

Nutrient Enrichment of Phytoplankton in the Chesapeake Estuary A MWEE for 9th Grade Environmental Science Classes

Fifth Day of PLANS – Teacher –led classroom activity supported by PLANS staff via online resources.

Overview

During this class period your students complete the Model Enrichment Experiment by filtering their samples and comparing the filters to the color comparator to semi-quantitatively determine the degree of algal growth. Soil extracts will then be used by students to set up the second enrichment bioassay and make initial observations of their tubes.

Objectives

- Students will learn how to syringe filter to collect algae from their bioassay samples.
- Students will compare the color of their filters to the color comparator chart and interpret the results of their model enrichment experiment.
- Students will use their soil extracts, which represent various land uses, to set up a second enrichment experiment.

Background

Some background concepts for this period’s activities have been discussed in previous teacher guides and so will not be reiterated here.

The important point to make with the students during this period is that in completing the Model Enrichment Bioassay, they now have observed the growth response of algae to known concentrations of a plant fertilizer. Certainly more fertilizer (at least up to a point) should cause greater algal growth. If their data were graphed, it might show a trend similar to the one seen in Figure 1.

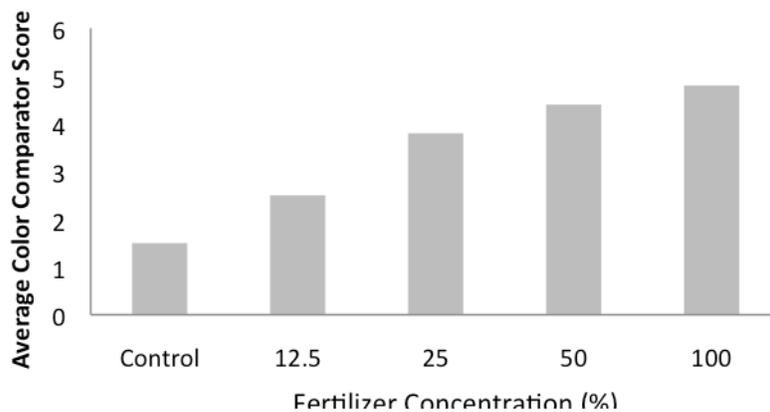


Figure 1. Algal growth response to varying levels of plant fertilizer.

These growth levels will be compared to those elicited by the students’ soil extracts at the completion of the next enrichment bioassay.

Completing the Model Enrichment Bioassay

Materials Needed (per student group)

- 1 - Syringe filter holder
- 5 - Whatman No. 5 filter paper discs
- 1 – 100 ml graduated cylinder
- 1 – 200 or 400 ml beaker
- 1 – Color Comparator
- 1 – Pair of plastic forceps
- Distilled Water & dropper

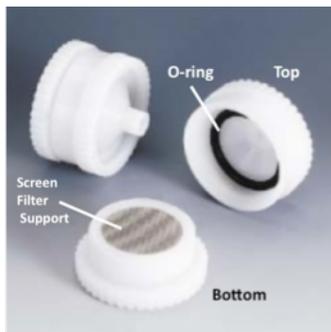
Preparation

- Make copies of the student handout: *Class Period 5*.
- Make a copy of the class data sheet

Procedure

1. Filtering algal cultures from the Model Enrichment Bioassay using a syringe filter.

Students will need to assemble the syringe filtration apparatus, filter their samples and collect the filters. Steps are provided below:



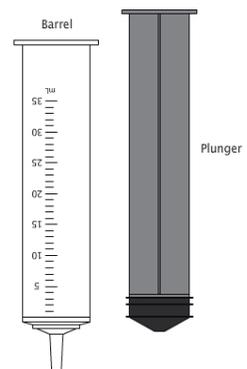
Assembling the Filter Apparatus -

- Open the syringe filter holder (Figure 2) by unscrewing the top from the bottom.
- Rinse filter chamber with distilled water.
- Carefully place the filter in the center of the bottom part of the chamber (the side with the

flat silver screen).

Figure 2. The syringe filter holder.

- Add 2-3 drops of distilled water onto the filter (enough so that the filter is completely wet).



- Place the black rubber gasket over the filter making sure both are centered.
- Carefully screw on the top part of the filter chamber (Make sure the chamber is flat while doing setting it down on the table helps).
- Remove the syringe plunger from the barrel of the syringe (Figure 3). Set the plunger aside.
- Attach the barrel of the syringe to the top of the assembled holder.

Figure 3. The syringe barrel and plunger.

Filtering a Sample -

- Add approximately 5 ml of distilled water to the syringe barrel and carefully insert the plunger into the syringe.
- **Slowly** push the plunger down until the filter holder is filled with water. Excess water can be caught in the beaker. (Look closely for air bubbles that may remain in the translucent filter holder. If you see some, filter another small volume of water.)
- Temporarily remove the barrel from the syringe holder and then remove the plunger from the barrel. Now replace the barrel on the holder. (Pulling up on the plunger without removing the barrel from the holder may cause a suction that alters the position of the filter in the holder.)
- Using a graduated cylinder, measure 10ml of algal culture from one of the treatments in the model enrichment bioassay. (**Note:** It is best to filter the algal cultures in the following order: spring water, 12.5%, 25%, 50% and then 100%.)
- Pour the 10 ml of culture into the syringe barrel and again carefully insert the plunger into the syringe.
- **Slowly** push the plunger down to filter the algal culture. Filtrate can be caught in the beaker. **IMPORTANT:** Be sure that you filter the culture **very slowly**, at about 1 drop per second. More rapid filtration may damage the filter and cause loss of algae. **Filter the entire 10ml of culture.**
- Detach the syringe barrel.
- Carefully unscrew the filter holder and remove the black rubber gasket.
- Using filter forceps, remove the filter and set aside on a paper towel.
- Rinse the syringe barrel and the filter holder thoroughly with distilled water before beginning filtration of the next sample.
- As each culture is successfully filtered, one member of the group can discard the remaining culture from the test tube and rinse the tube thoroughly with tap water. Carefully, shake the tube to remove excess

water. Then allow the tube to drain upside-down in the rack until it is used later in the period.

2. Interpreting the filter color.

- Before the filters dry completely, students should place each on the laminated color comparator and determine which portion of the comparator most closely matches the color of the filter and record the corresponding number value from the comparator.
- To insure that filters do not become mixed up, have students move each filter on to the correct circle on the data strip provided and make sure that they also record their comparator values on this sheet as well as the class data sheet.

Teacher	Control	12.5%	25%	50%	100%

Team					

Comparator #	_____	_____	_____	_____	_____

- Remind students that the reason for conducting the Model Enrichment Experiment is to demonstrate that the amount of plant fertilizer present directly affects the degree of growth stimulation of phytoplankton in the Bay. Additionally, the data gathered in this experiment can be used to compare the degree of growth response observed during the up-coming Soil Extract Bioassay.

Setting up the Soil Extract Bioassay

Materials Needed

- Enrichment Bioassay Kit(s) – provided by PLANS staff
- Constructed Light Box(es) – from previous experiment
- Digital Camera (optional)
- Student Handout for *Soil Extract Bioassay Procedure* - downloadable from the PLANS website, under “Teacher Resources”

- Group Data Sheet (for recording periodic observations during the Bioassay) - downloadable from the PLANS website, under “Teacher Resources”

Preparation

- Make copies of the student handout: *Class Period 5*.
- Make a copy of the class data sheet

Procedure

Set up the Bioassay:

Each team of students should set up one replicate of the control, one blank containing soil extract but no algae, and 3 replicates of the soil extract treatment (5 tubes in total). Provide copies of the student handout of the Soil Extract Bioassay Procedure to be used as a precise guide in setting up the experiment.

Have students transfer the soil extract made several days ago to a clean 50ml beaker without disturbing the particles that have settled to the bottom of the test tube.

Label 5 test tubes using tape and a marker. Label with the group letter (A, B, C, D or E) and the appropriate labels from Table 1 below. For example, the control tube for group A should be labeled “A-Control”, the blank tube should be labeled A-Blank, the first soil extract tubes should be label A-SE-1, A-SE-2, and A-SE-3

Then fill each tube with the following:

Tube 1 - Using a graduated cylinder measure 20ml of spring water and pour into the test tube labeled “Control”

Tube 2 - Using a graduated cylinder measure 5ml of soil extract and 15ml of spring water into the test tube labeled “Blank”

Tubes 3 to 5 - Using a graduated cylinder measure 5ml of soil extract and 15ml of spring water into each of the test tubes labeled “SE”

Now, in all but the blank test tube, add 2 ml of the algae stock to each test tube.

Tube	Test Tube Label	Volume of Soil Extract	Volume of Spring Water	Volume of Algal Culture
1	Control	0 ml	20 ml	2 ml
2	Blank	5 ml	15 ml	0 ml
3	SE - 1	5 ml	15 ml	2 ml
4	SE - 2	5 ml	15 ml	2 ml

5	SE - 3	5 ml	15 ml	2 ml
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Table 1: Lab Soil Extract enrichment set up

Cap each tube and invert it several times to mix the algae and soil extract solutions. Then return each tube to the rack and loosen the cap to allow for some exchange of air.

This bioassay will incubate in the light box for approximately 10 days.

Monitoring the Bioassay:

1. Have each team of students make their initial observations and record their findings on the group data sheet. Optionally, digital images can be taken of the tubes to record the initial coloration of the solutions.
2. Algae will settle to the bottom of the tubes, so students should invert tube daily or every other day to re-suspend them. Remind them to tighten caps before inverting and then loosen them again after algal re-suspension.
3. Students should observe the test tubes periodically (every 3rd or 4th day) during the experiment to note changes in coloration that might be developing. Measurements using the color comparator can be recorded. Encourage student to compare the colorations of their soil extract tubes with the control. Can they discern any trends? Which is lighter? Which is darker? Have them record their observations. Additionally, optional digital images may be taken at these times.

At the conclusion of the incubation, each algal suspension will be filtered and the color of the filters among the treatments will be compared using a color comparator. These methods will be described in detail in the Period 6 Teacher’s Guide.

Check for Understanding –

The following questions can be used to extend the class discussion of enrichment bioassays.

1. What differences did you observe in the amount of algal growth under different fertilizer concentrations? Is there a relationship between algal growth and fertilizer concentration? If so, describe that relationship. *(The general trend should be that there is an increasing level of growth with increasing fertilizer concentrations. This may be best seen by looking at the average growth response at each fertilizer levels rather than the results from individual groups.)*

2. Do you think that the color comparator method of assessing growth is sufficiently sensitive to differentiate between algal growth responses at different fertilizer levels? Why or why not? *(The color comparative method is not as sensitive as many other techniques for measuring algal density. Alternately, measuring chlorophyll content or conducting algal cell counts on the bioassay results could yield a greater ability to differentiate growth responses between fertilizer levels.)*

3. What is the function of the control and blank in the Soil Extract Bioassay experiment? *(The control can be used to 1) indicate algal growth in the absence of enrichment and 2) confirm that the growth responses of the Model Enrichment and the Soil Extract bioassays are comparable. The blank can be used to observe whether the color of the extract alone is influencing the observed coloration due to the growth of algae during the bioassay.)*