



CyanoHAB Analysis & Testing Methods from



Information Management
& Quality Assurance Center

State Water Resources Control Board

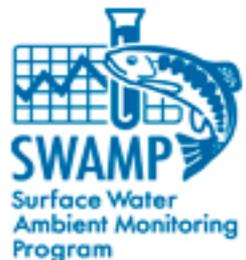
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Outline

- Field Based Analysis
- Laboratory Based Analysis
 - Toxin testing
 - Toxin genes detection
 - Cyanobacteria cell counts
- Response to Animal Illness/Death



Overview of Cyanotoxins

- Cyanobacteria capable of toxin production - contain toxins within *intact* cells
 - Exception for cylindrospermopsin producers
- Cyanotoxins can persist in water ways for weeks to months
- For cyanotoxin analysis, use cell “lysis” to release toxins
 - Lysis by chemicals or freeze/thaw cycles
- To detect and quantify cyanotoxins in water measure “total toxins”
 - Includes measurement of internal toxins and dissolved toxins

Collect HAB Sample

Whole water sample includes combination of water with particulates (visible colonies, scums, and/or algal mats)



Categories of Cyanotoxins

Common cyanotoxins include:

➤ Hepatotoxins

– **Microcystins**

- Class consists of 100+ variants (also called congeners)
- Variants have different toxicity
- Commonly found MC-LR, MC-LA, MC-RR, MC-YR

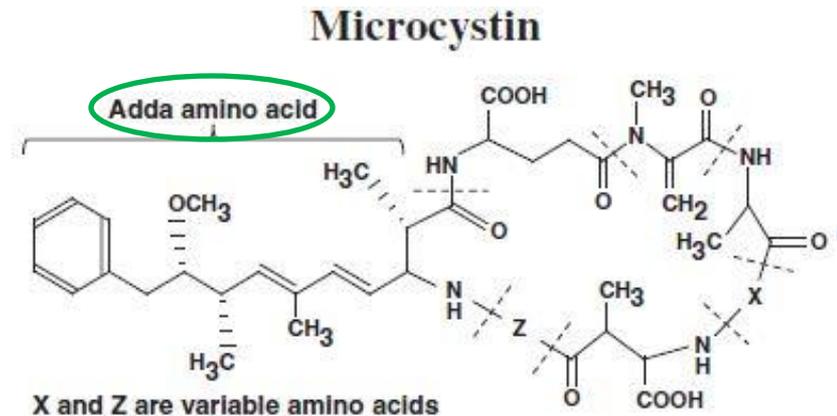
– **Cylindrospermopsin**

– **Nodularin**

➤ Neurotoxins

– **Anatoxin-a**

– **Saxitoxin**



Cyanobacteria Genera & Toxins

Cyanobacteria Genera	Hepatotoxins		Neurotoxins				Dermatoxins		
	CYN	MC	NOD	ATX	BMAA	NEO	SAX	LYN	LPS
Anabaena (Dolichospermum)	X	X		X	X	X	X		X
Anabaenopsis		X							X
Aphanizomenon	X	X		X	X	X	X		X
Aphanocapsa		X							X
Coelosphaerium (Woronichinia)		X							
Cylindrospermopsis	X	X		X	X		X		X
Gloeotrichia		X							
Limnothrix		X							
Lyngbya	X	X		X	X		X	X	
Microcystis		X			X				X
Nodularia			X		X				X
Nostoc		X	X		X				
Oscillatoria (Planktothrix)	X	X		X	X		X	X	X
Phormidium		X		X	X				
Planktolyngbya							X	X	
Pseudanabaena		X		X					X
Raphidiopsis	X			X					X
Synechococcus		X			X				X
Synechocystis		X			X				X
Woronichinia		X		X					

Cyanotoxins: LYN, lyngbyatoxin-a; LPS, lipopolysaccharides; CYL, cylindrospermopsins; MC, microcystins; NOD, nodularins; ATX, anatoxins; BMAA, β -N-methylamino-L-alanine; NEO, neosaxitoxins; SAX, saxitoxins.

Field Tools

Field cyanobacteria identification

- Capture digital images of algal material to aid identification
- CyanoScope – offers starter kit ~\$500 and collaborative group to aid ID

https://github.com/cyanoScope/cyanoscope.github.io/raw/master/assets/NEAEB_poster_2016_final.pdf

- Phoneskope - gadget to mount smartphone to microscope ~\$100

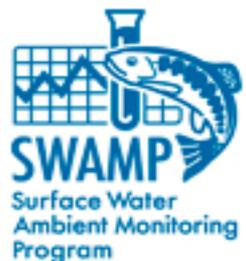
<http://www.phoneskope.com/microscope-mount-smart-phone/>



Field Tools

Field cyanotoxin detection

- Commercially available field kits based on ELISA technology – strip or tube based systems
 - Useful for confirming presence & absence
 - Results in ~45 minutes
 - Costs \$6-25 per test
 - Available for microcystins, anatoxin-a, and cylindrospermopsin



Field Tools

➤ Abraxis @

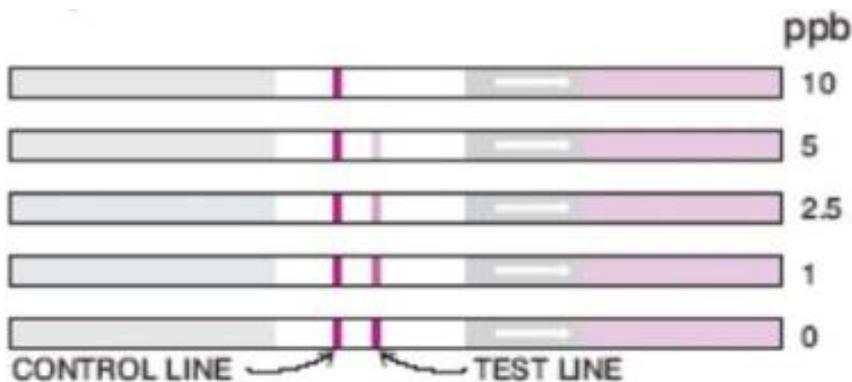
<http://www.abraxiskits.com/products/algal-toxins/>

- Strip tests; recreational water w/lysis (1-10 ug/L)
- Adding anatoxin-a & cylindrospermopsin mid-May

➤ Beacon Analytical Systems @

<http://www.beaconkits.com/welcome/category/algal-toxins>

- Microcystin ELISA tube kits (0.3-5 ug/L)



Laboratory Cyanotoxin Analysis

Method	Advantages	Disadvantages
Enzyme Linked Immunosorbent Assay (ELISA)	<ul style="list-style-type: none"> -Fast -Low detection limits -Economical -Kits available, relatively easy to use 	<ul style="list-style-type: none"> -Data interpretation somewhat difficult -High rate of false positives, recommend 2nd confirmation test
Liquid Chromatography – Mass Spectroscopy (LC-MS)	<ul style="list-style-type: none"> -Slower sample processing -Low detection limits -Good ability to differentiate among similar toxins 	<ul style="list-style-type: none"> -More expensive -Requires high tech instruments & experienced operators
Liquid Chromatography – tandem Mass Spectroscopy (LC-MS/MS)	<ul style="list-style-type: none"> -Slower sample processing -Low detection limits -Best ability to distinguish among similar toxins 	<ul style="list-style-type: none"> -Often most expensive, unless multi-toxin analysis method available -Requires high tech instruments & experienced operators

Laboratory Cyanotoxin Analysis

ELISA Method

What does it measure? Reacts to a chemical group found on similar compounds thus it is less selective for target compound/toxin

- microcystins detection specific to *MC-LR* or *ADDA* subgroup

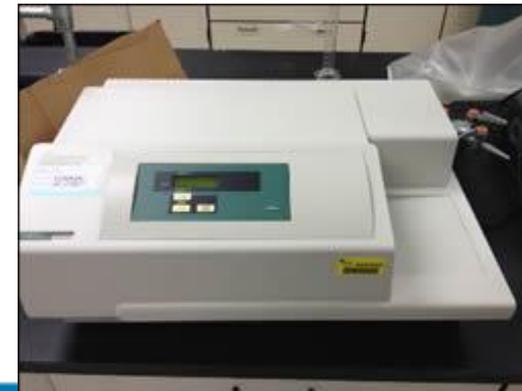
What are the results? Range of 0.01-5.0 ug/L or ppb

Microcystins test special considerations:

- Results in **units** of “total MCs” or “MC-LR equivalents” depending on ELISA kit design
- Kits have variable reactivity to all MC variants (see cross reactivity data)



ELISA analysis for microcystin-LR



Laboratory Cyanotoxin Analysis

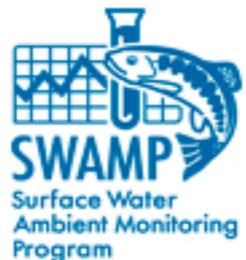
LC-MS and LC-MS/MS Methods

What does it measure? Separates and detects target compounds, response compared to standard curves

What are the results? Concentration of *individual* compounds/toxins
- detection limit 0.1 ug/L or ppb & upper limit based on lab method

Microcystins test special considerations:

- Analysis requires reference standards, only 4-12 MC variants
- Measures **individual** MC variants
- Results are not directly comparable with ELISA results because of different units



Laboratory Toxin Gene Analysis

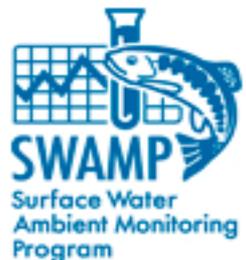
qPCR Methods

What is it? Uses quantitative Polymerase Chain Reaction (PCR), quick method to replicate target genetic material

What does it measure? Quantifies toxin producing genes

What are the results? Number of gene copies per volume (copies/mL)

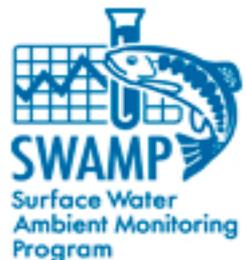
- Positive result – gene is available and may be activated to produce toxin
- Negative result – gene is not in bloom material
- Greater number of gene copies, greater capability of toxin production
- May correlate results to toxin concentration & cell counts



Laboratory Toxin Gene Analysis

How is qPCR applied to monitoring?

- Quick, large batch screening to assess which toxins can be produced by bloom
 - Informs toxin testing based on detected/non-detected genes
- Bloom forecasting
 - Correlate level of toxin concentration or cell counts to # toxin gene copies
- Characterize cyanobacteria blooms, informs risk assessments
 - What genera/strains make up the bloom?
 - What toxins can the bloom produce?
 - Locate source of toxic cyanobacteria



Laboratory Cyanobacteria Counts

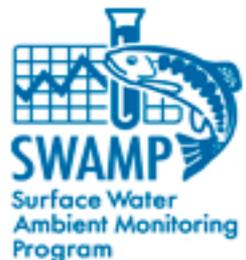
Cyanobacteria enumeration - to assess amount of bloom formation & rate

By Microscopy

- Lab technician counts cells using microscope microscopy
 - Units of cells/mL or natural units/mL
- Individual sample processing

By qPCR

- Instrument estimates total number of cyanobacteria by measuring # of nucleic acid copies (16S rRNA) in a sample
 - Units of copies/mL
- Multiple sample processing
- Often faster & economical alternative



Animal Illness/Death

Dog Exposure from Swimming and Drinking

- Severe symptoms within minutes/hours of exposure
 - loss of appetite, loss of energy, vomiting, foaming at mouth, diarrhea, convulsions, excessive drooling, tremors, after contact with water
 - More than one cyanotoxin possible in bloom, impacts different organs
- If veterinarian suspects cyanotoxin-caused illness:
 - Collect stomach contents (primary), feces and vomitus (secondary)
 - Treatment possible w/activated charcoal slurry (no antidotes)
 - If deceased, request necropsy from Vet prior to preservation & collect samples for toxin testing
 - Include Gross Necropsy Report (visual observations & interpretation)
 - Stomach contents or digestive tract (whole or 20g; freezing possible)
 - Liver and kidney (whole or 20g; no freezing required for histology)
- Submit samples ASAP to animal testing lab (see [Lab Services List](#) for location)

Animal Illness/Death

Reporting

- Report all suspected incidents to County Public Health Office and State Water Agency @ CyanoHAB.Reports@waterboards.ca.gov

Livestock & Wildlife are also susceptible to cyanotoxins

Report incidents to above plus CA Dept. of Fish & Wildlife Lab

- [Water Pollution Control Laboratory](#) (good contact for all types)
2005 Nimbus Road, Rancho Cordova, CA 95670
[Daniel Orr](#) (916) 358-2807 | General (916) 358-2859
- [Wildlife Investigations Lab](#)
1701 Nimbus Road Suite D, Rancho Cordova, CA 95670
[Stella McMillin](#) (916) 358-2954 | General (916) 358-2790
- [Marine Wildlife Veterinary Care and Research Center](#)
1451 Shaffer Rd, Santa Cruz, California 95060
[Melissa Miller DVM](#), PhD (831) 469-1746

Additional Information

- Regional Laboratory Services List @ http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/cyano_handout.pdf
- Interested in recommended quality assurance (QA/QC) & sample handling guidelines?
www.waterboards.ca.gov/water_issues/programs/swamp/mqo.shtml
- Interested in further discussion on lab analysis or interested in collaborating with laboratories?
 - To participate in the CA Cyanobacteria Lab Network email

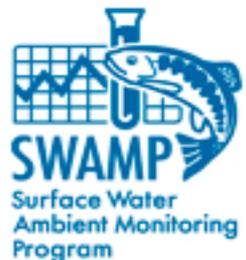
Marisa.vandyke@waterboards.ca.gov or
CaCyanoLabNetwork@gmail.com

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CA Environmental Protection Agency
CA State Water Resources Control Board

Lab Analysis – Quick Tips

- Maintain communication with lab, contact lab prior to sampling
- Identify what fraction of water sample should be tested - total fraction or dissolved & intracellular
- Request information about lab's QA program
- Discuss how results are reported in lab reports
 - Reporting % error of toxin concentration is important when comparing results alongside action thresholds (e.g. results of $0.8\mu\text{g}/\text{L} \pm 0.2$ compared with $1\mu\text{g}/\text{L}$ threshold)



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