Harmful Algal Blooms & Analysis

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HAB 101 :

- Blue Green Algae are actually bacteria called cyanobacteria.
- They possess chlorophyll- a and phycocyanin both of which fluoresce.
- They occur mainly in marine and freshwater.
- They may or may not produce cyanotoxins.
- Under certain favorable conditions cyanobacteria may multiply rapidly resulting in a bloom.
- If the bloom is producing cyanotoxins, then the bloom is considered a harmful algal bloom or HAB.

Favorable conditions for algal growth

- Temperature
- Rainfall and drought fluctuations
- Nutrient Levels – total phosphorus and nitrogen (also iron, silica)
- Stagnation
- Fulvic and humic acids
- Sunlight
- Carbon Dioxide

Monitoring of Source Water:

Monitoring or screening these parameters as indicators for a potential bloom:

- pH
- Temperature
- Turbidity
- Total phosphate
- Total nitrogen (Nitrate/nitrite)
- Secchi depth (Water Clarity)
- Dissolved Oxygen
- Suspended Solids
- Chlorophyll-a
- Phytoplankton

Monitoring of Source Water:

- Grab Samples
- Cell Counts
- Online Monitoring: pH, temperature, turbidity etc.
- Fluorescense of phycocyanin in cyanobacteria
- Multi-parameter probes

Indicators of HAB during treatment:

- Increased chlorine demand
- pH changes
- Filter clogging and reduced filter run times
- Increased amount of coagulant
- Taste and Odor Issues

Determine the following:

- Are the conditions favorable for an algal bloom a HAB?
- Which of those conditions can be monitored and used as indicators?
- Have any indicators been triggered?
- If triggered, can Cyanobacteria be identified - Cell Count (Microscopic analyses)?
- Presence of cyanotoxins? Screening.
- Quantification and identification of the cyanotoxins.

Are cyanobacteria/cyanotoxins present?

World Health Organization Cyanobacteria Cell Count Action Levels:

Species	Action Level
***Microcystis* spp.**	2,000 cells/mL
***Anabaena* spp.**	15,000 cells/mL
***Aphanizomenon* spp.**	15,000 cells/mL
Combination of all potentially toxic cyanobacteria species present	15,000 cells/mL

ELISA Test Strips to determine if cyanotoxins present or the benchtop ELISA method.

Geosmin and MIB

- Low molecular weight tertiary alcohols
- Geosmin is 1,2,7,7 tetramethyl-2-norborneol and has an earthy smell associated with fresh turned dirt.
- MIB or 2-methylisoborneol has a musty taste and odor
- Produced by some species of cyanobacteria
- Have low odor thresholds for humans
- Method used for analysis is SM 6040D
- If Geosmin and/or MIB are determined to be greater than 10 ppt treatment should be initiated to remove such as PAC.

Triggers for more targeted analyses:

- Total Phosphorus (P) - Possible trigger value to treat the source water could be 0.05 mg/L
- Chlorophyll a - Possible trigger to perform an algae or cyanobacteria cell count could be 20-30 ug/L
- Water Clarity (Secchi Depth) - Possible trigger to perform an algae or cyanobacteria cell count would be less than one meter.
- Blue-Green Algae Counts - Possible trigger to treat the source water could be 5000 cells/mL.

Included in CCL3 and proposed for UCMR4:

- Microcystins, cylindrospermopsin, anatoxin-a and are cyanotoxins that are prevalent in freshwater and can possibly impact drinking water surface water sources.
- There are at least 80 known variants or congeners of microcystins. They are hepatoxins.
- Cylindrospermopsin is a hepatoxin.
- Anatoxin-a is a neurotoxin.

Before discussing analytical methods……

- Cyanobacteria that produce cyanotoxins contain them within their cell walls (intracellular) and also release the toxins to the water (extracellular).
- Intracellular toxins are released when the cell dies.
- Many analytical methods include a lysing step to rupture the cell membrane in order to release any toxins.
- Extracellular microcystins typically make up less than 30% of the total microcystin concentration in source water.

Microcystin Analyses

- HPLC coupled with MS or tandem MS
- HPLC coupled with UV/PDA
- Enzyme Linked Immunosorbent Assay (ELISA)
- Protein Phosphatase Inhibition Assay (PPIR)

Microcystin Analyses

PPIR Protein Phosphatase Inhibition Assay

- Useful as a screening tool
- Use a variety of detection methods and substrates
- Total microcystins
- DL of about 0.1 ug/L when using radiometric protein phosphatase assays (as microcystin –LR equivalents)
- DL is 0.01-0.02 ug/L when using colorimetric PP1 inhibition assays
- Adapted for field testing but do not have sensitivity below 1 ug/L and should be used qualitatively to determine presence or absence.

Microcystin Analyses

- ELISA- Enzyme Linked Immunosorbent Assay
- Various commercial products by vendors where MDLs range from 0.04 – 0.2 ug/L as Microcystin LR
- Result of total microcystins.
- The Abraxis ELISA-ADDA Analytical method has a working range of 0.3 ug/L to 5 ug/L and a Reporting Limit of 0.3 ug/L. It is used for the determination of MC in surface water, ground water and finished water.
- Benchtop and Mobile units

Microcystin Analysis

EPA Method 544: Determination of MC and Nodularin in Drinking Water by SPE and LC/Tandem MS (LC/MS/MS) Version 1.0 February 2015

- LC/MS/MS Method for microcystins and nodularin in drinking water.
- Microcystin LR, LA LF, LY RR and YR and nodularin R
- MRLs range from 2.9 to 22 ng/L.
- Requires an extraction surrogate
- Concentration of each analyte is determined by external standard calibration.
- Proposed for use with the UCMR4
- EPA is presently modifying this method for ambient water.

Microcystin Analysis

Being developed by EPA……

Simple and fast providing that you have a laser diode array thermal desorption atmospheric pressure chemical ionization interface coupled to tandem mass spectrometry (LDTD-APCI-MS/MS)

Oxidize the toxins using potassium permanganate under alkaline conditions to 2- methyl-3- methoxy- 4 -phenylbutyric acid (MMBP).

MMBP is extracted and injected directly into the instrument to give a total microcystin value. Does not identify the individual MC congeners. The MDL is 0.2 ug/L and the LOQ is 0.9 ug/L.

Microcystin Analysis

Field Tests

- Rapid
- Presence Absence to determine if bloom is toxic and if treatment of source water needs to be adjusted.
- Only sensitive to about 1 ug/L so should not be used for treated water analyses.
- Adapted using PPIR, ELISA
- Some do not include a lysing agent therefore only giving extracellular MC results. (consult manufacturer if total MC is required)
- Example Abraxis Microcystins Strip Test Product No. 520019 and 520020 "Immunochromatographic Strip Test for the Detection of Microcystins and Nodularins in Source Drinking Water at 1 ppb."

Cylindrospermopsin Analysis

- LC coupled with UV/PDA detector. LOQs range from 4 ug/L to less than 0.1 ug/L depending on the instrumental setup and preconcentration steps.
- LC-ESI/MS/MS LC coupled with electrospray ionization tandem MS (EPA 545)
- ELISA kits from various vendors available as semi-quantitative and quantitative. Can be adapted for field or screening measurements. Working concentration range of 0.05 ug/L to 2 ug/L.

Cylindrospermopsin Analysis

- **Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS)**
- Published April 2015
- Uses internal standards
- LCMRL was 0.063 ug/L and the average of 4 LCMRLs was 0.083 ug/L.
- Proposed for use with UCMR4
- EPA is presently modifying the method for ambient water.

UCMR4 and Cyanotoxin Monitoring

- The December 11, 2015 Federal Register proposed the details of the UCMR4.
- Includes Testing for microcystins, cylindrospermopsin and anatoxin-a. This would be required for UCMR4 systems that are surface water or ground water under the influence.
- Monitoring would be required 2X a month for 4 consecutive months between March and November. Sampling would not occur in the months of December, January or February.

1. The monitoring of MC deviates from the usual List 1 Assessment monitoring. It requires that source water samples be collected at the intake with POE samples. The source water would be tested using a method similar to that used by Ohio EPA's ELISA Abraxis method. The pH and temperature would also be measured at the intake.

UCMR4 and Cyanotoxin Monitoring

2. Phased sample analysis for MC: Based on the mycrocystin result of the intake, the POE sample would either not need to be analyzed, only analyzed by ELISA method or analyzed by both ELISA and EPA 544.

3. The MCs to be analyzed using 544 are microcystin –LA, LF, LR, LY RR, YR. In addition to the MCs, analysis of nodularin was proposed.

The POE would be sampled for cylindrospermopsin and anatoxin-a and analyzed using EPA 545. Source water monitoring would not be required with these analytes.

HAB and Analyses

THE END

- Questions?
- Discussion?