Beyond Fecals

Advanced Analytical Techniques to Evaluate Hazardous Algal Blooms

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Background

- The only contamination levels for microbial contaminants in recreational and source waters are coliforms and the fecal bacteria *E. coli* and *Enterococci sp.*
- With the threats to public health caused by emerging contaminants in these waters, development of advanced analytical testing is necessary.
- Harmful Algal Blooms (HABs) are becoming more prevalent in waters and pose risk to fish, other animals, and humans whom consume or come into contact with affected waters.
- Cyanotoxins have been proposed in the UCMR 4.
- Advisory levels have been created for cyanobacteria as well as several cyanotoxins.

WHO Advisory Levels (1998)

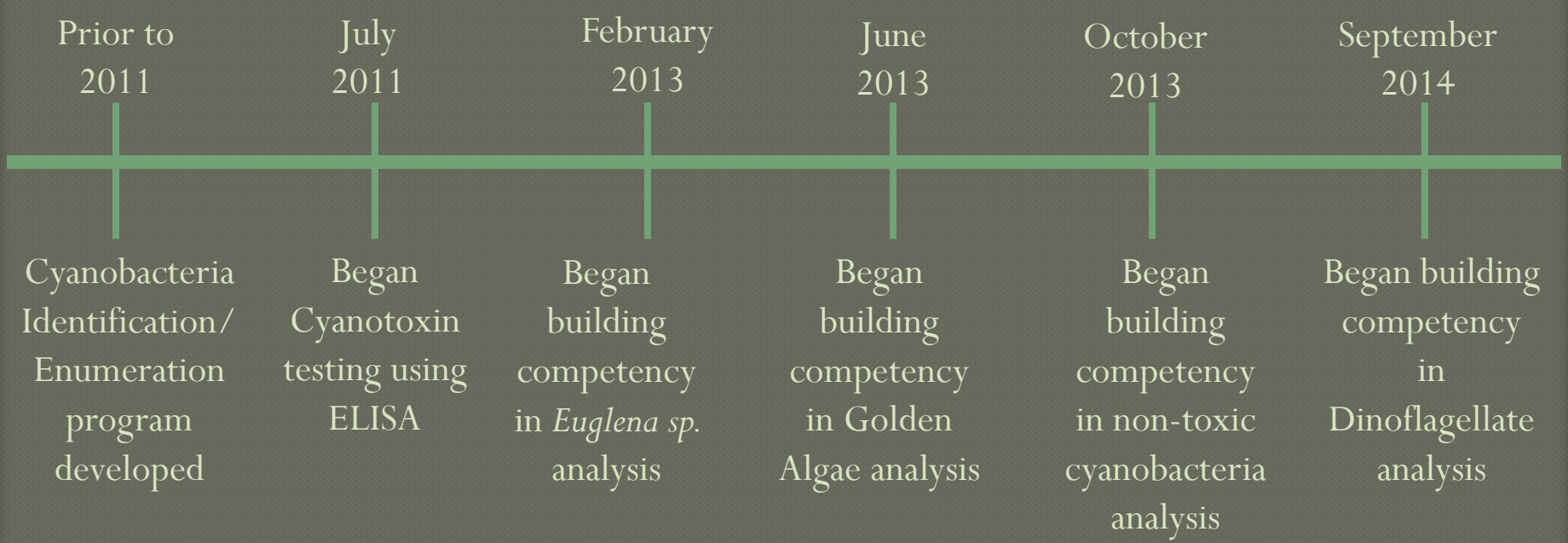
Risk of Adverse Health Effect	Level of Cyanobacterial Cells	Level of Microcystin	Recommended Action
Recreational Water Limits			
Low	20,000 cells/mL	4µg/L	Provide info to public, post advisory
Medium	100,000 cells/mL	20µg/L	Restrict bathing, post warning signs
High	Scum formation	N/A	Consider further restrictions
Drinking Water Limits			
High	No level determined	1.0µg/L	Prohibit swimming in source water, inform public & authorities

EPA Advisory Levels (2015)

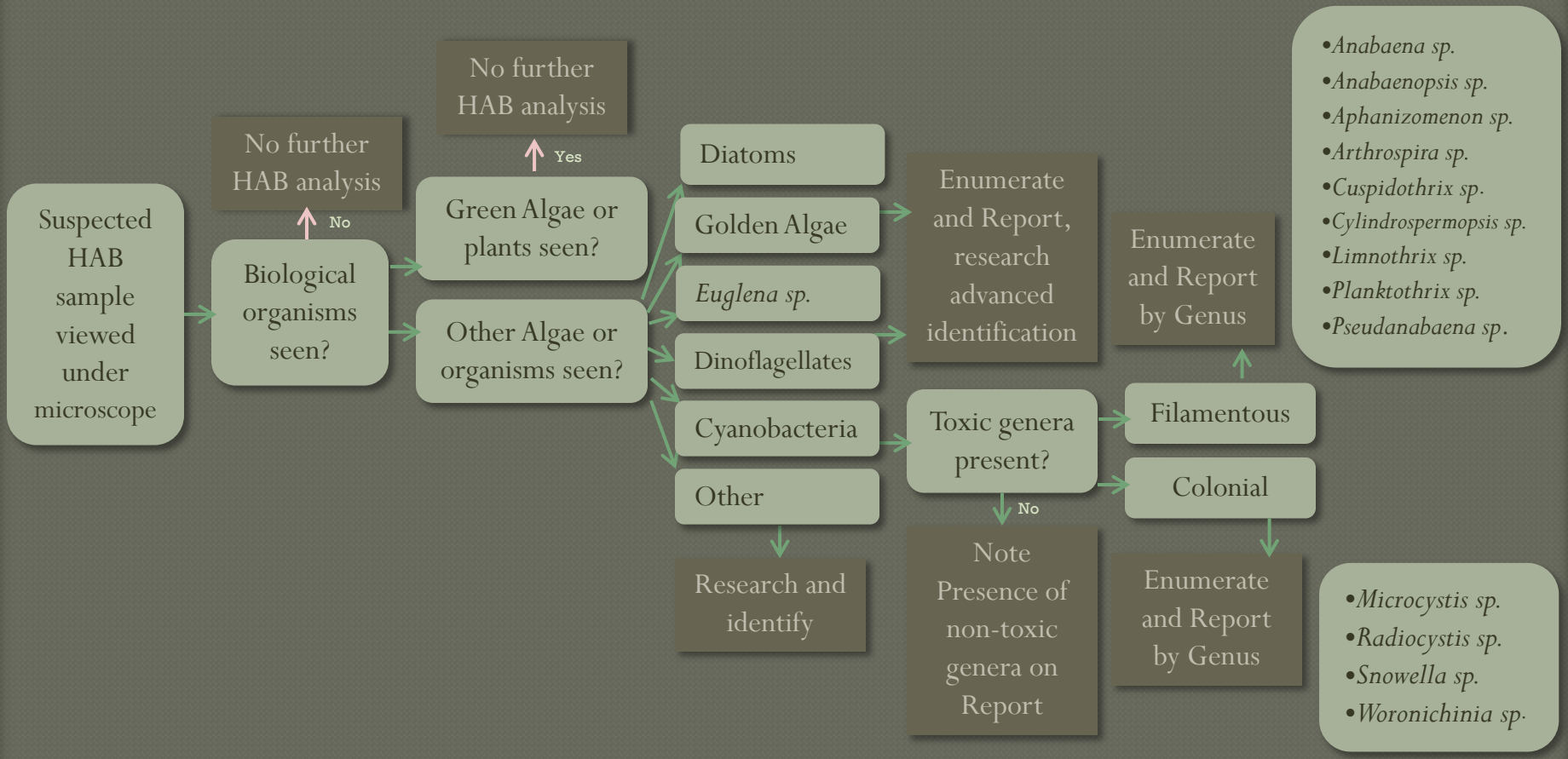
- Ongoing study with numerous agencies since 2012.
- Different communication, treatment, and monitoring actions are available based on the varying toxin levels.

Cyanotoxin	Bottle-fed infants and pre-school aged children	School-aged children and adults
10 Day Advisory Level Drinking Water		
Microcystins	0.3µg/L	1.6µg/L
Cylindrospermopsin	0.7µg/L	3µg/L

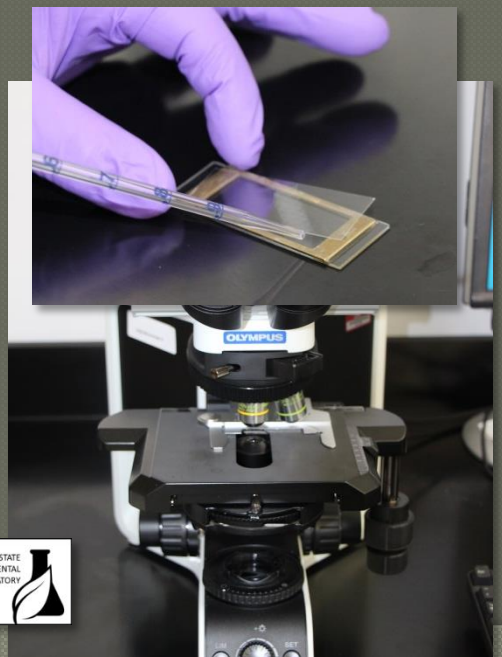
ODEQ HAB Program Development



ODEQ HAB Analytics Algorithm

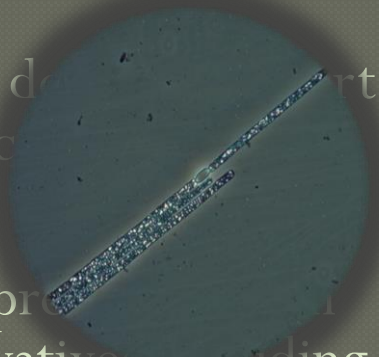


Microscopy



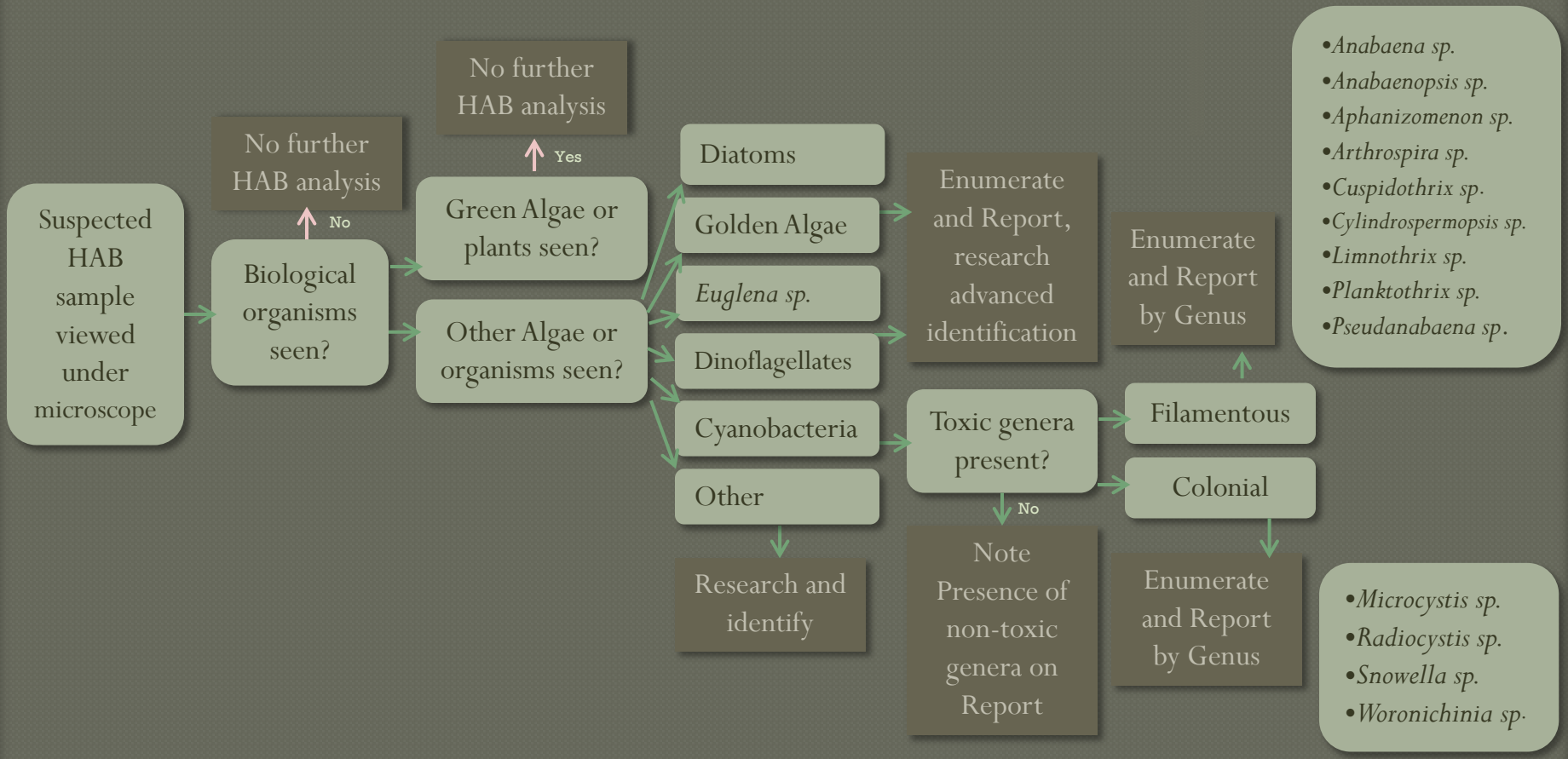
- A Sedgwick-Rafter Cell and a Whipple Grid are used for enumeration of most organisms. A hemacytometer can also be used.
- Identification and Enumeration are done at the same time, recording a count of each genus found.
- [illegible] are d[illegible]rt to c[illegible]
- [illegible] be pr[illegible] different preservatives including Lugol's Iodine and Acidic Lugol's Iodine.

Bright Field Transmitted Light Microscopy

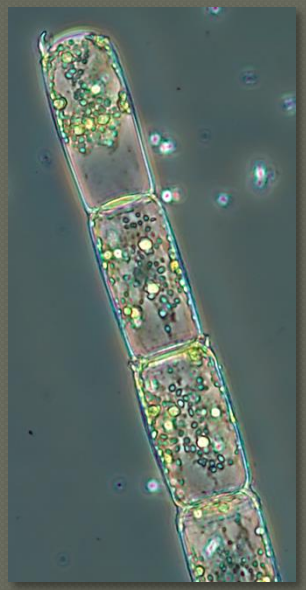


Phase Contrast Microscopy

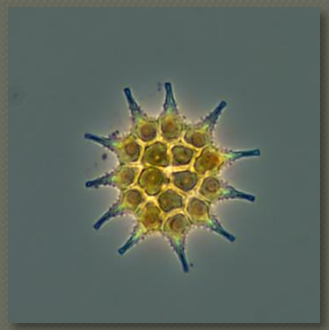
ODEQ HAB Analytics Algorithm

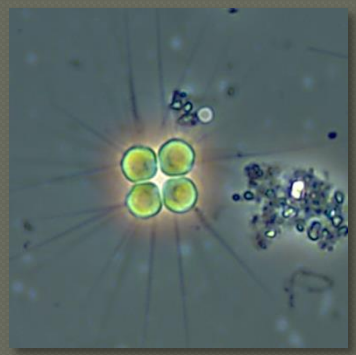


Green Algae and Plants



Green Algae





Plants

- Green algae are common in waters and do not pose health threats
- Plants are often mistaken for algae. Plants are not microscopic

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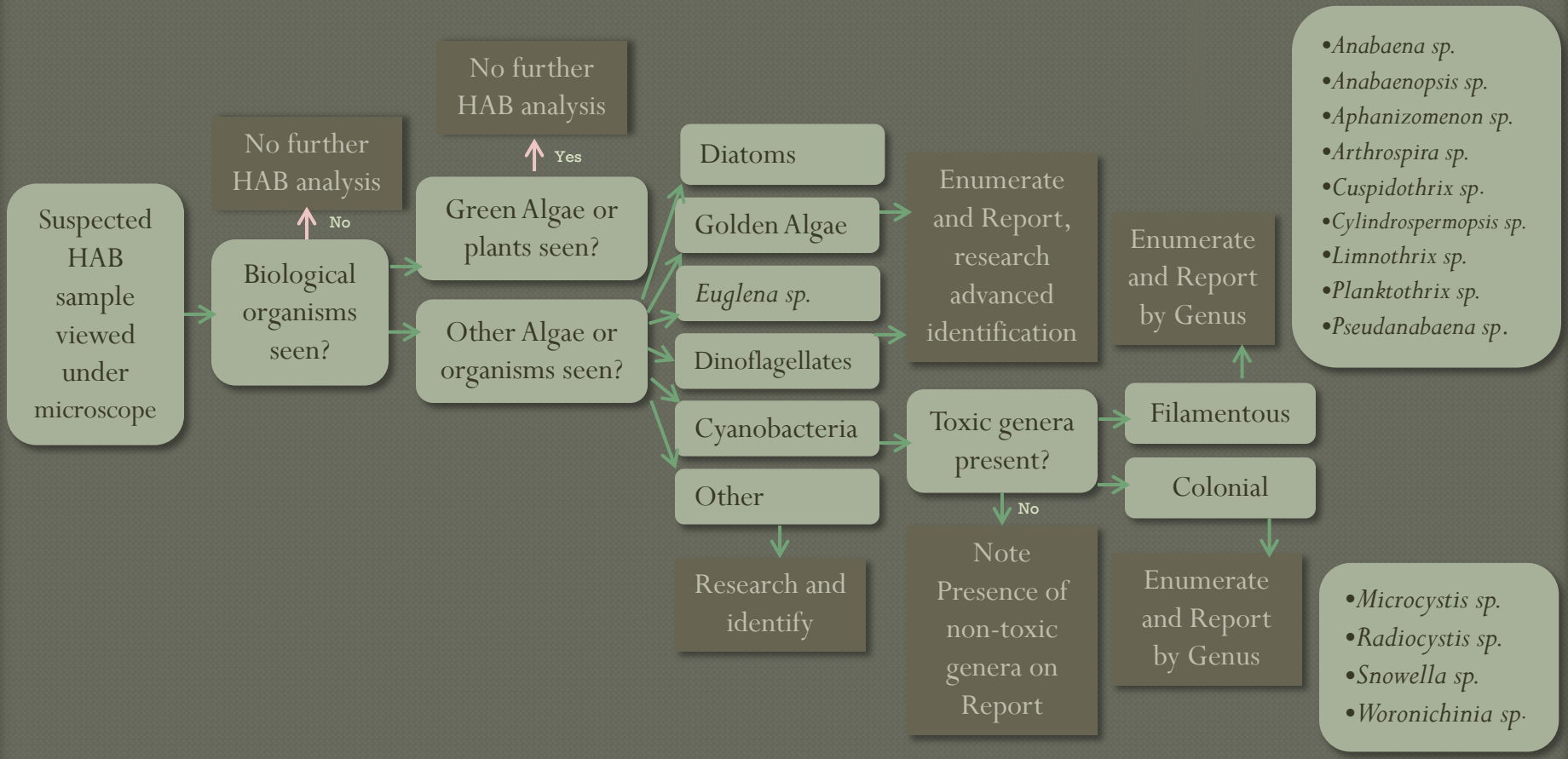
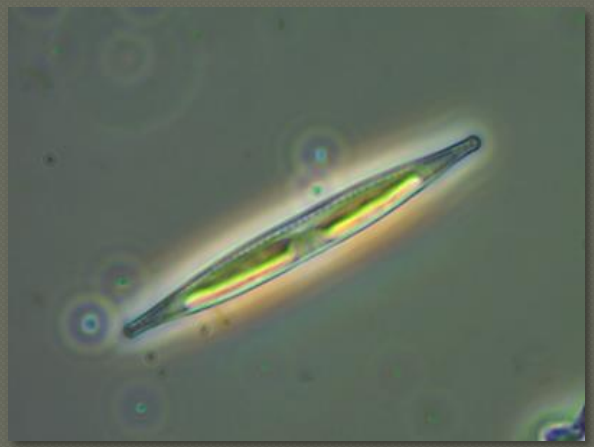
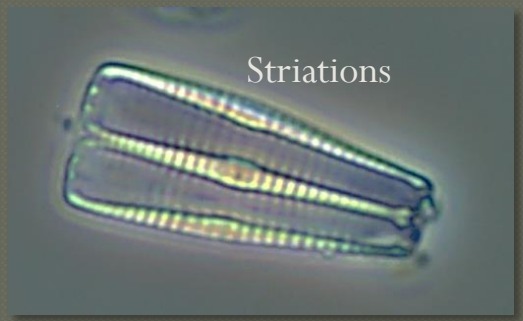


Diatoms





- Diatoms have visible striations
- Are bilaterally or radially symmetrical
- Naturally found in waters in low amounts
- Certain species are known for producing toxins that can kill birds

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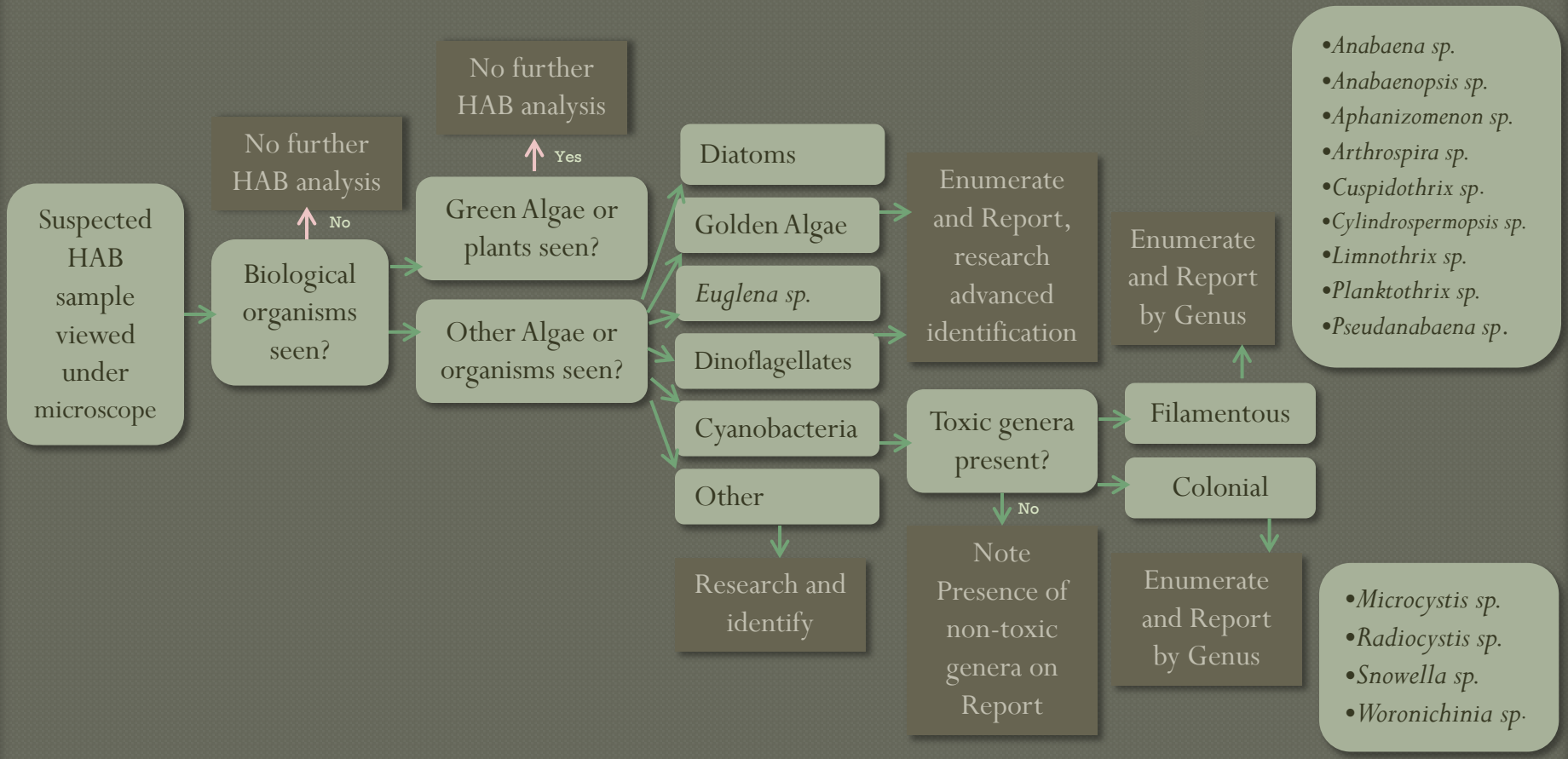
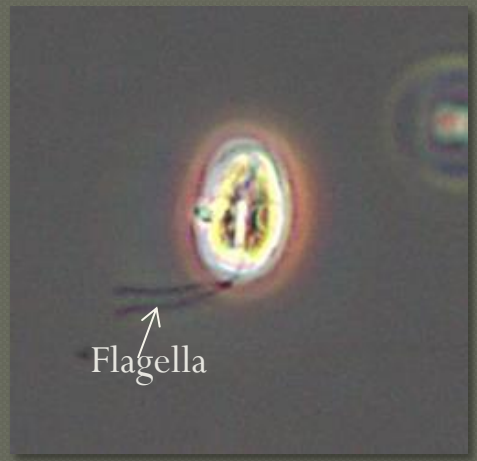


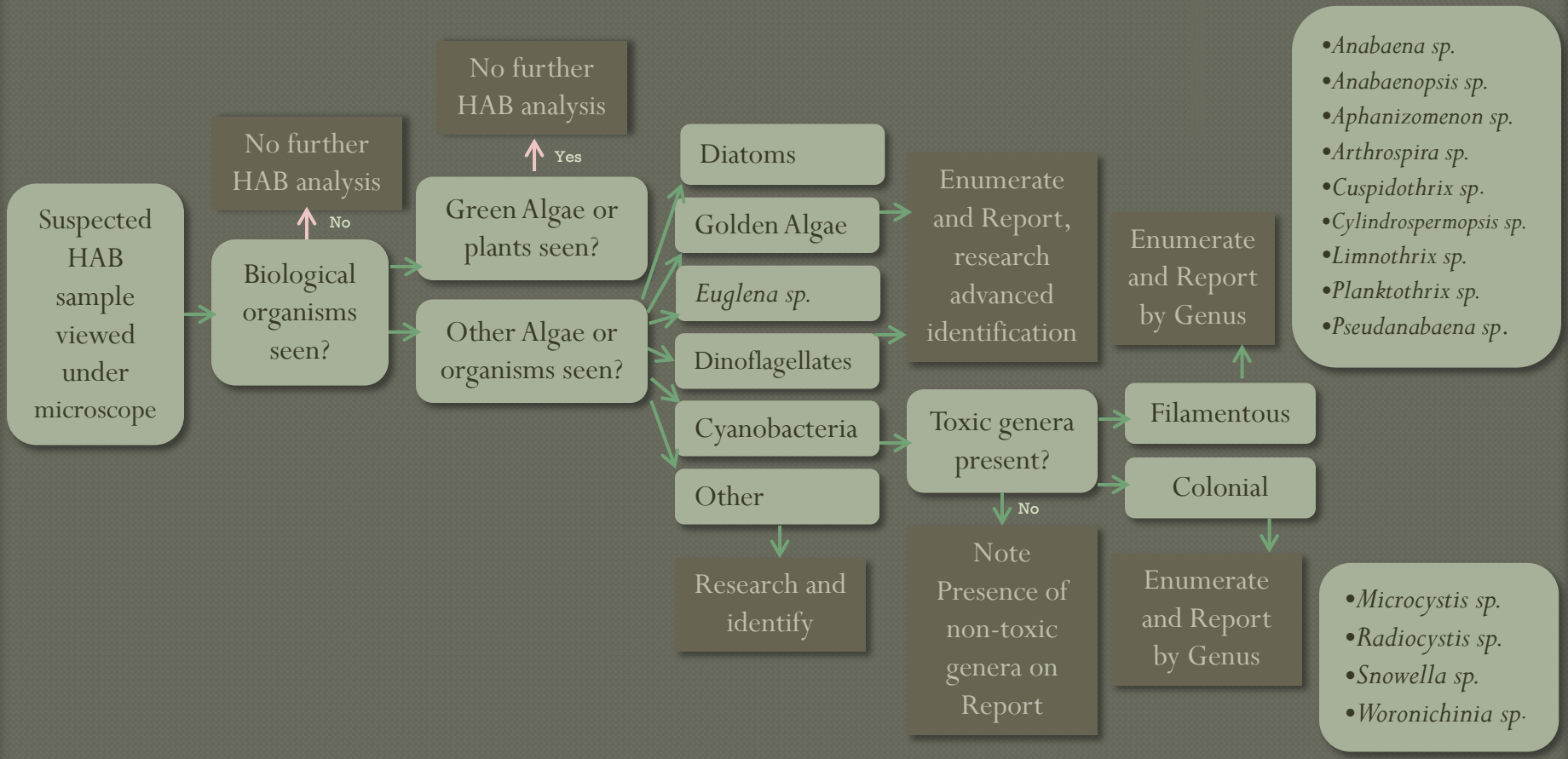
Golden Algae



Prymnesium parvum

- Golden Algae have two flagella, two C-shaped chloroplasts and one haptonema
- Can cause water to turn brownish, yellowish, or tea colored
- *Prymnesium parvum* is known for producing prymnesin toxin, which can cause fish kills

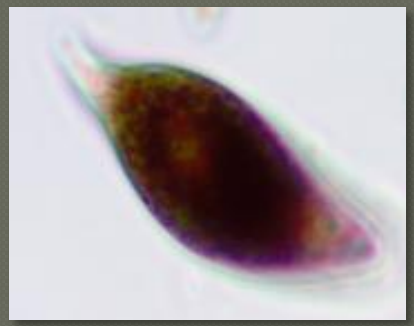
ODEQ HAB Analytics Algorithm



Euglena sp.

Euglena agilis

Euglena sanguinea bloom



Euglena agilis

- *Euglena sp.* have a red eyespot and visible chloroplasts
- *Euglena sanguinea* blooms can cause the water to turn pink or red.
- *Euglena sanguinea* is known for producing Euglenophycin toxin, which can cause fish kills

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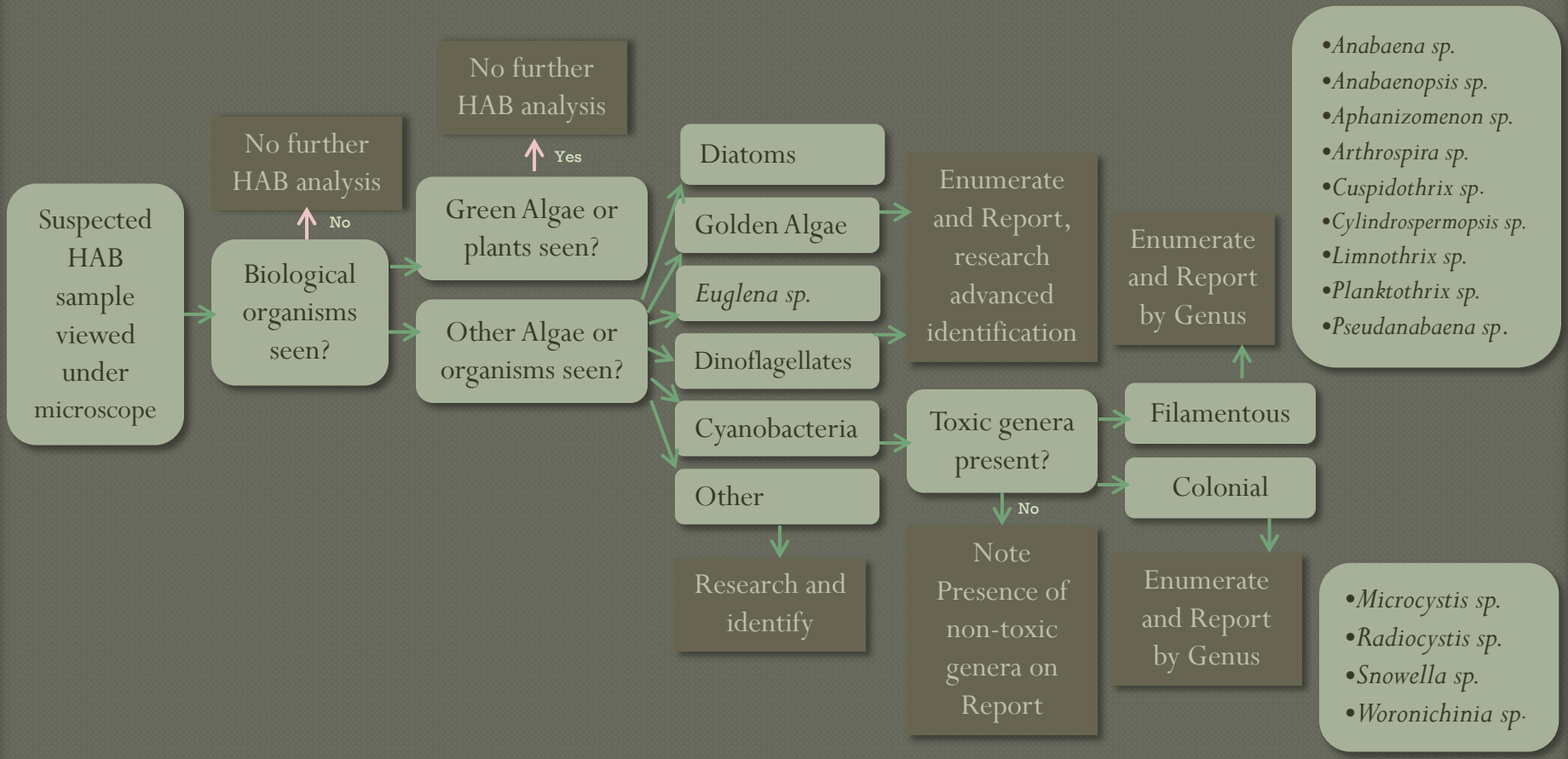
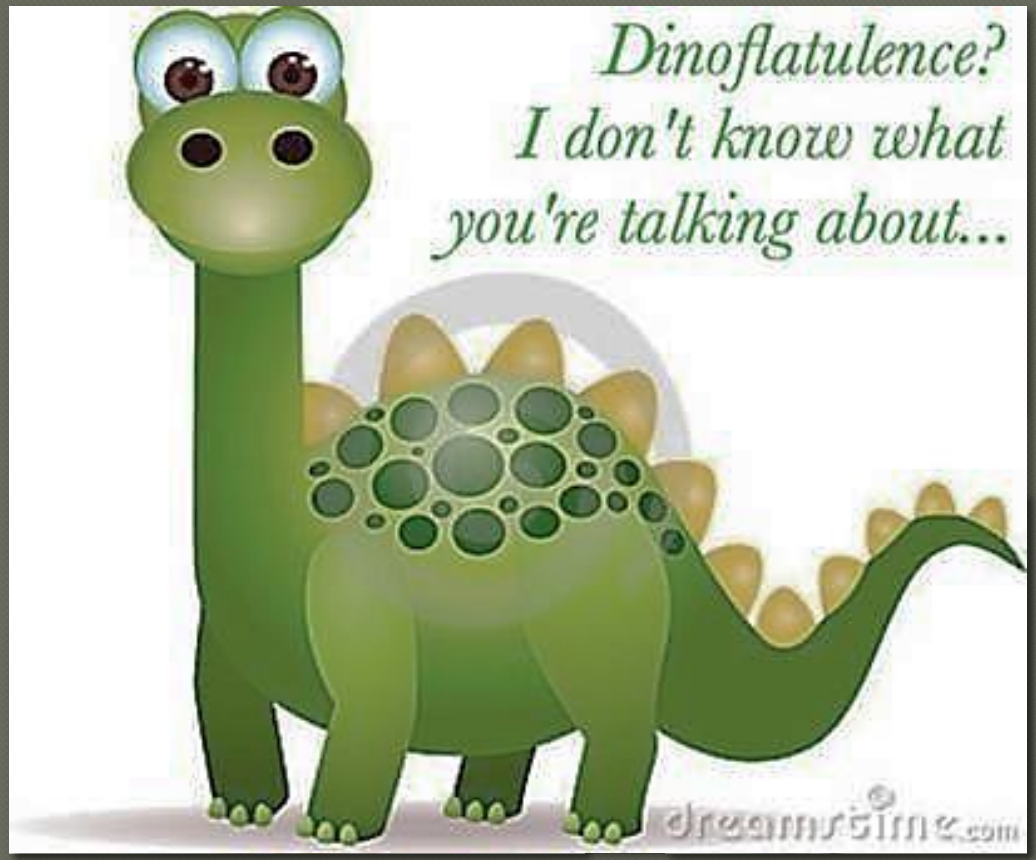


Dinoflagellates



Tovellia sp.

Peridinium sp.

- Dinoflagellates have thecal plates that look like armor
- Some species produce 23% lipids by mass
- Can turn water red or brown, and can leave an oily residue
- Can produce toxins that can kill fish or contaminate shellfish

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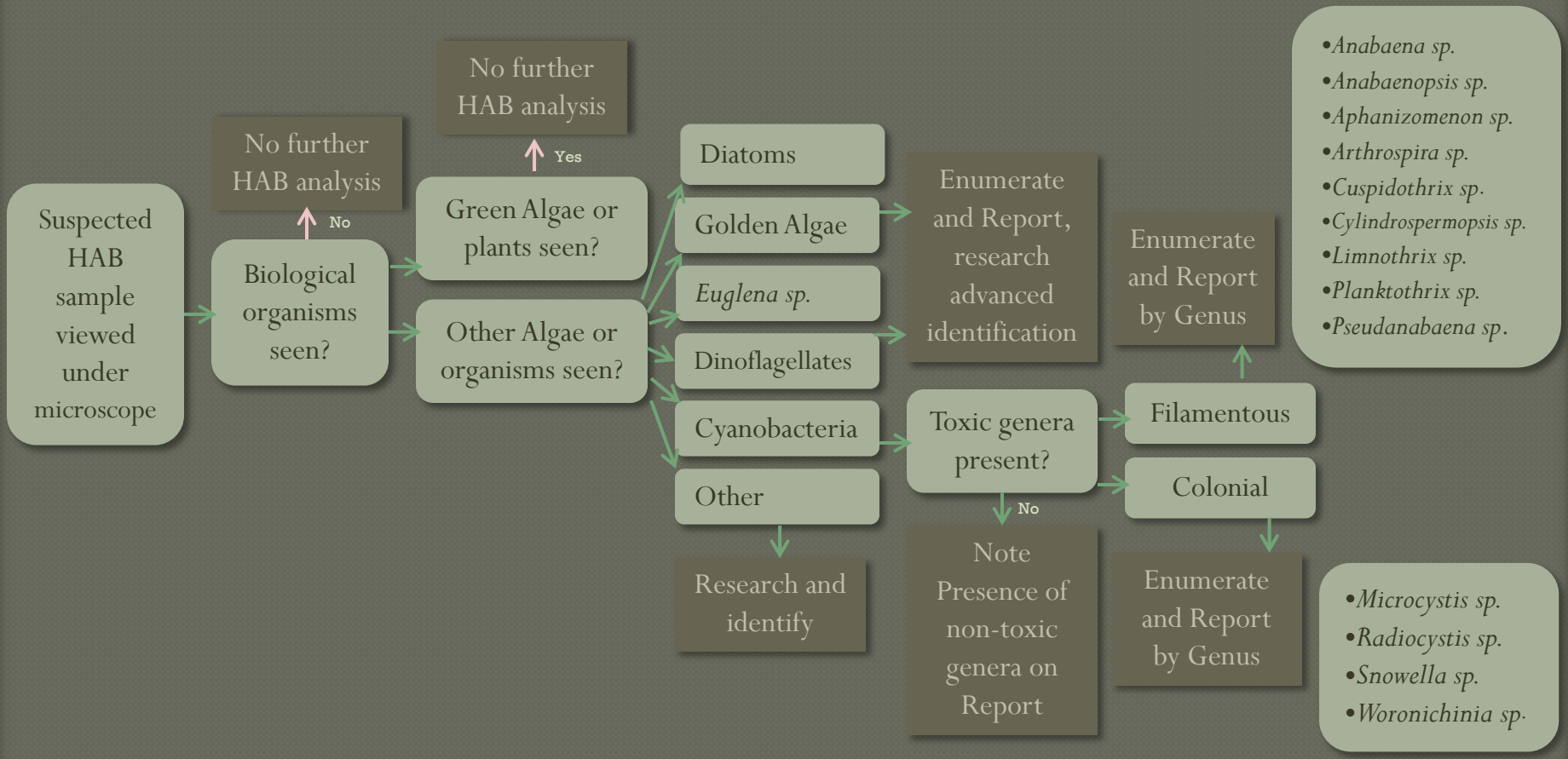

Cyanobacteria

- Cyanobacteria are also called blue-green algae
- Cyanobacteria blooms often cause the water to turn green or greenish-blue
- The lipopolysaccharides in the cell walls are a dermatoxin
- Many genera produce cyanotoxins that are toxic to fish, livestock, other animals and humans

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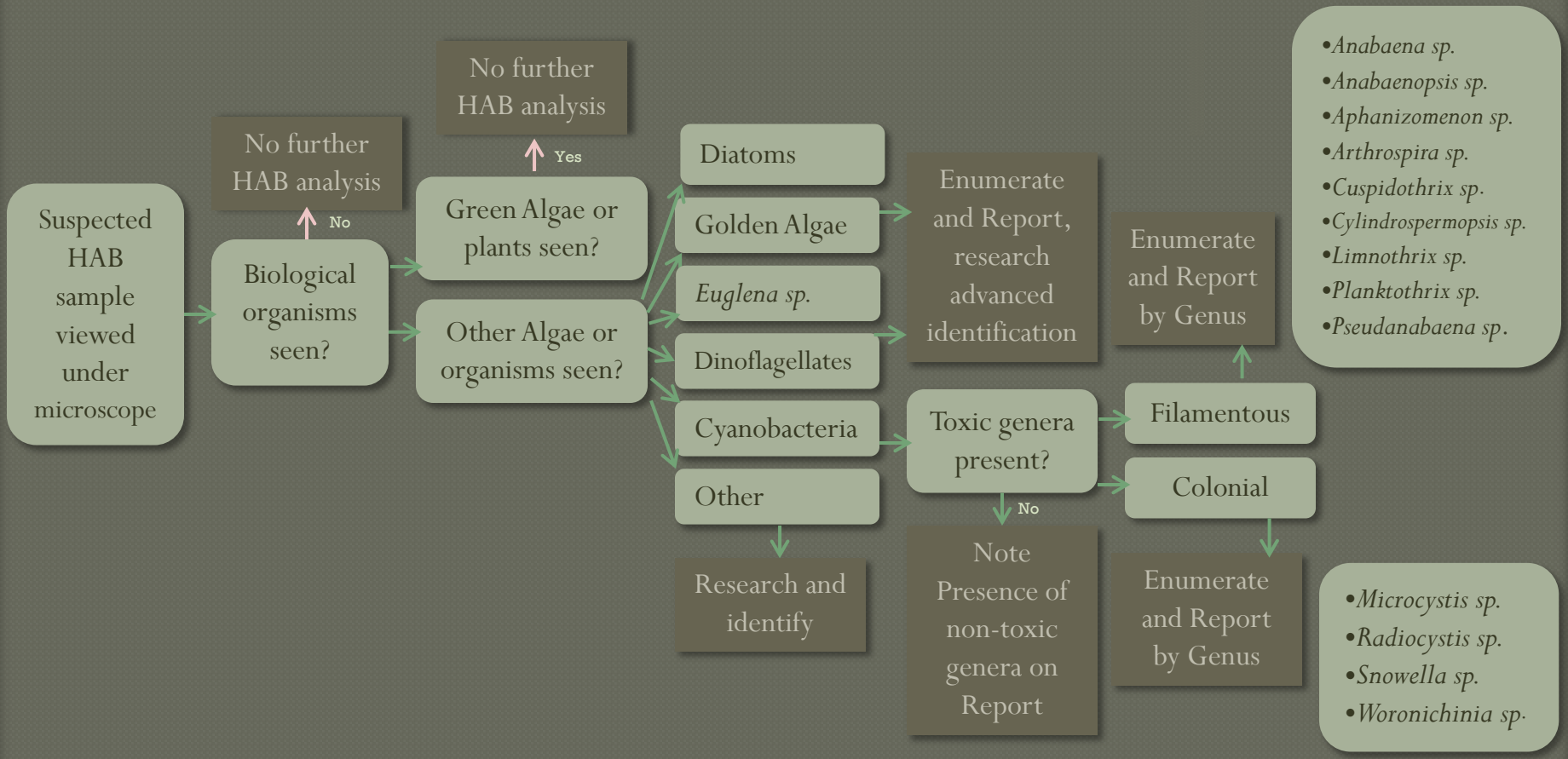
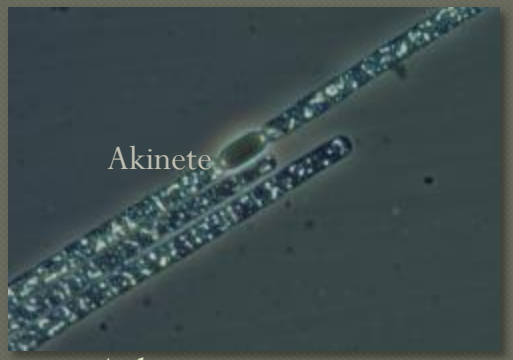


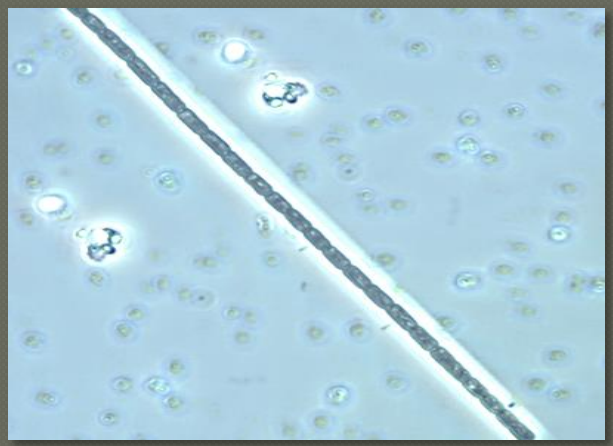
Filamentous Cyanobacteria



Aphanizomenon sp.

Planktothrix sp.

Cylindrospermopsis sp.



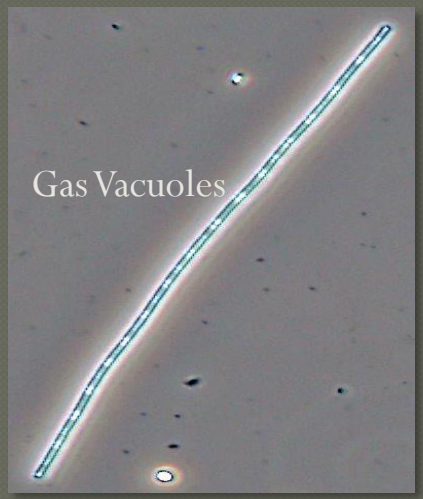
Psuedanabaena sp.

- Filamentous Cyanobacteria consist of chains of individual cells called trichomes
- Many genera have specialized cells:
  - -akinetes-used for reproduction
  - -gas vacuoles-allow movement up and down the water column
  - -heterocysts-used for nitrogen fixation

Filamentous Cyanobacteria

Anabaena sp.

Anabaenopsis sp.



Limnothrix sp.

Cuspidothrix sp.

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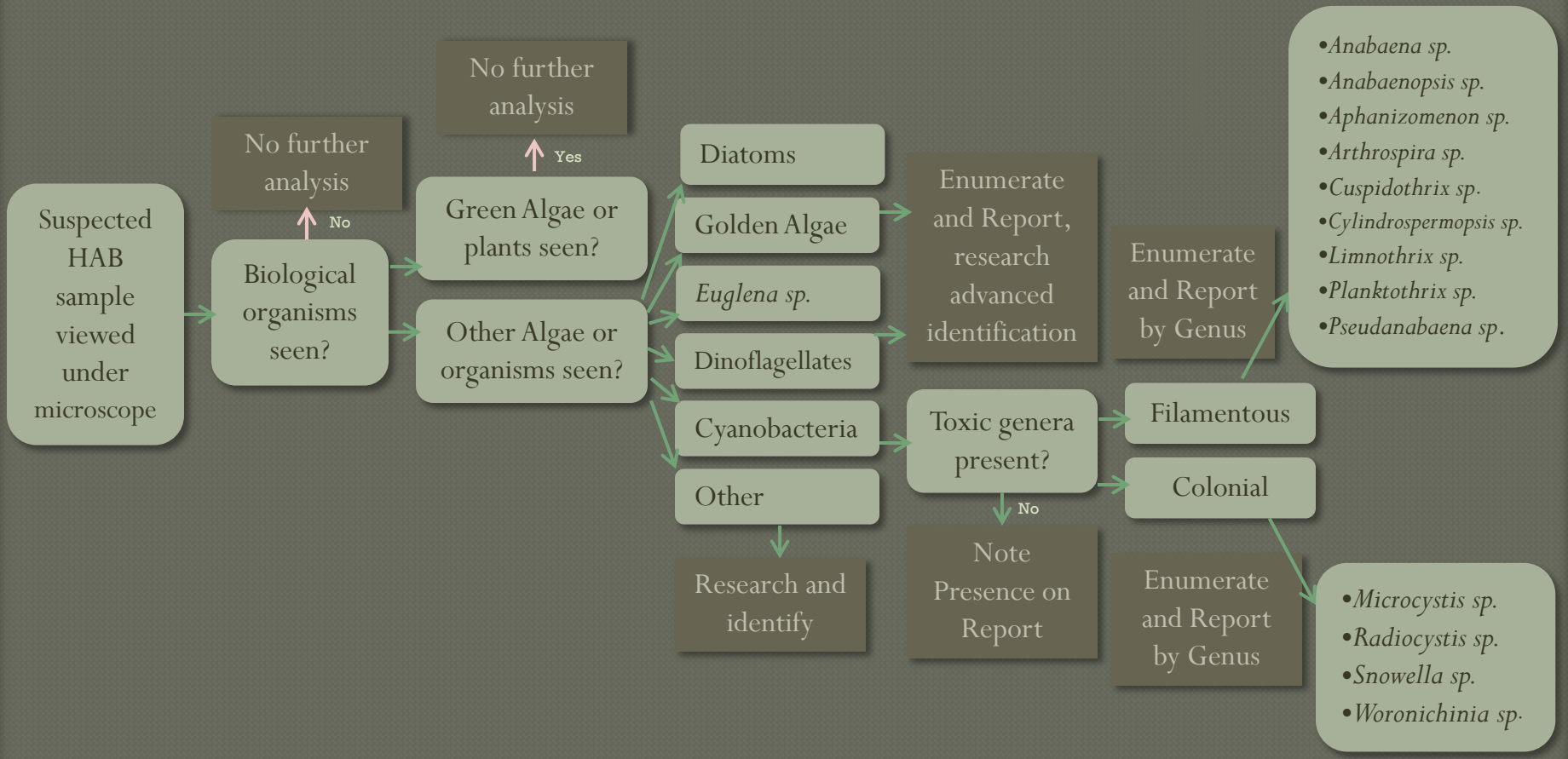
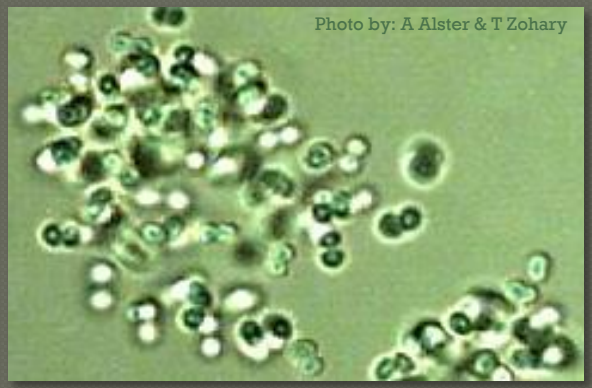


Colonial Cyanobacteria



Radiocystis sp.

Snowella sp.

Microcystis sp.

Woronichinia sp.

- Colonial Cyanobacteria consist of groups of individual cells
- Many genera have specialized structures:
  - -gelatinous stalks-in center of colony, bears cells at ends
  - -gelatinous mucilage-envelopes and holds cells together in colony
- Colonies can be confluent or hollow in center

Future

- EPA plans to develop advisory level criteria for recreational waters in 2016 as well as advisory levels for lifetime exposure of cyanotoxins in drinking water.
- Harmful Algal Bloom and Hypoxia by research and Control Amendments Act (S. 1254) of 2014 requires the National Oceanic and Atmospheric Administration to have primary responsibility in advancing the scientific understanding and ability to detect, monitor, assess, and predict HAB and hypoxia events in marine and freshwater. This effort is funded until 2018.
- Expansion of state monitoring efforts for cyanobacteria and cyanotoxin preparation, observation, response and monitoring.
- ODEQ will continue to maintain and expand analytical abilities in HAB analysis

Helpful Links

- EPA's Website on Cyanobacteria HABs (information on cyanobacteria/cyanotoxins, detection, health/ecological effects, research/news, causes/prevention, control/treatments, guidelines/recommendations, resources)

  https://www.epa.gov/nutrient-policy-data/cyanohabs

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