Detection of Cyanobacteria and Cyanotoxins: Current Methodologies

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Analytical Techniques

- Microscopy
- Mouse bioassay
- Protein phosphatase inhibition assay
- Chlorophyll-*a*
- ELISA
- PCR
- HPLC

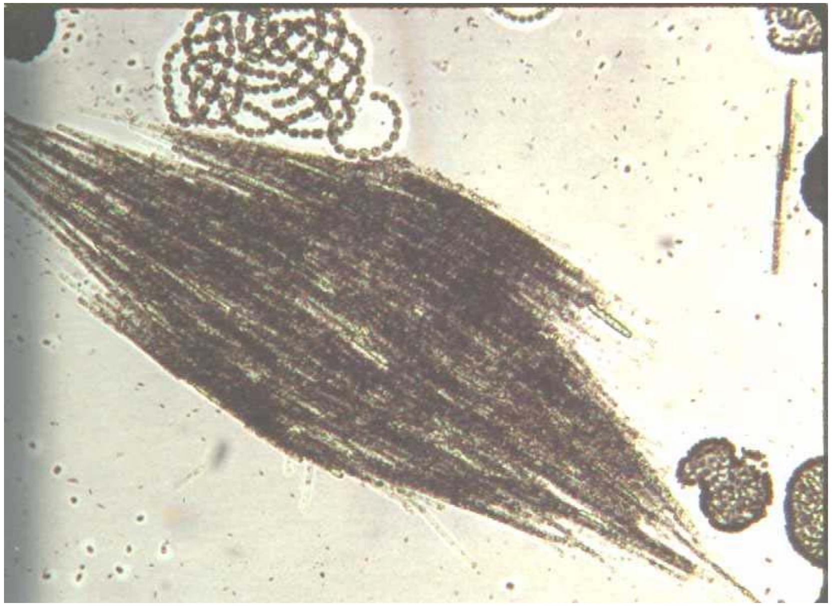
Microscopic Examination

Advantages

- Presence or absence of cyanobacteria (density, diversity and species composition)
- Quick and inexpensive
- Low detection level (cells/mL)

Disadvantages

- Presumptive for the presence of toxins
- Need trained personnel



Mouse Bioassay

Advantages

- Bioactivity of toxins can be determined
- Gold standard in vertebrate testing
- Show lethal and sub-lethal toxin effects

Disadvantages

- Non-specific response
- Labor intensive
- Working with live animals
- Special licensing and training needed

Protein Phosphatase Inhibition Assay

Advantages

- Rapid assay
- Relatively inexpensive
- Sensitive (0.3 µg/L)
- Measures total hepatotoxin

Disadvantages

- For hepatotoxins only (microcystin & nodularin)
- Can not identify hepatotoxin variants
- Non-cyanobacterial interferences can occur (e.g. okadaic acid)

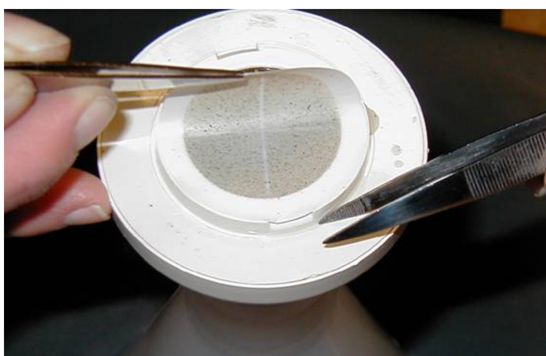
Chlorophyll-*a*

Advantages

- Quick
- Inexpensive

Disadvantages

- Measures all algal biomass (cyanobacteria and non-cyanobacteria)
- Presumptive for toxins



ELISA
(enzyme-linked immunosorbent assay)

Advantages

- Rapid assay
- Sensitive (0.1 µg/L)
- Commercialized kits
- Cost effective
- Cross-reactive to variants (total toxin)

Disadvantages

- Non-specific for compounds present
- Only available for microcystin, nodularin, saxitoxin and cylindrospermopsin (no anatoxin kits)
- Limited shelf life

PCR (polymerase chain reaction)

Advantages

- Presence of *mcy* DNA fragment shows possible toxin production
- Highly specific and sensitive (0.001 µg/L)
- Fairly rapid
- Fairly inexpensive
- Can be a quantitative assay

Disadvantages

- For microcystin producing cyanobacteria only
- False positives possible

HPLC (high performance liquid chromatography)

Advantages

- High selectivity
- Multi-toxin analysis possible
- Low detection levels
- Capable of quantification and confirmation in a single analysis

Disadvantages

- Requires costly equipment & supplies
- Requires high level of analyst training
- Per sample cost is high
- Multiple reference standards needed

Ranking the Methods
(e.g. Microcystin toxin analysis)

Method	*Selectivity*	*Cost*	*Time*	*Training*	*Overall Rank* *
PPi	63	19	25	20	1
ELISA	50	42	25	20	2
PCR	56	9	50	29	3
Microscopy	100	3	50	42	4
HPLC/LC	38	63	50	74	5
Mouse	75	13	100	42	6

Adapted from Hawkins et al. 2005. *J. of Water Supply: Research and Technology-AQUA*, 54:509-518.

* Lower score corresponds to the highest ranking and most preferred method.

Method Summary

- Microscopy – very good screening method for potential toxic cyanobacteria
- ELISA & PPi– very good for screening environmental water samples (reliable and highly specific)
- PCR – highly sensitive (low detection limit) and low cost
- HPLC – good for final confirmation and/or to examine more than 1 toxin at same time

HAB Projects at WSLH

Historical Perspective

1878*	Livestock	Australia
1931	Livestock	Australia
1979	Humans	Australia
1983	Swine	Illinois
1985	Cattle	Wisconsin
1985	Dogs	Wisconsin
1996	Humans	Brazil
2004	Dogs	Wisconsin

* Francis, G. 1878. Poisonous Australian Lake. *Nature*, 18:11

1986 – Wisconsin Cyanotoxin Occurrence Study

- 286 samples analyzed from 102 sites
- 28% tested positive for acute toxicity via mouse assay
  - *Microcystis, Anabaena, Aphanizomenon, Planktothrix*, & *Lyngbya* identified in toxic samples
  - Acute hepatotoxicity was always associated with the occurrence of *Microcystis*
  - Acute neurotoxicity was always associated with *Anabaena* occurrence

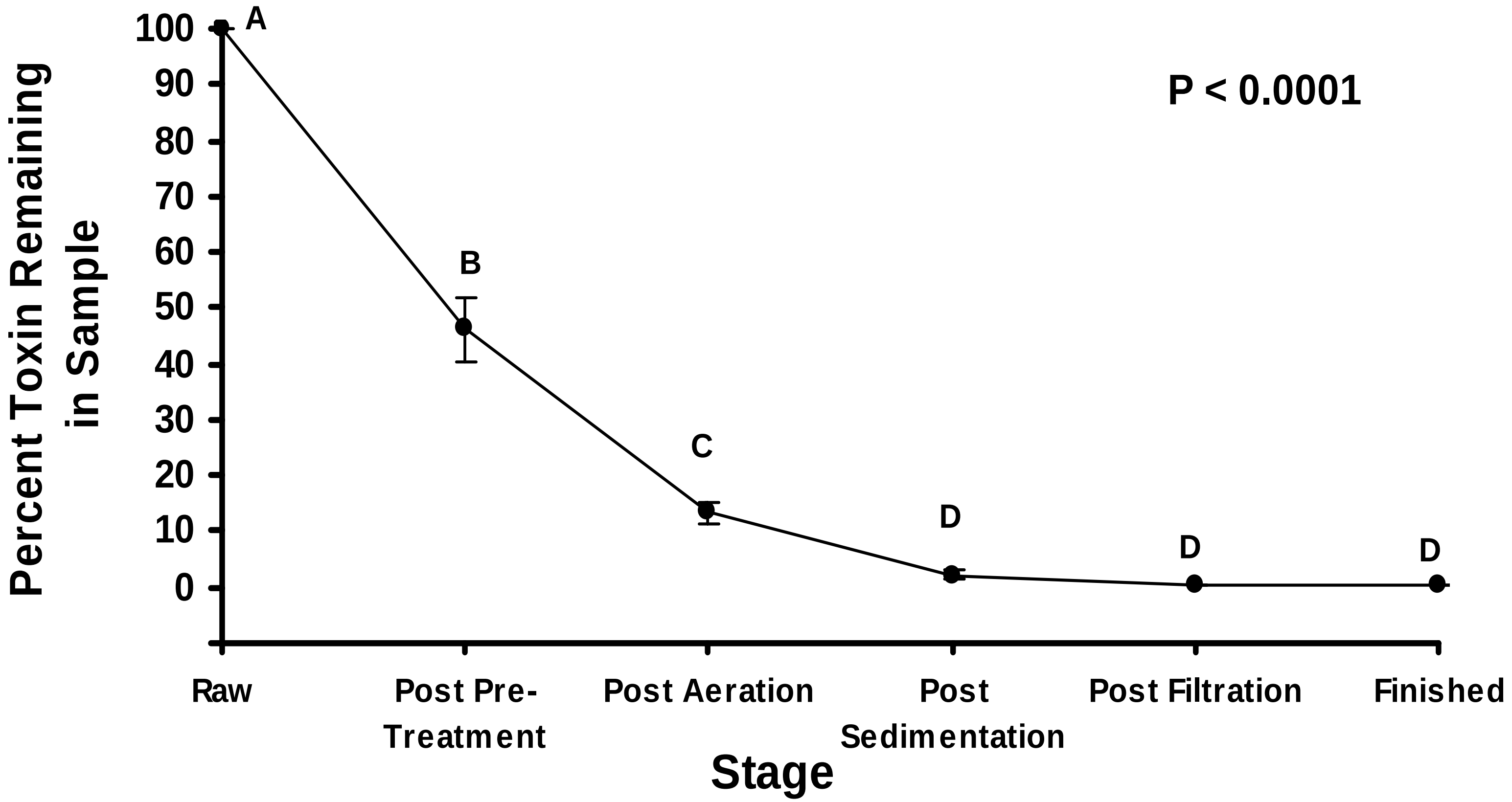
Repavich, WM et al. 1990. Cyanobacteria (blue-green algae) in Wisconsin Waters - Acute & Chronic Toxicity. *Water Research*. 24:225-231

1998 and 1999 Microcystin Project

- Studied microcystin concentrations in Lake Winnebago & Rainbow Lake
  - Followed drinking water treatment facilities processes to examine toxin removal
  - Used ELISA assay to analyze for Microcystin
  - 1998 - 107 out of 289 Lake Winnebago samples (37%) exceeded the WHO guideline of 1.0 µg/L Microcystin-LR
  - 1999 - toxin levels in Lake Winnebago never exceeded the WHO guideline (highest was 0.99 µg/L at Neenah)
  - All facilities showed significant toxin reduction of 2-3 logs to safe levels

Karner, DA et al. 2001. Microcystin algal toxins in source and finished drinking water. *JAWWA*. 93:72-81

1999 Menasha Drinking Water Treatment Facility - Microcystin Removal

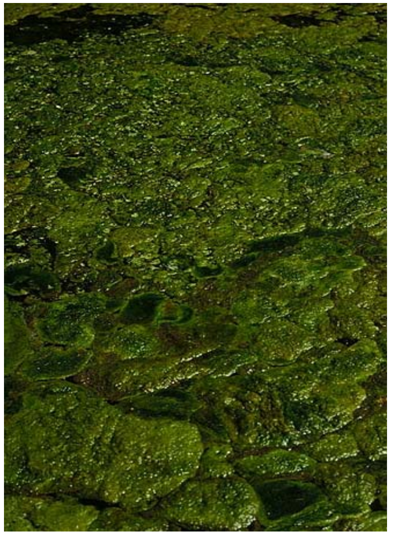


2004 – 2006 Monitoring

- Lakes
  - Focused on lakes with history of cyanobacteria blooms (eutrophic)
  - Recreational use
  - Beaches present
  - Not treated with herbicides
- Ponds
  - Eutrophic
  - History of blooms
  - Not treated with herbicides
- Natural Ponds
  - Beach present and used for recreation
- Detentions Ponds
  - Located in residential areas
- Golf Course Ponds
  - Accessible to public (municipal)

2004 - 2006 Monitoring Continued

- Summer 2004
  - WDNR collected 187 samples throughout Wisconsin
    - 31 lakes (148 samples)
    - 10 Ponds (38 samples)
    - 1 River (1 sample)
- Summer 2005
  - WDNR collected 194 samples
    - 38 Lakes (154 samples)
    - 8 Ponds (35 samples)
    - 1 River (5 samples)



- Summer 2006
  - WDNR collected 40 samples (mostly in June 2006)
    - 29 Lakes (33 samples)
    - 5 Ponds (5 samples)
    - 2 Rivers (2 samples)

2004 - 2006 Monitoring Continued

- Samples analyzed at the Wisconsin State Lab of Hygiene for:
  - Cyanobacteria microscopic ID and enumeration
  - Chlorophyll *a*
  - Toxins (selected samples) via HPLC/MS/MS:
    - Microcystin-LR
    - Anatoxin-a
    - Cylindrospermopsin

2004 - 2006 Overall Results

- 2004 Results
  - Cyanobacteria detected in 138/187 samples = **74%**
  - 31/45 samples analyzed tested positive for toxins
- 2005 Results
  - Cyanobacteria detected in 143/194 samples = **74%**
  - 14/34 samples analyzed tested positive for toxins
- 2006 Results
  - Cyanobacteria detected in 27/40 samples = **68%**
  - 5/9 samples analyzed tested positive for toxins

2004 - 2006 Cyanobacteria Results

- Cyanobacteria blooms occur throughout WI
  - Most severe in South-Central (near Madison) and West Central (near Menomonie)
- Concentrations frequently above the WHO level (100,000 cells/mL)
- Large blooms of *Anabaena*, *Aphanizomenon* and *Microcystis* occur throughout the summer
- Blooms of *Anabaena* sp. occur early (beginning of June)
- *Cylindrospermopsis* sp. not detected until August and September

Cyano Resources

- **World Health Organization**
  - *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management (edited by Chorus and Bartram)*
    - http://www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/index.html
    - Second edition currently being written
- **NOAA Center of Excellence for Great Lakes and Human Health (CEGLHH)**
  - http://www.glerl.noaa.gov/res/Centers/HumanHealth/ceglhh.html
- **CyanoNet** **www.cyanonet.org**
  - A project by the United Nations Educational, Scientific and Cultural Organization (UNESCO) International Hydrology Programme (IHP)
  - "A global network for the hazard management of cyanobacterial blooms and toxins in water resources"
- **EPA: International Symposium on Cyanobacterial Harmful Algal Blooms**, September 2005 in RTP, NC
  - http://www.epa.gov/cyano_habs_symposium/
  - Monograph will be available through Springer Press


QUESTIONS??