producing cyanobacteria blooms are not visually discernable, underscoring need for routine monitoring.
- Cyanobacteria can occur year-round, even in colder climates.
- Each analytical method has limitations and benefits.
- Current data support use of the ELISA MC-ADDA method for the detection of total microcystins.
- LC-MS/MS Individual Variant Analysis is appropriate for saxitoxins, cylindrospermopsin, anatoxin-a and individual microcystin variants, but it (U.S. EPA method 544) may under-report total microcystins.
- Methods continue to advance.
- Remote sensing and source water monitoring can be used to direct reservoir management strategies and trigger treatment optimization.
- Treatment optimization can reduce potential for cyanotoxin breakthrough.
- Consider proactive approaches: monitor source water, evaluate optimization options & update contingency plans to increase preparedness for a HAB.

Ohio Environmental Protection Agency

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