Comparison of Methods for the Measurement of Cyanotoxins in Recreational Water

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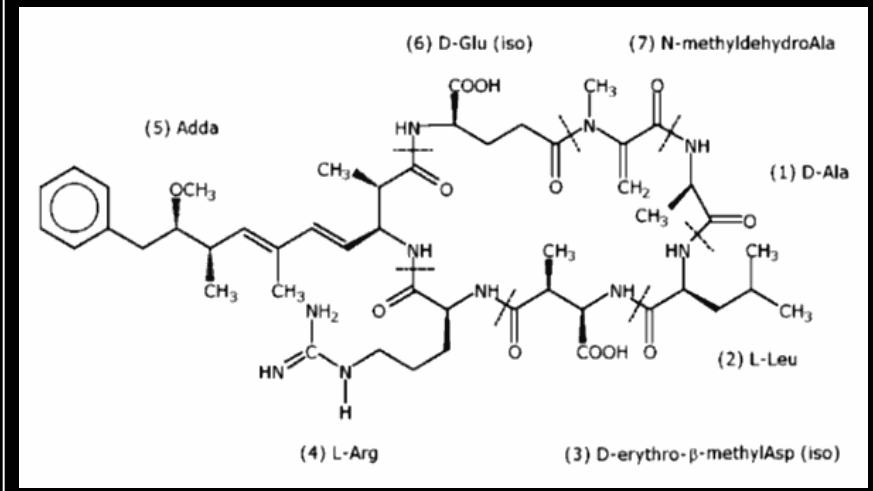
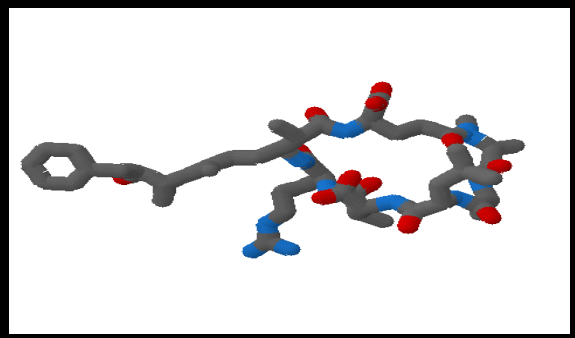
Cyanobacteria

- **Oxygen producing prokaryotes**
- **3.5 billion years old**
- **Adapted to extreme environments; desiccation, permafrost, high UV, low light, low nutrient, and wide temperature range environments**
- **Unicellular, colonial, filamentous**
- **30% can fix nitrogen**
- **Primitive organism – complex biochemistry**
- **Produce a variety of hepatotoxins and neurotoxins**

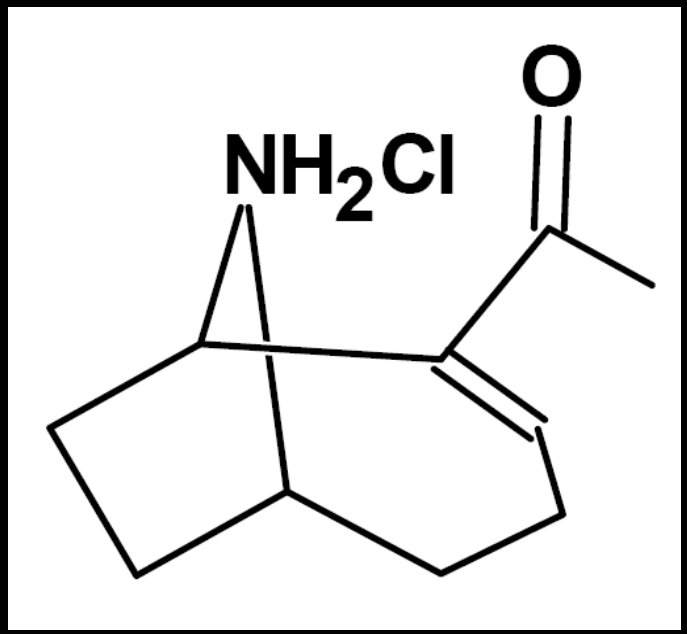
Microcystins





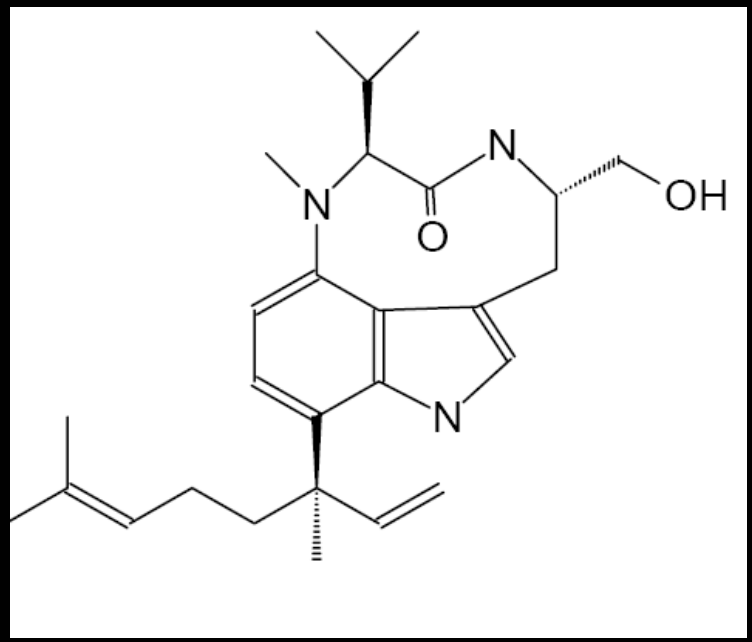
- Polypeptide
- 90 congeners (LR, RR, YR, etc), 200 related compounds
- Produced by *Microcystis* and other genera
- LD_{50} 25-60 *u*g/kg (cyanide 4 mg/kg)
- Hepatotoxin and tumor promoter

Anatoxin



- Alkaloid
- 3 common variants
- Produced by *Anabaena* and other genera
- LD_{50} 200 *u*g/kg
- Neurotoxin

Cylindrospermopsin



- Alkaloid
- Produced by *Cylindrospermopsis*
- LD_{50} 300 *u*g/kg
- Hepatotoxin and Neurotoxin
- Subtropical species recently reported in Michigan

Recreational Water

- Human exposure from ingestion, dermal contact, and inhalation
- Wildlife contact from ingestion
- WHO Guidelines:

Advisory	Microcystin LR	Chlor *a*	#/ml
Moderate	20 ug/l	50 ug/l	10^5
High	100 ug/l	100 ug/l	10^7

Methods

General

- **Visual**
- **Chlorophyll *a***
- **Plankton Counts**

Specific

- **Enzyme-Linked Immunosorbent Assay ELISA**
- **Chromatographic Methods (LC/UV or LC/MS)**

Activity Based

- **Protein Phosphatase Inhibition Assay (PPIA)**

Visual-Chlorophyll-Plankton Counts

- Easy to tell *Cladaphora* from cyanobacteria
- Many species of cyanobacteria do not produce toxins
- Strains capable of toxin production may not actually produce toxins

ELISA Methods

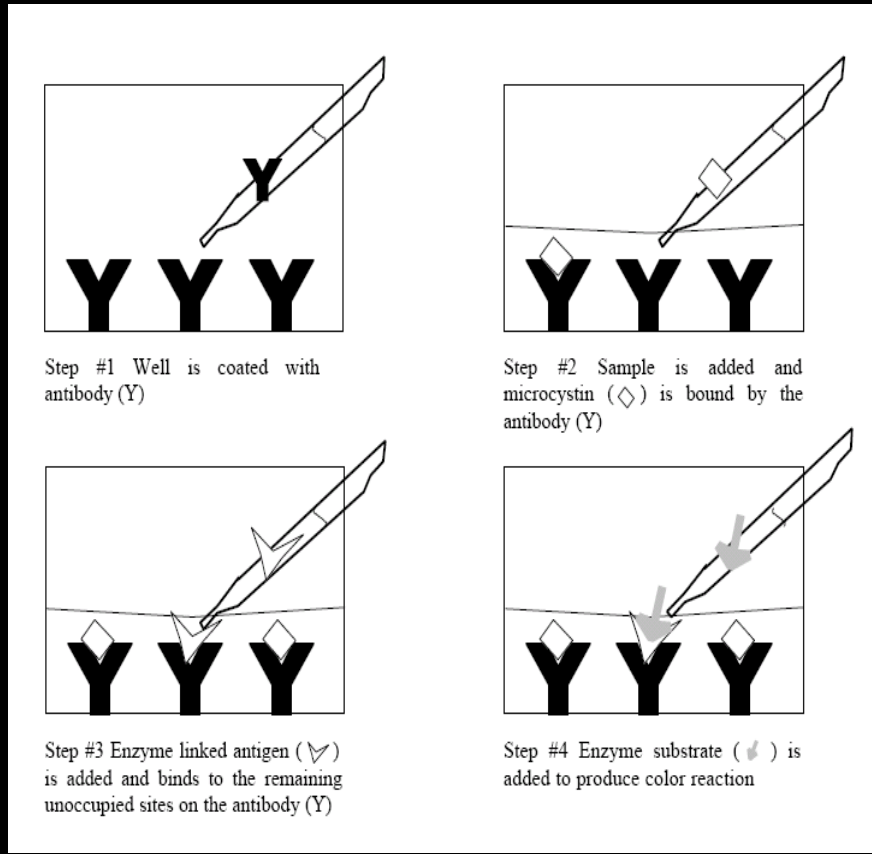
Based on structure

Rapid results at low cost ($10)

Test reports in LR equivalents

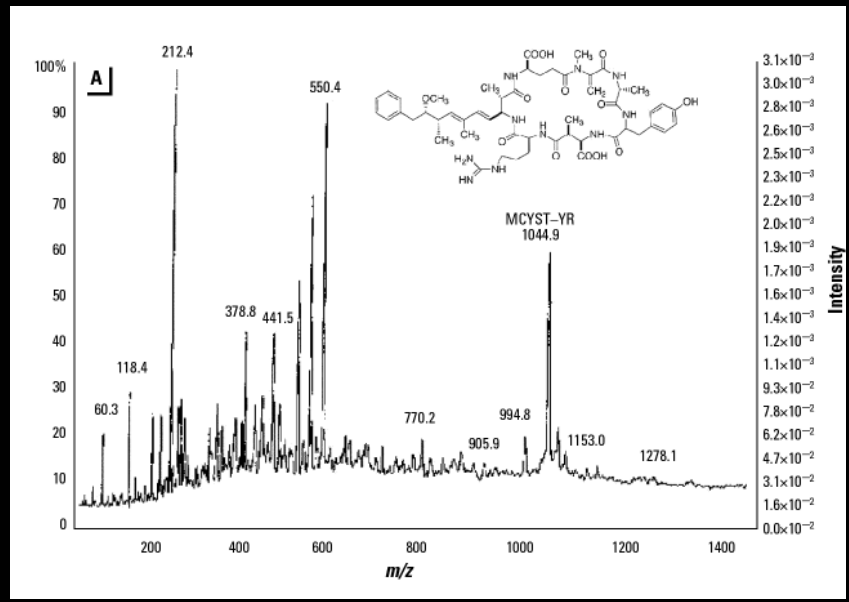
Potential problems from

- **Pipetting errors**
- **Contact with plastic**
- **Methanol concentration**
- **Cross reactivity**



Chromatographic Methods

- Expensive equipment ($50-$150K)
- Few standards are commercially available
- Arginine based congeners behave differently from non arginine analogs
- Precise and accurate results for a limited number of compounds



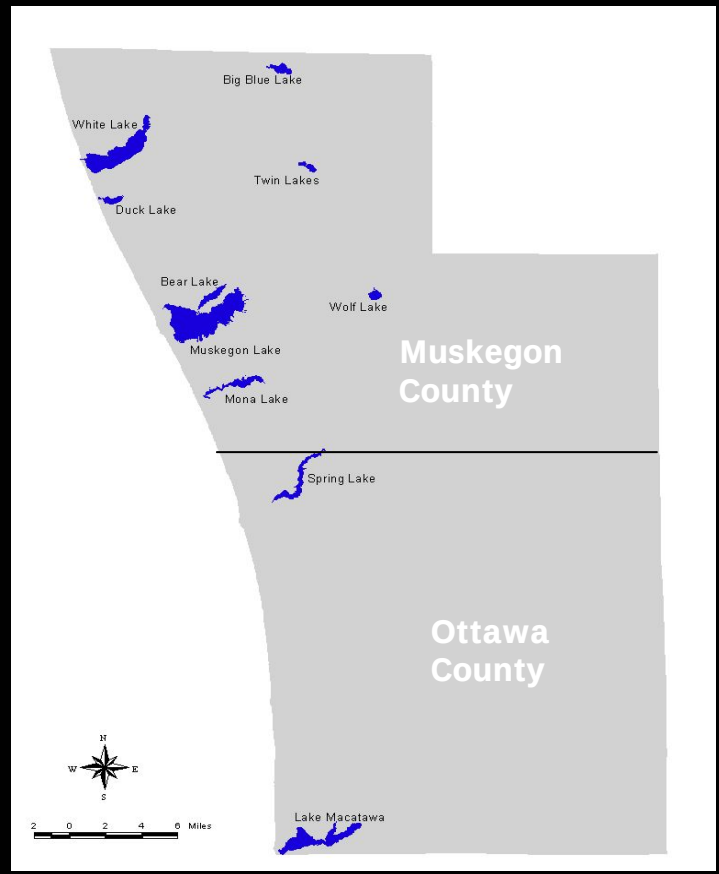
LC/MS has a 100X better detection Limit than LC/UV

PPIA Method

- Based on the toxic effects of microcystins to the Protein Phosphatase system
- Colorimetric method
- Gives an indication of overall activity
- Inexpensive ($20-$30/ test)
- Results not expressed in terms of Microcystin LR

Investigation of Cyanobacteria and Their Associated Toxins in 7 West Michigan Lakes

- Gradient of mesotrophic-hypereutrophic lakes with histories of cyanobacteria blooms
- 3 open water and 3 beach sites 2x/mo for 2 months
- ELISA, LC/UV,LC/MS, Chlorophyll a, and plankton counts



Results of Cyanotoxin Analysis in 7 West Michigan Lakes

Microcystin LR by ELISA	Bear Lake	Duck Lake	Lake Macatawa	Mona Lake	Muskegon Lake	Spring Lake	White Lake
# Analyzed	29	28	30	28	30	29	28
> 0.01 *u*g/L	29	28	30	28	30	29	28
> 0.1 *u*g/L	29	0	30	11	20	5	11
> 1 *u*g/L	28	0	2	0	7	0	0
>20 *u*g/L	0	0	0	0	0	0	0

Anatoxin-a and Cylindrospermopsin were not detected

Results of Cyanotoxin Analysis in 7 West Michigan Lakes

	>1 ug/l Microcystin LR	Dominant Cyanobacteria Species (#/ml)	Chlor a (ug/l)
Bear Lake	27/28	*Microcystis aeruginosa* (1.2E+07)	85
Muskegon	7/30	*Microcystis aeruginosa* (1.1E+06)	10
White Lake	0	*Microcystis wesenbergii* (1.86E+07)	35
Spring Lake	0	*Limnothrix* (1.2E+07)	70
Duck Lake	0	*Aphanocapsa conferat* (1.1E+04)	2

Results of Cyanotoxin Analysis in 7 West Michigan Lakes

- ELISA results for Microcystin LR were 1.5X-2X higher if other congeners were present
- 25% of all samples had >50 ug/l chlorophyll a
- 75% of all samples had cyanobacteria cell counts >100,000 ml
- 15% of all samples had cyanobacteria cell counts >10,000,000 ml
- The lakes with the highest levels of Microcystin LR had *Microcystis aeruginosa* as the dominant phytoplankton

Conclusions

- LC/MS yields the most accurate results
- ELISA is a good screening tool and very accurate if only LR is present
- Chlor a and #/ml are not reliable indicators
- Cyanobacteria populations are very different in similar lakes
- With one exception, the LR/RR ratio was consistent for 2 months

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