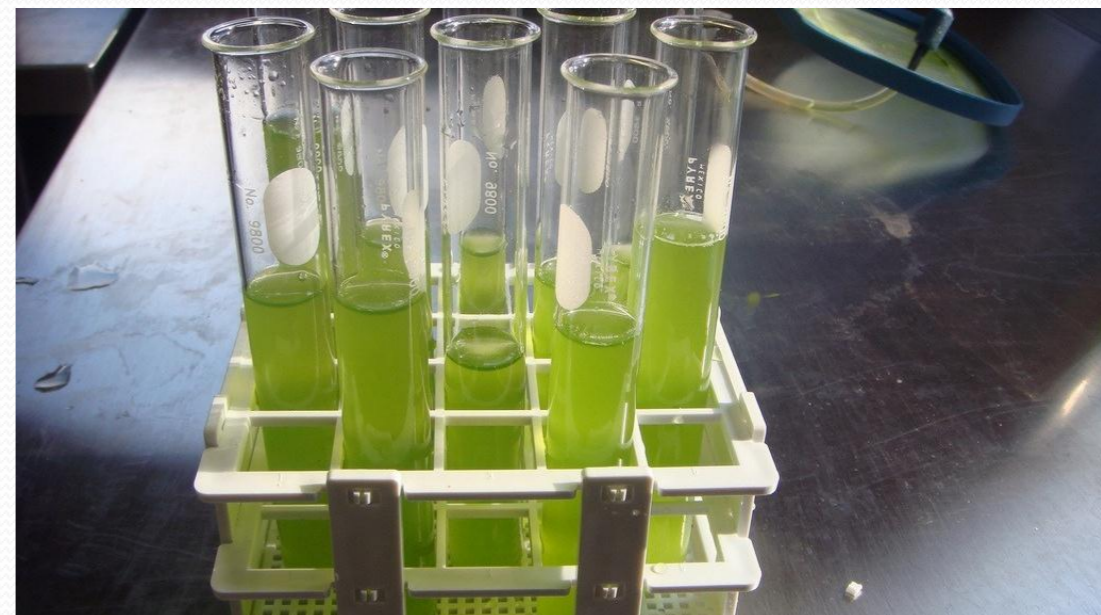


Chlorophyll Analysis



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Organization of Presentation

- What is Chlorophyll?
- Why Test For It?
- Types of Algae
- Detection Methods
- Summary

Quick Definitions

- **Chlorophyll** is a magnesium based green protein that turns blue and red light into electron movement, which turns water and carbon dioxide into sugar.
 - There are a few types of chlorophyll: A, B, C, D & F.
 - Most labs focus on the most common type, A.
- **Phaeophytin** is Chlorophyll without the magnesium.
“Dead Chlorophyll”
 - **If you want to stump your biologist, ask them for their phaeophytin numbers.**

More Definitions

- **Bluegreen Algae (Cyanobacteria)** are photosynthetic bacteria. Some produce toxins and some fix N_2 from the air.
- **Diatoms** are single celled algae that have a silica shell
- **Green algae** are filamentous or flagellated algae
- **Dinoflagellates** are single celled algae that cause red tides in marine environments



Why are chlorophyll A levels important?

- Nutrient level indicator
 - Chlorophyll A levels increase with nutrient levels
- Indicates water clarity – total suspended solids, turbidity
- Chlorophyll A is half of the story
 - Different nutrient levels will support different types of algae.
 - Different Algae will support different aquatic consumers
- Harmful Algal Blooms
 - Toxic algae
 - Oxygen depletion
 - Aesthetics and increased bacteria levels at beaches

Lake Erie Has Algae

- Is this Fish food or Fish poison
- Chlorophyll A alone will not tell us

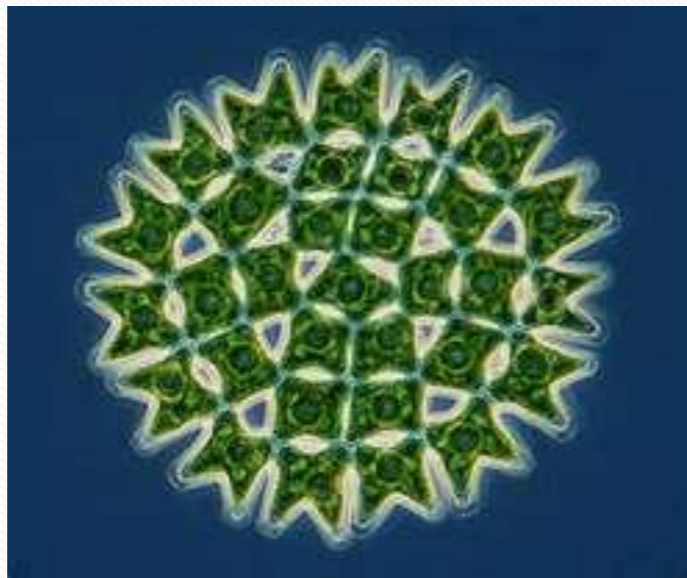
About The Algae: Diatoms

- The Golden Algae-brown slippery rocks
- Single celled and filamentous
- Cell wall is made of 2 silica shells (like a pill box)
- Store food as lipids, not starch
 - Great source of food for fish fry and zooplankton
- Diatom presence is indicative of clean and clear lakes
- Diatomaceous earth



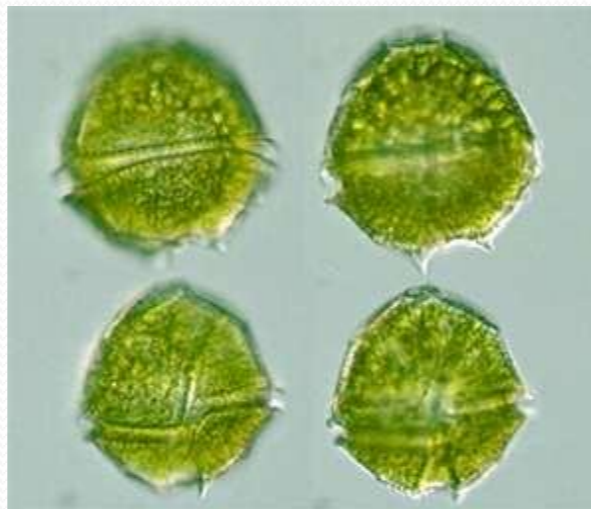
About The Algae: Green Algae

- Filamentous, single celled and flagellated
- Store food as starch
- Cell wall is made of cellulose, like land plants
- Good source of food for fish fry and zooplankton
- Green algae are found in all environments
- Can cause severe algal blooms



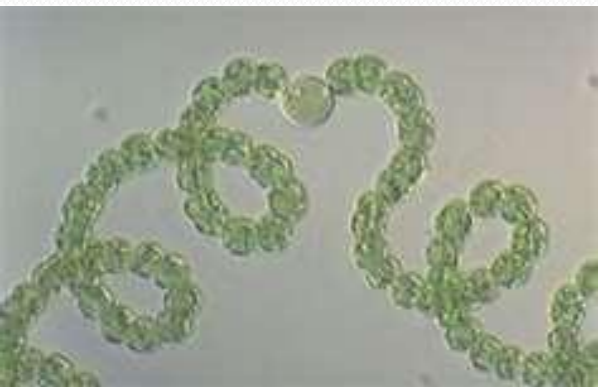
About The Algae: Dinoflagellates

- Single celled and flagellated
 - Two flagella, one around the waist and one on the end
- Found in abundance in impaired waters.
- Cell wall is made of rigid plates
- Some are not photosynthetic
- In marine environments, they make coral.
- During a bloom, they cause red tide events



About The Algae: Cyanobacteria

- Bluegreen algae, It's photosynthetic bacteria
- Some can fix N₂
- In waters with high phosphorus levels, N₂ fixing cyanos will outcompete the green algae and diatoms
- A few strains are toxic
 - Generally they are difficult to digest
- Cell wall is made of peptidoglycan.
- Nutrient supplement



Toxins related to freshwater blue green algae

Neurotoxins

Anabaena

Planktothrix

Aphanizomenon

Lyngbya

Hepatotoxins

Microcystus

Planktothrix

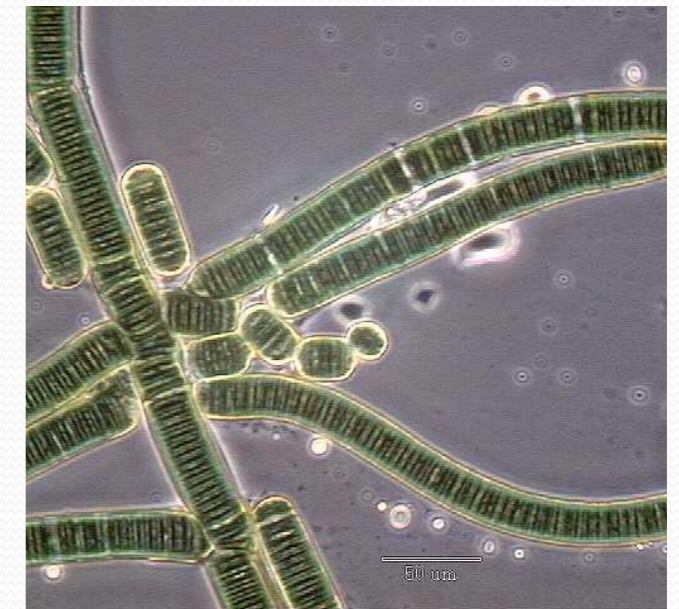
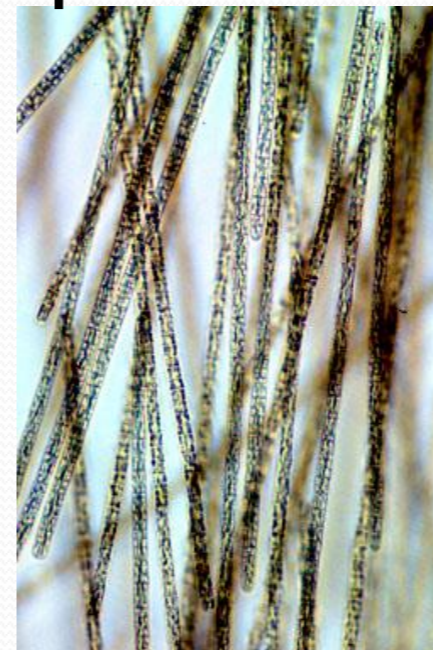
Aphanizomenon

Cylindrospermopsis

Skin Toxins

Lyngbya

Not every strain can produce toxins



Toxic Algae Can Be a Public Hazard

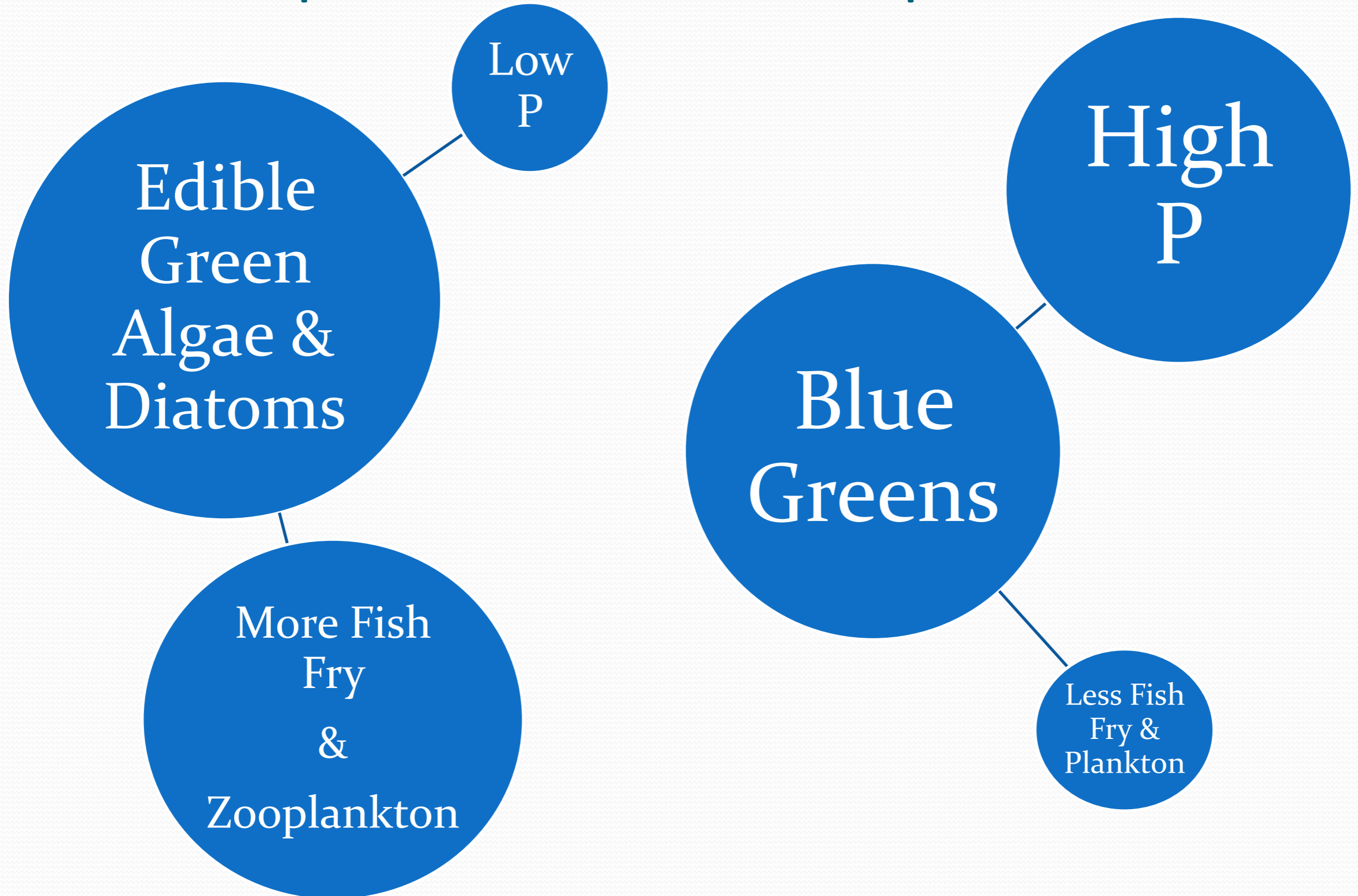
- We have all heard of fish kills caused by cyanobacteria
- Every year, hunting dogs die from water that contained cyanotoxins.
- In 2002, two boys in Madison, WI died after swimming in, and ingesting water from a golf course pond.

The presence of N₂ fixing Heterocysts are indicative of High phosphorus levels

- High phosphorus levels will select for blue-green algae that can fix nitrogen from the air and possibly be toxic
 - *Anabaena*
 - *Oscillatoria*
 - *Aphanizomenon*



How Phosphorus Affects the Aquatic Food Web



Algal Blooms and Recreation

- Non toxic algal blooms also promote bacteria growth, reduce water quality, and discourage beach use.



Why Measure Chlorophyll A?

- Knowing the Chlorophyll A and phaeophytin levels can give us a general idea about the health of our system.
- There are two accepted techniques for measuring Chlorophyll A.

Chlorophyll Detection Techniques

- Spectrophotometric Technique

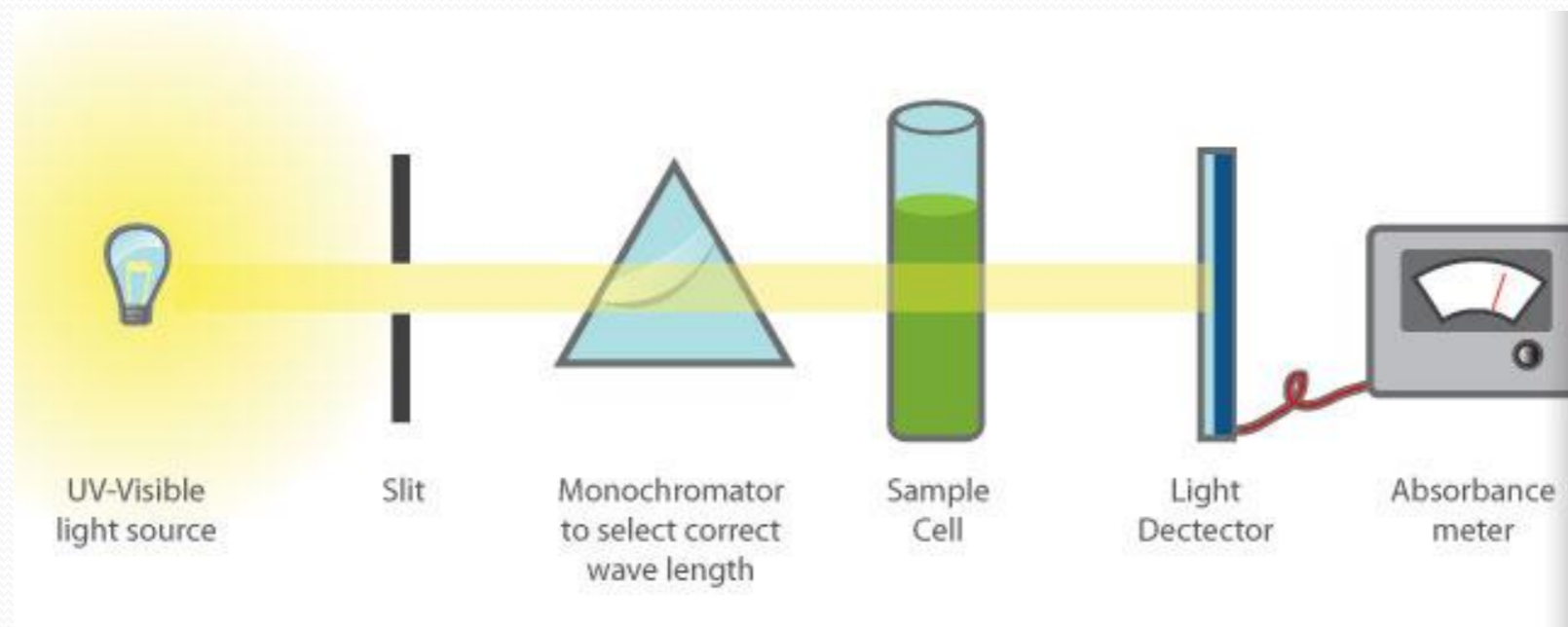


- Fluorometric Technique



Spectrophotometric Technique

- For water samples with high levels chlorophyll.



Red Light
(~665nm) into the
Sample

Red Light
(~665nm) into the
Detector

Equipment for Spec Method

- Spectrophotometer –
- Tissue Grinder
- Centrifuge
- Dark room
- 47 mm glass fiber filters
- Forceps
- Filtration manifold
- Filtration funnels and bases
- 15 mL conical tubes
- Volumetric or auto-pipettes w/tips
- Aluminum foil
- Freezer (-30 Celcius)

Reagents and Standards

- 90% acetone
- DI water
- Standards
 - Calibration
 - ICV – independent calibration verification
 - CCV – continuing calibration verification
 - CCB – continuing calibration blank
- Spinach
- Standards PPM

Overview of the Spectrophotometric Method

- US EPA method 150.1
- The Spec method is used when chlorophyll levels are very high.
- Step 1: Collect water sample
- Step 2: Filter known quantity of water onto a 47 mm glass fiber filter
- Put the filter in a known volume of an acetone solution and grind the filter with a tissue grinder.
 - This releases the chloroplasts from the algae and filter



Overview of the Spectrophotometric Method

- In a darkened room
- Centrifuge the sample to settle the filter and debris and aspirate the supernatant
 - Or just pour the solution through a paper filter
- Deliver a portion of the chlorophyll solution into a cuvette and place the sample into the spectrophotometer

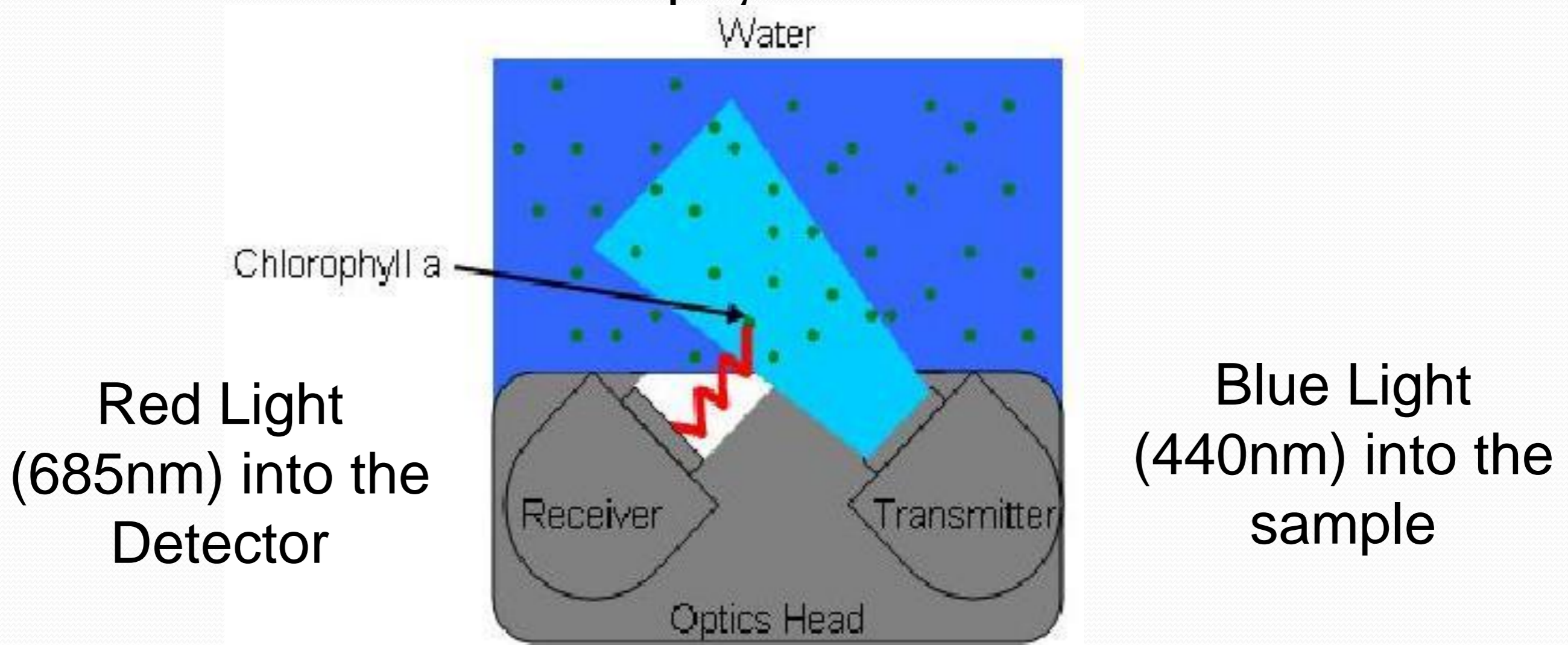


Overview of the Spectrophotometric Method

- Read the absorbance at 665 nm
- Compare this value to your standard curve and account for the dilution factors.
- The method to manually calculate the concentration is in US EPA method 150.1
- Most labs use the Fluorometric method

Fluorometric Technique

- More sensitive than spectrophotometric techniques for low levels of chlorophyll.



Equipment for Laboratory Fluorometric Method

- Sample preparation is the same as the spec method
- The fluorometer is more sensitive, so a very green sample may need to be diluted.
- Most fluorometers today will read chlorophyll out in mg/L.



Equipment for Field Fluorometric Method

- Fluorometer attached to a sonde
- Technician
- The sonde will read out the concentration
- This method is not reliable when algae physically floats in mats.



Summary

- Chlorophyll A levels
 - Give us a snapshot of the algal biomass in the water
 - This correlates with the nutrient load
- Chlorophyll A is only half of the story
 - The types of algae can tell us if our system is potentially toxic
 - If there is too much P or N
- Not all strains of cyanobacteria are toxic
- With minimal training, anyone that can microscopically analyze a sludge sample can analyze algae in a water sample.