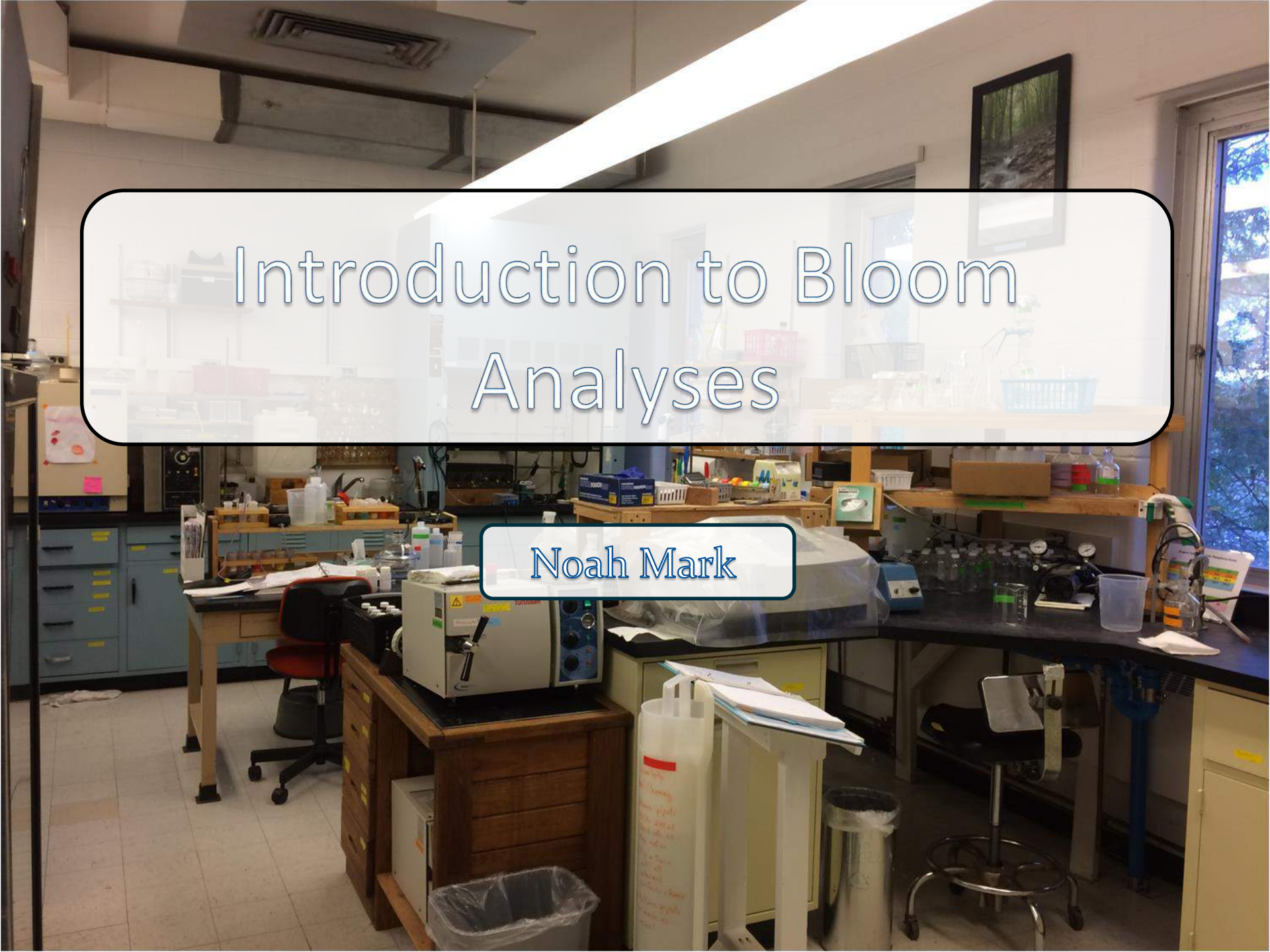


Introduction to Bloom Analyses

Noah Mark

Effects of Cyanotoxin Exposure

Humans

- 1930: *Microcystis* bloom on Ohio and Potomac Rivers caused instestinal illness in 5000-8000 people
- 1980: Several cases of illness in PA following a bloom
- 2004: 50 people reported illness following exposure to toxic bloom in Nebraska lakes and reservoirs
- 2010: 7 people ended up in the Toledo hospital following exposure to toxic cyanobacteria in Grant Lake St. Marys, OH

Boyer (2014)

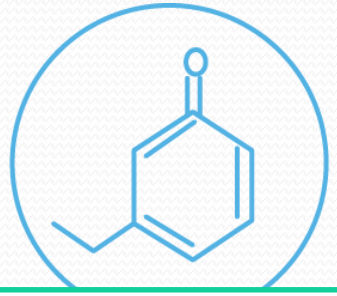
Wildlife

Storm Lake, Iowa, 1952

"…associated with the *Anabaena Flosaquae* were estimated 5000-7000 gulls, 560 ducks, 400 coots, 200 pheasants 50 squirrels, 18 muskrats, 15 dogs, 4 cats, 2 hogs, 2 hawks, 1 skunk, 1 mink, plus numerous song birds."

Rose (1953)

Cyanotoxins are natural compounds either excreted from the cell or released from within the cell upon lysis. Three overarching structural groups of cyanotoxins:

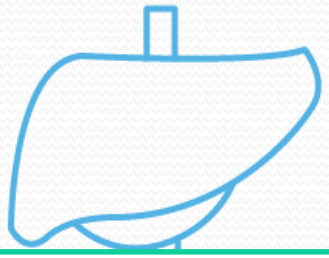


Cyclic Peptides

Alkaloids

Lipopolysaccharides

We can further breakdown these groups looking at their impact on cell types and organs within the body:

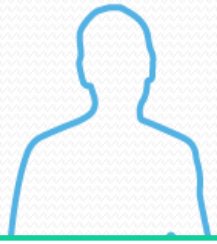


Hepatotoxins

affecting the liver

Neurotoxins

affecting nerve tissue



Dermatoxins

affecting skin, mucous membranes &tissue

Cytotoxins

affecting cell function

https://www.bluegreentest.com.au/discovering-cyanobacteria-cyanotoxins-and-how-to-detect-them/

Microcystin

- Amino acids form ring-shape (cyclic peptide)
- Hepatotoxin (liver toxin)
  - Inhibits an enzyme required for cell regulation
- Most commonly detected cyanotoxin (NYSDEC, USEPA)
- Produced by several genera of cyanobacteria
- NYSDOH guidance values
  - Drinking water: 0.3 µg/L
  - Recreational water: 4 µg/L

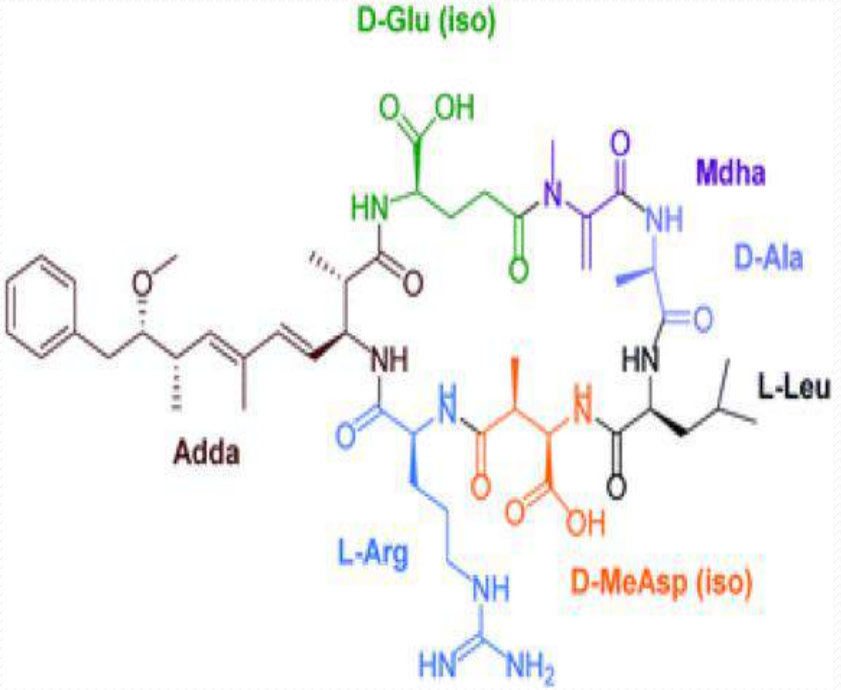
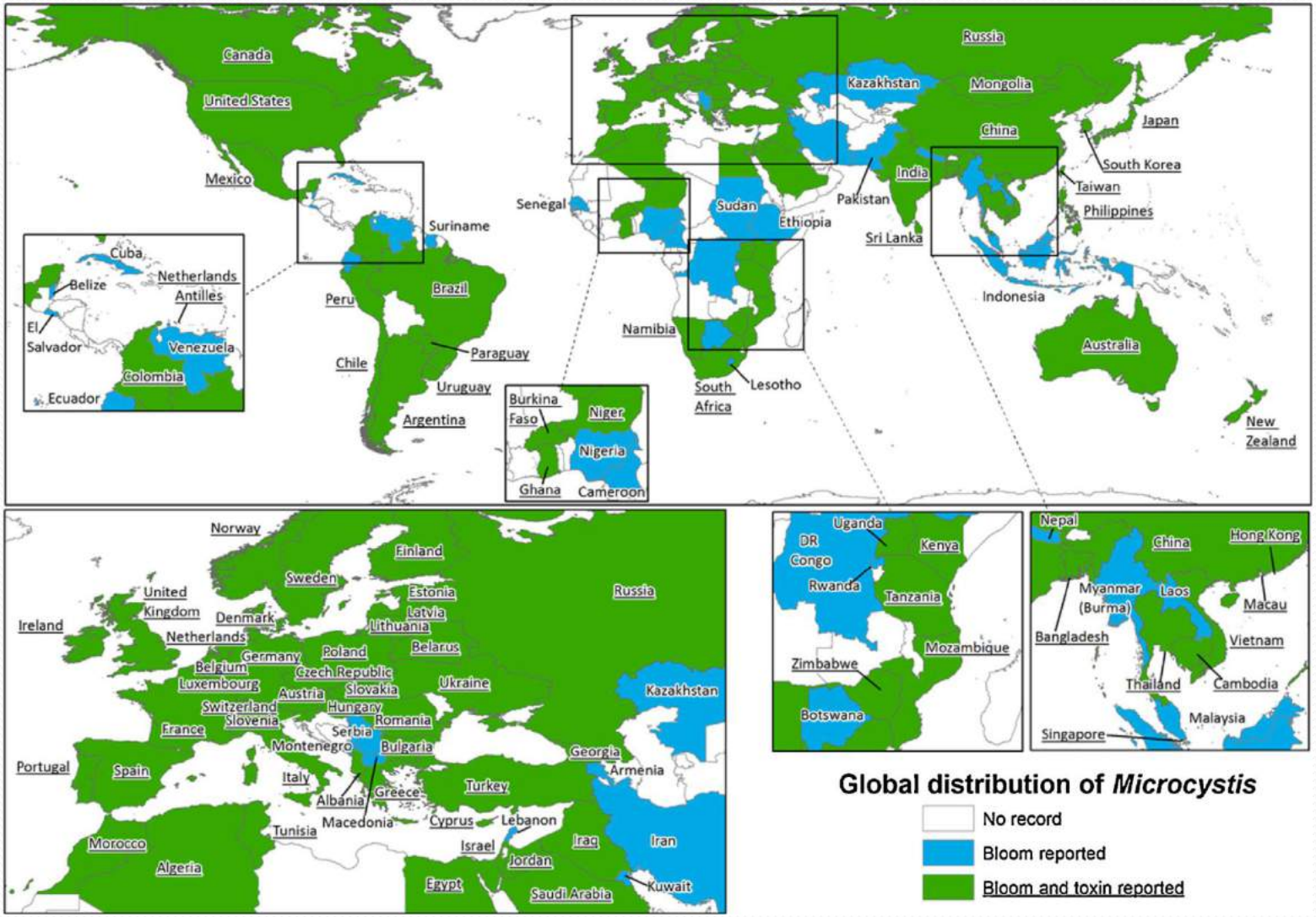


Image from www.bluegreentest.com.au

(Buratti, 2017)



Global occurrence of *Microcystis* blooms and microcystin as determined through literature searches for records of *Microcystis* blooms from 257 countries and territories.

(Harke et al., 2016)

Lab Analyses

Microscopy

Image from bolioptics.com

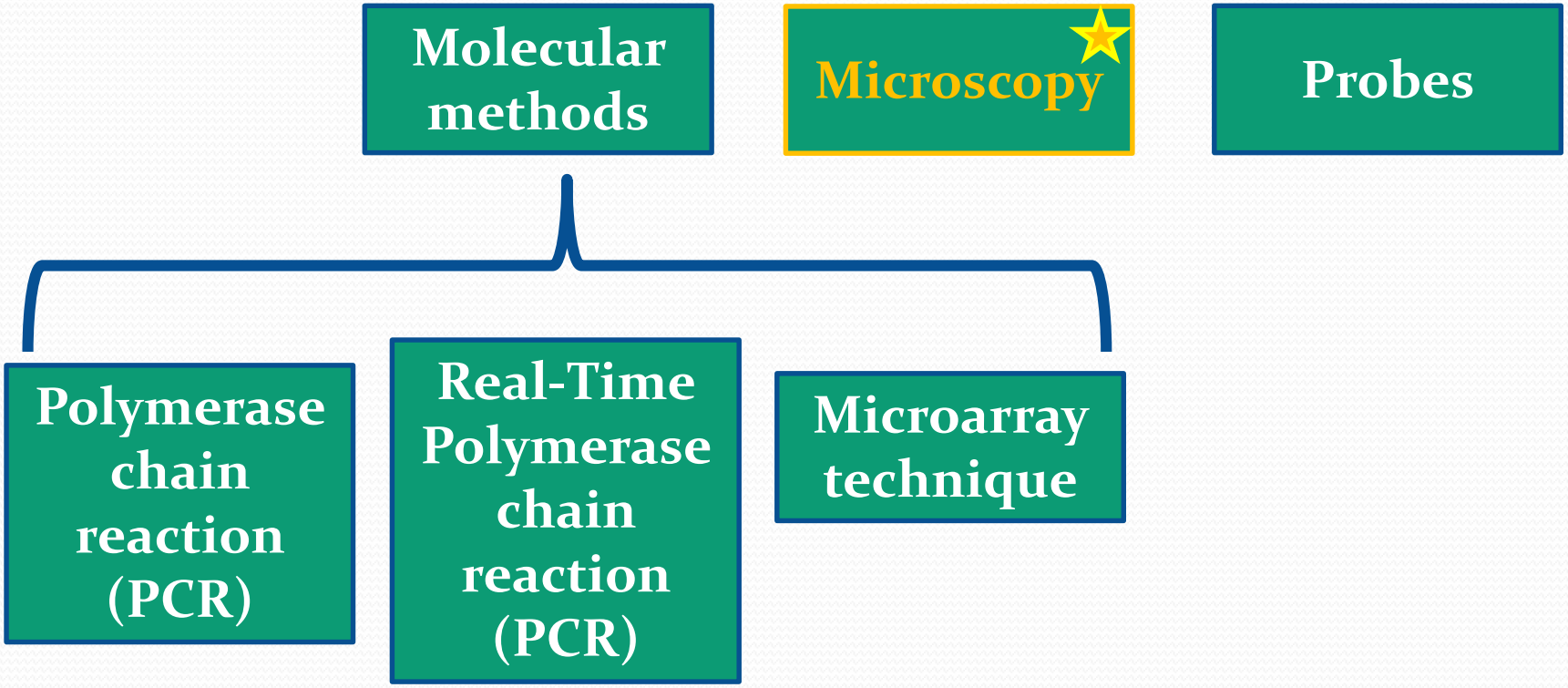
Total Microcystin (ADDA)

Image from orbitbiotech.com

Total Chlorophyll a

Image from www.hunterlab.com

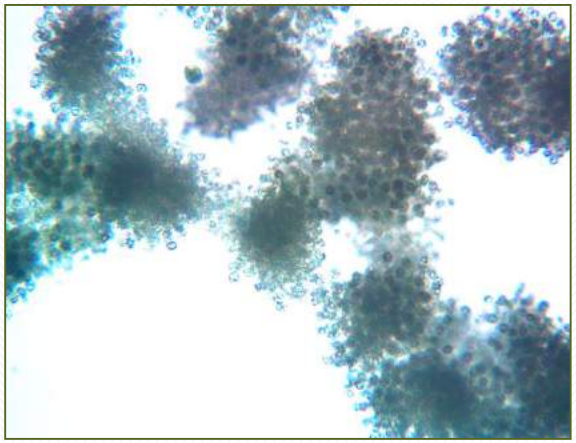
Detection of Potentially Toxic Cyanobacteria



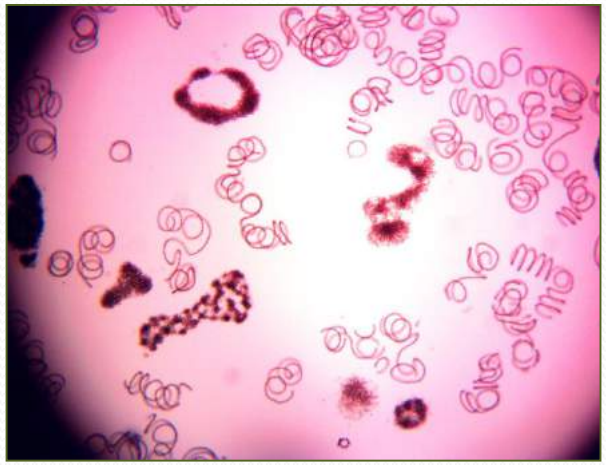
(Kaushik, 2013)

Light Microscopy

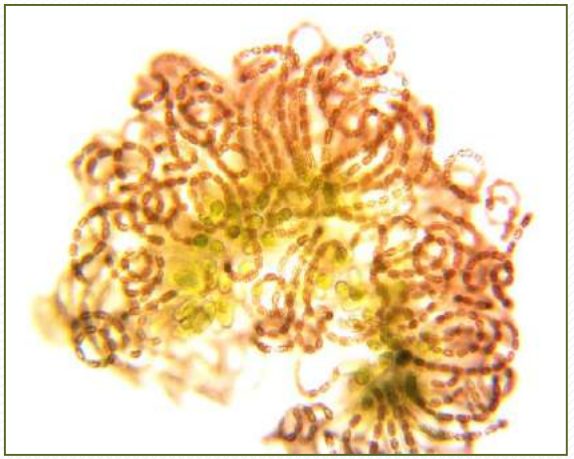
- Survey dominant phytoplankton taxa
- Relative abundance of major genera



Microcystis spp
•Amorphous colonies
•Microcystin



Both *Microcystis spp* and *Dolichospermum spp* present

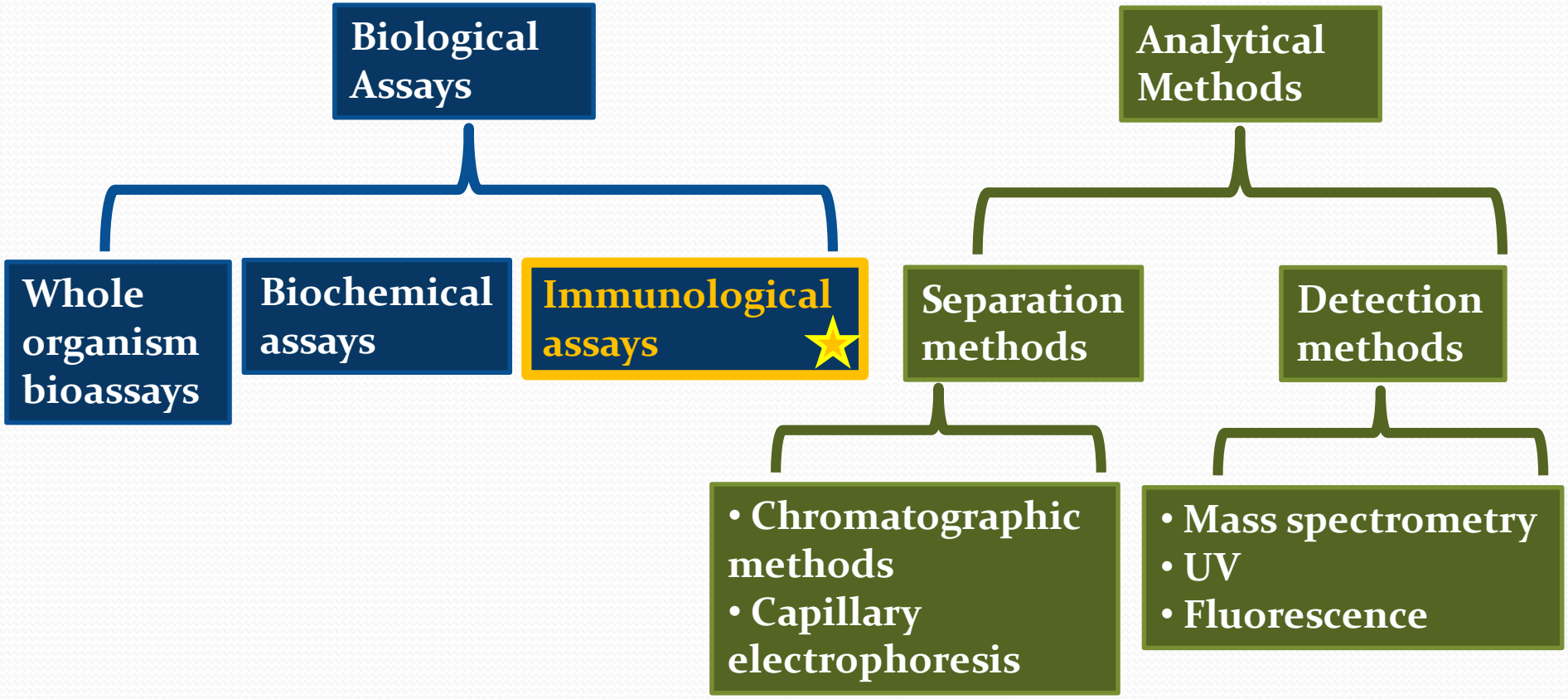


Dolichospermum spp
•Unbranched filaments
•Heterocytes fix N_2
•Anatoxin, microcystin

Total Chlorophyll a

- Chlorophyll a is the most dominant chlorophyll pigment
  - Often used as a direct estimate of algal biomass
- Pass known volume of sample through glass-fiber filter
- Extract chl. a with 90% acetone and quantify using spectrophotometer

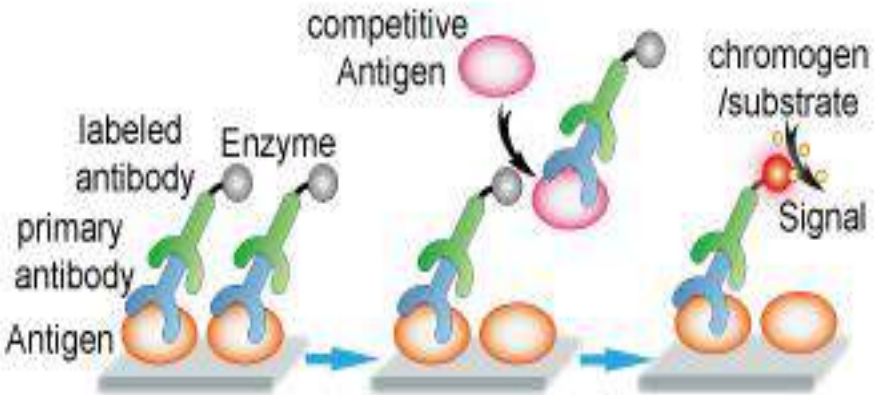
Detection of Cyanotoxins



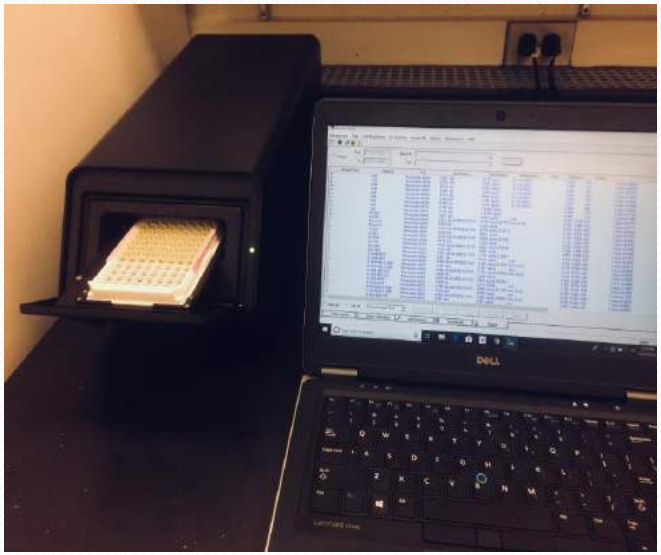
(Kaushik, 2013)

EPA Method 546

- Total microcystin and nodularins with amino acid ADDA
- Enzyme-Linked Immunosorbent Assay (ELISA)
- Detection range: 0.3 – 5 µg/L
  - Dilutions used for >5 µg/L
- Quick turnaround: takes ~6 hours to the perform assay



https://www.creative-diagnostics.com/ELISA-guide.htm



EPA Method 546

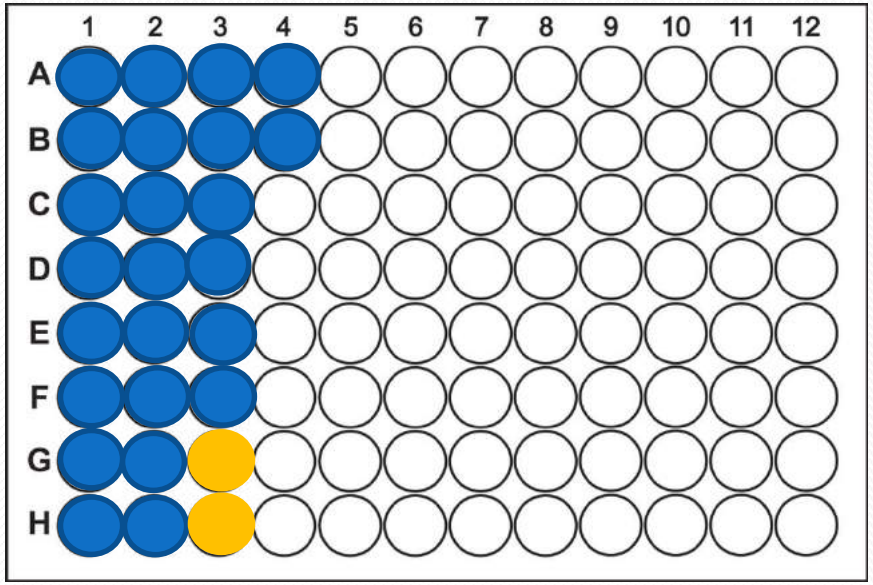


Image from http://www.cellsignet.com/media/templ.html

Calibration Curve & Quality Control

Sample

- Requires extensive quality control
  - Typically 24 wells on a plate (up to 30 wells if >20 samples)
- Cannot discriminate between microcystin variants (>100 structural variants)
- Very costly to client and lab

Regulating Cyanotoxins

- DEC detects microcystin and anatoxin-a (nerve toxin) most often
  - Both listed on USEPA's Contaminant Candidate List
- WHO has not set guidelines for other known cyanotoxins
  - Nevertheless, some states are setting action levels for other toxins
- NYS is being proactive about HABs!

World Health Organization

Image from https://www.who.int/

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Image from https://www.epa.gov/

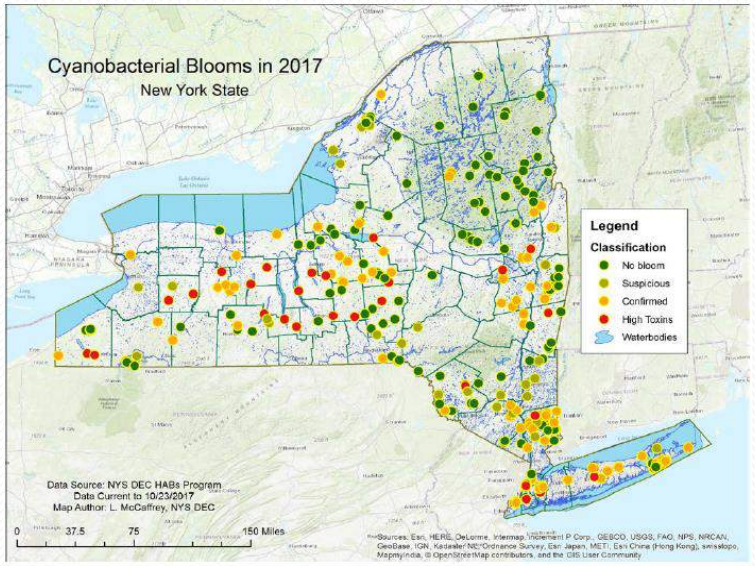


Image from "HABs in New York," McCaffrey, 2017

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