Supplemental Materials for:

Green Plants, Red Glow – Looking at chlorophyll's red fluorescence as an exercise in exploring photosynthesis, agriculture, and global ecology

Table of Contents

Laboratory Instruction Sheet: Green Plants, Red Glow

Part 1: Extract Preparation

(1) Pick 5-7 green leaves of spinach.
(2) Break the leaves into small pieces by hand or scissors.
(3) Place the pieces into the mortar (or bowl).
(4) Using a pestle (or spoon), thoroughly grind the leaves.
(5) Fill the mortar with rubbing alcohol to less than half full.
(6) Repeat the grinding without spilling the mixture.
(7) Place two unfolded napkins over the mouth of the funnel.
(8) Place a small vial directly underneath the opening of the funnel.
    - ❖ Make sure the vial and funnel do not tip over.
(9) Pour the mixture over the napkins inside the funnel and strain.
    - ❖ Make sure leaf pieces are not included in the vial.

Stop here and wait for instructions

Part 2: Visualization

(1) Bring your sample to the nearest light box and place it into the slot in the front.
(2) With the flashlight on, place your sample near the light.
(3) Use the slot at the top of the light box in order to look inside.
    - ❖ You may need to move your sample back and forth from the light.
(4) Return to your seat, record what you saw, and write why this may have happened.

Observations and Assessment: Green Plants, Red Glow

Question 1. What molecule makes leaves green, and what is its function?

Question 2. Where is chlorophyll located within a plant?

Question 3. Where does the red glow come from?

Question 4. Why is the red glow used as a measure of plant health?

Question 5. What would farmers be able to learn from images taken by satellites?

Observation Space. Please use this area to make observations during the activity.

3. Additional Information for Instructors

A. Why Plants? – the following is provided to spark classroom discussion

1. What do plants give us directly?

- Food – vegetables, fruits
- Wood – for furniture, buildings, basketball courts, etc.
- Flowers
- Fragrances – rose, lavender, anise, etc.
- Cotton and clothes made of cotton
- Coffee and tea
- Sugar
- Spices – cinnamon, chili, clove, curry, eucalyptus, ginger, vanilla, etc.
- Oxygen, from photosynthesis
- Shade
- Emerald green planet – beauty
- Habitat for birds and other living things
- Food for insects

2. What do plants give us indirectly?

- Gasoline (derived from oil, which is produced by decay of plants over millions of years)
- Food (such as bread, from wheat; meat, from animals eating plants; fish, from fish eating phytoplankton)
- Medicines (the majority of all medicines are derived from plants)
- Removal of carbon dioxide from the atmosphere
- Capture of energy (from the sun) to feed almost the entire biosphere

B. Rubbing alcohol

Rubbing alcohol is a term that refers to various types of solutions, including those that contain 95% ethanol, 70% isopropyl alcohol, or 91% isopropyl alcohol, with the remainder in each case comprised of water as well as trace quantities of bitter-tasting additives to discourage ingestion. For this activity, rubbing alcohol refers to 91% isopropyl alcohol purchased from a local drugstore. While 95% ethanol (sometimes termed "surgical spirit B. P.") appears equally effective, use of ethanol may not be appropriate for a classroom setting. The results with 70% isopropyl alcohol were adequate but less effective than with 91% rubbing alcohol.

Among all possible solvents, we chose "isopropyl rubbing alcohol" (~91% isopropyl alcohol, ~9% water, and bitter additives) for the following reasons:

- It is sold in most grocery stores, pharmacies, and discount stores.
- It is relatively non-hazardous, particularly compared with many other solvents; diethyl ether (flammable), for example, is used in research labs to extract chlorophyll from plants, but is inappropriate for educational activities in a classroom setting.
- It may be disposed of in a ventilated sink by flushing with excess amounts of water.
- It contains bitter additives to prevent intentional ingestion.

C. Solar Induced Fluorescence

What is SIF? Solar-Induced Fluorescence is the emission of photons by the chlorophyll molecules in plants upon illumination by the sun. "Solar-induced" contrasts the emission with other types of illumination, say by use of lasers in the field to observe fluorescence. Regardless of light source (sun or laser or flashlight), the photons emitted from chlorophyll can be easily observed by fluorimeters (fluorescence detection instruments) that are tuned to the same wavelength. We do not see the stars (other than the sun) during daytime because the light from the sun is so overwhelming. So how is solar-induced fluorescence detected out in the bright light during daytime?

It turns out that the sun emits light at all wavelengths – all colors of the rainbow. But the gases that surround the sun absorb very narrow slices of the rainbow. So, the sunlight reaching

Earth has all colors of the rainbow minus a set of very narrow slices of light that has been filtered out by the gases surrounding the sun. Those slices are referred to as Fraunhofer lines – particular wavelengths where there is no light from the sun. If we were to look for light in one of these narrow slices, we should find none, because none is coming from the sun. But in fact, there is some light in the Fraunhofer lines in the red region, due to the glow of chlorophyll. A satellite with appropriate detectors can peer into the Fraunhofer lines and measure any light that is there. In this manner, the red glow from plants on Earth can be detected in bright sunlight. The more red glow, the more extensive the photosynthesis, and the more abundant the plants.

It warrants mention that maps of SIF can be displayed in a variety of formats. Displays to emphasize the relative intensity of light often use so-called false-color, where different colors convey different intensities. Here, Figure 1 simply uses the observed red at different intensities.

Monitoring the Amazon Rainforest: Rainforests are believed to play a key role in global ecology through the abundant photosynthesis in these warm wet regions. As the world's largest rainforest and home to the greatest variety of living organisms on earth, it is reasonable to monitor the photosynthetic activity of the Amazon ecosystem.

Measuring the wet season: The length of the annual wet season is directly associated with rates of photosynthesis. Extended dry seasons decrease rates of photosynthesis, which can be gauged by analyzing SIF. During the wet season, atmospheric carbon dioxide levels are measurably lessened due to increased rates of carbon fixation and photosynthesis. There is an association between measured SIF and carbon dioxide concentrations, but it is weak and inversely associated; meaning that as carbon dioxide concentrations decrease due to increased photosynthetic activity there will be a corresponding increase in fluorescence (De Sousa *et al.*, 2017, p. 4).

D. Observations from Prior Implementation of This Activity

- Teachers may wish to preface the extraction portion of the demonstration with some background information so that students can understand the activity before they begin the extraction procedure.

- The demonstration portion of this activity is best completed in groups of anywhere from 1–4 students. The opportunity to work in small groups is a necessary condition to promote critical thinking and collaboration reflective of a research environment. When filling out the summative review document, students should be allowed to work together and share the observations that they made in their respective groups. Group size can be increased if obtaining supplies is an obstacle, but individual involvement in each step decreases with every group member added. A collaborative environment is key to allow students to construct explanations and solutions to any obstacles that they might come across (MS-LS1-5).
- This demonstration was carried out in a classroom setting that varied from twenty-three to twenty-eight students. Two instructors, one teacher and one scientist, facilitated the extraction and visualization stages much to the enjoyment of the participants. This can be completed with one teacher.
- Background information on the organization of plant tissue structure and factors contributing to chlorophyll fluorescence was given before students assembled into groups.
- After the observation stage, the significance and possible applications of chlorophyll fluorescence were relayed to the entire class. Students then spent time contemplating the role of chlorophyll in photosynthesis, and reflecting on the significance of what they accomplished.
- Possible extensions of this activity may include students bringing leaves from around their home or school and comparing the visual fluorescence between the leaves they have selected and the spinach provided. Note that all plants contain chlorophyll, and following extraction, the chlorophyll should glow red upon illumination. However, some plants have leaves with tough exteriors, making extraction difficult.
- The chlorophyll solution and/or extract resulting from the demonstration will stain objects a very dark green, so students should use caution to avoid spilling the solution on their clothes and hands.
- Spills most often occur during the grinding and pouring stages of the activity.
- If not enough spinach or other type of leaf tissue is used, red fluorescence may not be so readily observable, as the concentration of chlorophyll may vary.

- If the red fluorescence is not immediately visible following completion of all listed steps, allow the solution to sit in the dark for ten minutes (so any poorly filtered material can settle out) before exciting with a bright flashlight.
- If an ample number of mortars and pestles is not available, teachers may either allow for chlorophyll to be extracted from the leaves on standing over time. Leaves may also be placed in a blender to shred.
- Students sometimes misunderstand that chlorophyll is "turning red"; in fact chlorophyll remains green, but only releases some of the absorbed light energy as red light.

4. Extension 1 – Green Food Coloring as a Negative Control

Experimental controls are necessary to establish baseline values for comparative results of experimentation. A negative control is developed with the intent to ensure that the dependent variable is truly the cause of the experimental results. One classic example is pharmaceutical trials that use sugar pills as a placebo group. The sugar pills do not contain the drug, but the subjects go through the same experimental process. The results from the two (or more) groups are then compared. A single experimental variable, the independent variable, is changed between the two groups. The inclusion of a negative control provides a wonderful opportunity to explain how and why scientific experimentation is seen as significant and trusted. An understanding of science as a process generally follows a clear grasp of the concepts of independent/dependent variables and experimental controls.

In the context of the chlorophyll extraction activity, one suitable negative control uses green food coloring (see Figure S-1). Students may have simply attributed the fluorescence to the fact that the chlorophyll is green. If this is the case, a dropwise addition of green food coloring to a similar volume of rubbing alcohol should create an observationally similar green solution. The apparent "greenness" of the two solutions should be as similar as possible. Green food coloring is not fluorescent, so when the two are compared with a flashlight, it should be apparent that the significance of chlorophyll fluorescence is beyond the presence of a green color. This extension activity may be useful to complete before, after, or alongside the other activities described. As a preceding activity, a negative control may deepen the impact that the initial observation of chlorophyll fluorescence has for each student. Providing the negative control after the chlorophyll extraction could be effective at dispelling inaccurate conclusions and cementing the significance of chlorophyll and its fluorescence.

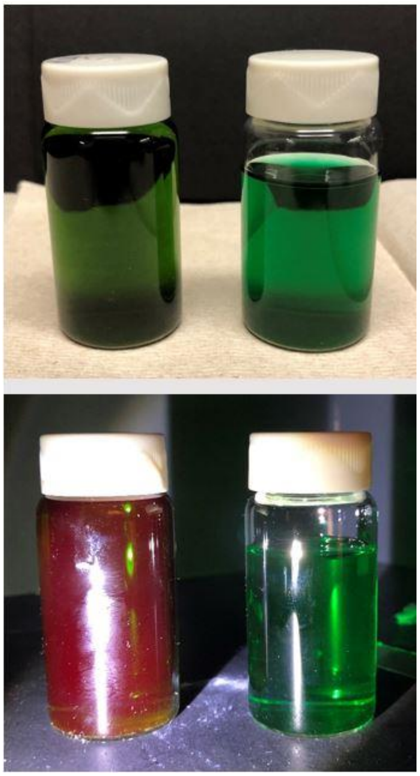

Figure S-1. Chlorophyll extract (left-hand side) and green food coloring (right-hand side) in natural light (top across) and with flashlight illumination (bottom across). The green food coloring does not fluoresce; chlorophyll in solution fluoresces strongly.

Materials Supply List for Green Food Coloring as a Negative Control:

(i) Green food coloring (Fast Green FCF) – avoid contact with skin
(ii) ~30 mL of rubbing alcohol or water
(iii) ~25 mL clear container
(iv) A dark space for visualization
(v) Sample produced from the Chlorophyll Extraction Activity
(vi) Bright flashlight

Green Food Coloring as a Negative Control

Observations: Use this space to record your findings as you complete the activity.

Question 1. What is a negative control?

Question 2. What are the observable differences between chlorophyll and the negative control group?

Question 3. How does this information from the negative control pertain to SIF?

5. Extension 2 – Fluorescent Materials Activity

What constitutes fluorescence? Fluorescence is not the reflection of light, but the absorption and subsequent emission of light. In general, the light absorbed is at a shorter wavelength and the light emitted is at a longer wavelength. Students can find examples of this fluorescent behavior in other classroom and household objects as well. Objects such as highlighters display fluorescent behavior. Other products that exploit the principle of fluorescence include high visibility vests and tapes.

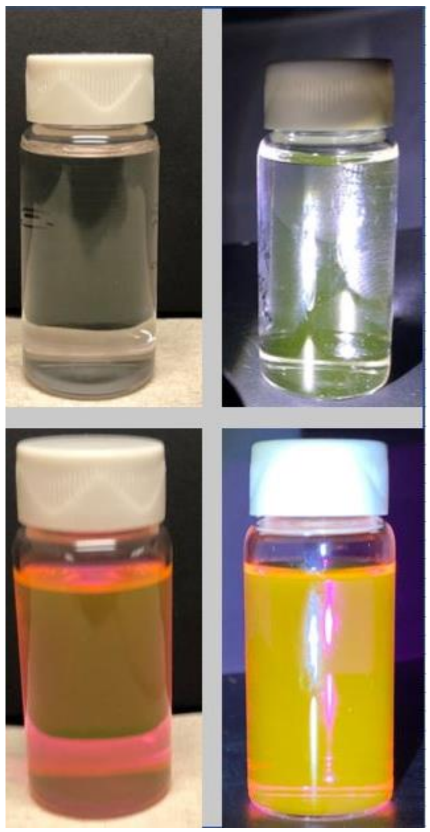

Figure S-2. Orange highlighter extract solution (top across); pink highlighter extract (bottom across). Pictures on the right-hand side are illuminated in a light box. The pink highlighter solution (bottom right) is highly fluorescent; the orange highlighter solution (top right) is not.

What materials fluoresce under a flashlight? This extension activity examines alternative materials that display fluorescent properties. Highlighters are quite accessible, and most students should be familiar with them while perhaps unaware of their fluorescent properties. Some of these materials display different properties in their typically used state than when treated with rubbing alcohol in a similar manner to the chlorophyll extraction experiment. For this extension the highlighters selected should fluoresce with light from a handheld flashlight (see Figure S-2). The fluorescence displayed in a few of the highlighter colors is not the same as that of chlorophyll. Pink and orange highlighters contain fluorescent rhodamine dyes. The tips of most highlighters can be easily removed and placed in rubbing alcohol to create a solution. The pink highlighter shown here produced a pink solution that emitted an intense yellow fluorescence when excited. The orange highlighter solution was clear in natural light and produced a green fluorescent glow upon excitation.

Materials Supply List for the Fluorescent Materials Activity:

- (i) Pink and/or orange highlighters
- (ii) Rubbing alcohol
- (iii) Multiple clear containers
- (iv) A dark space for visualization
- (v) A bright flashlight

Fluorescent Materials Activity

Observations. Use this space to record your findings throughout the activity.

Question 1. What are the advantages of using fluorescent materials in everyday objects?

Question 2. What are the observable differences between the fluorescence in the highlighters and the fluorescence seen in chlorophyll?

Question 3. If you could make one item fluorescent for practical reasons, what would it be and why?

6. Extension 3 – An Additional Leaf

Students were asked to bring leaves, or were provided with leaves other than spinach. When working with the additional leaves, students took additional data on leaf texture, the ease of tearing, and the color in comparison to spinach. In some cases, the additional leaf (e.g., holly leaves from outside the school) were hard, more difficult to tear, and not as green. This information was taken into consideration when extracting chlorophyll, as students had to use more force when grinding. When the additional leaf suspension was illuminated, students noticed that it did not glow the same deep red as the spinach. This observation led students to dive deeper into the reasoning. They discussed how all plants have chlorophyll, but the structure of the holly leaf must be slightly different. Students were able to conclude that since a holly leaf is tougher to tear and has adapted to the harsh outdoor environment it takes more force to break down the leaf structure to extract the chlorophyll. As a class, students then discussed the importance of adaptations in the natural world. It warrants emphasis that the difficulty in extraction of chlorophyll reflects the texture of the sheath of the leaf, not the nature of the photosynthetic process or the red glow from the chlorophyll in the leaf.

7. Everything Light Box

Simply turning off the lights in your classroom may not be enough to effectively visualize the chlorophyll fluorescence in the activity. To deliver the best results for the visualization stages for all of the previously described activities, an inexpensive homemade lightbox can be constructed from materials that are readily available (see Figure S-3). A medium-sized cardboard box should suffice, but ensure that your box is an appropriate size for whatever containers hold your resulting solutions. Using a ruler, measure the sides of your box. It is recommended that the interior of the box is measured. If desired, the interior of your box can be coated with a matte black paper. This can be cut from a larger piece of black poster paper or from individual 8.5" x 11" pieces of construction paper. Painting the interior is also an option, but paper requires no drying time and requires less space to complete. At least two flaps should be cut into the box. The first flap will be on the horizontal facing side that is closest to the viewer. Cut a square, but make sure that the bottom portion of the square is not cut in order to make a flap that folds down. Corrugated cardboard boxes allow the flap to stay shut and maintain shape. Another flap can be cut open on the vertical side of the box. Keep this flap to a size that will allow students to be able to see through while minimizing the amount of ambient light let into the box. The final step is to mount your high intensity flashlight in a way that does not point the light source in the direct path of the eyes. This can be quickly done with tape before sealing the top of the box, or through the front-facing flap.

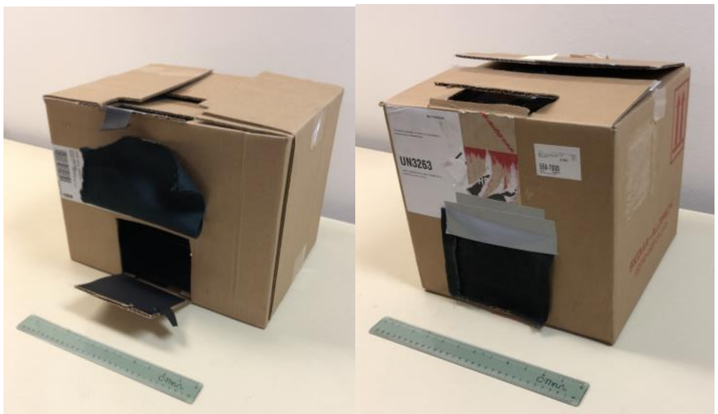

Figure S-3. Two light box models of varying size and modifications are shown. Any box with an adequately spacious and dark interior should suffice.

Materials Supply List for the Light Box

(i) Medium sized cardboard box
(ii) Matte black poster board or construction paper (optional)
(iii) Ruler
(iv) Scissors
(v) Tape and/or glue
(vi) Bright flashlight