Chlorophyll *a* Lab

Date: October 4, 2024

Write up due: October 9, 2024

**Overview:** Measurements of the concentration of photosynthetic pigments of algae and cyanobacteria can be used to estimate the composite biomass of phytoplankton populations. Although pigment concentrations generally are correlated to the biomass of phytoplankton, pigment concentrations of algae can vary widely depending on metabolism, light, temperature, nutrient availability, and many other factors. Plant pigments of algae and cyanobacteria consist of the chlorophylls and carotenoids. The three major chlorophylls, *a, b*, and *c*, absorb light maximally at specific wavelengths when dissolved in organic solvents. From these absorption characteristics an estimate can be made of the concentrations of pigments. Chlorophyll a is by far the most dominant chlorophyll pigment and occurs in greatest abundance. Thus, often chlorophyll *a* alone is used to estimate algal biomass. A Fluorimeter can be used to estimate chlorophyll but requires fluorescence to be measured both prior to and following acidification in order to correct for degradation products of chlorophyll that may be present.

The mini-lecture (Chl\_Lab\_MiniLecture), available on Canvas, is also an informative reference to help you complete this lab.

**Summary:** Because the extraction process takes 24 hours, we have already placed the filters you collected in Horsetooth Reservoir in acetone the day before the lab. Briefly, you will need to find and measure your sample before and after the addition of HCl. Once you have taken your first measurements and are satisfied with it add 2-3 drop of HCL to your sample. Wait 30 minutes and then repeat your measurement. Then use the worksheet below to calculate Chla values for your Horsetooth sample.

**Procedure:**

1. Obtain one of your group’s glass-fiber GFF filters from the Horsetooth Reservoir trip, that has been sitting in acetone for the past 24 hours.
2. Fill a cuvette with distilled water (a blank) and obtain a measurement for it from the fluorometer. Use the following procedure for using the fluorometer, for this step and subsequent steps:
   1. Read each cuvette twice, giving the cuvette a slight turn between readings.
   2. If difference between readings is more than 10 percent, repeat reading and keep two most similar readings.
3. Fill a cuvette with your group’s chlorophyll sample and obtain measurements for it from the fluorometer. Enter them in the table below (#1). Repeat for your second filter.
4. After the first measurement, add 2-3 drops of HCl to both samples. Wait 30 minutes for the acidification to take effect.
5. Fill another cuvette with your group’s sample and obtain measurements for it from the fluorometer. Enter them in the table below (#2)
6. Repeat steps 1 to 5 for your group’s other GFF filter. Fill in the rest of the table below.

**Results:**

Your group number: \_\_\_\_\_\_\_

Chlorophyll *a* Calculations

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| # | Variable | Units | Results for your group’s first filter | Results for your group’s second filter |
| 1 | Before acidification value (HB) from fluorometer | µgL-1 |  |  |
| 2 | After acidification value (HA) from fluorometer | µgL-1 |  |  |
| 3 | Volume of acetone used in the extraction (VAcetone): | ml | 10 | 10 |
| 4 | Volume of water filtered (Vwater): | ml |  |  |
| 5 | Vwater / VAcetone (aka Concentration Factor) |  |  |  |
| 6 | HB / Concentration Factor  *[This gives you combined phaeophytin and chl a]* | µgL-1 |  |  |
| 7 | (HB - HA)/ Concentration Factor *[This gives you just chl a]* | µgL-1 |  |  |

Calculate the following:

1. Mean of chlorophyll *a* of your two filters: \_\_\_\_\_\_\_\_\_\_
2. Standard deviation of chlorophyll *a* of your two filters: \_\_\_\_\_\_\_\_\_\_

**Questions**

1. Was your standard deviation high or low? If it was high, why do you think your samples were dissimilar?
2. One of the hardest things about collecting samples in the field (especially for the first time) is understanding why specific protocols are necessary and which details matter and why. Now having measured chlorophyll a in the lab, is there anything you would have done differently when collecting your samples?

**To Be Completed At Home**

We have posted results from the Lake Yojoa nutrient enrichment bioassay from January on Canvas. Please use that data set to answer the following questions.

1. What was the average chlorophyll content for the control bottles? Nitrogen addition? Phosphorus? Nitrogen and Phosphorus? What is the standard error of each? Results may be displayed in a table.
2. Based on your calculations, what appears to be the primary source of limitation in Lake Yojoa in January: Nitrogen, Phosphorus or Co-limitation?
3. What are other potential sources of limitation not accounted for in this experiment?
4. Produce one figure or statistical test of your choice that you feel best supports your conclusions in question 2 (i.e. bar chat with error bars, box plots, ANOVA etc.)