IMHOTEP'S LEGACY ACADEMY

9.2 Discovering Plant Pigments

Grade 9 Activity Plan

Reviews and Updates

9.2 Discovering Plant Pigments

Objectives:

1. To know how plants nourish themselves through photosynthesis. This includes knowledge of the process of photosynthesis: how plants convert light energy into chemical energy in the form of sugar such as starch.
2. To learn that photosynthesis takes place in the chloroplast and that chlorophyll is vital for photosynthesis.
3. To understand what is chlorophyll and that it is one of many colour pigments found in plant leaves.
4. To understand how paper chromatography is used to study plant pigments.

Keywords/concepts: chlorophyll, chloroplast, photosynthesis, autotrophy, pigments, plant cell, chromatography.

Take-home product: sample of separated pigments on chromatography paper.

Segment	Details
African Proverb and Cultural Relevance (5 min.)	"It is the chef who knows the contents of the pot best." **South Africa**
Pre-test (5 min.)	Ask probing questions on students' knowledge of growth in plants; the organelles responsible'.
Background (10 min.)	Discuss plants and their importance to life. Discuss photosynthesis and the associated basic concepts and processes including the relevance of pigments (chlorophyll). Connect the concept of chromatography to the activity.
Activity 1 (15 min.)	To determine the solubility of chlorophyll in water and alcohol using paper chromatography
Activity 2 (45 min.)	Demonstration to explain how chlorophyll absorbs certain wavelengths of light, and converts light into chemical energy.
Follow-up (5 min.)	What were everyone's results? What were all the variables? What effect could/did these variables have on the results?
Post-test (5 min.)	Calculation of R_f value. By calculating the R_f, students should understand the correlation between the retention factor and the distance travelled by each pigment.

Possible interpretation of proverb: In this lesson we must become the "chef" in order to discover the pigments contained in the spinach leaves, and their special properties.

Cultural Relevance

Benjamin Solomon Carson was born in Detroit, Michigan. His mother, Sonya Carson, one of 24 children, had dropped out of school in the third grade. She later married Robert Solomon Carson, a much older Baptist minister from Tennessee, when she was only thirteen. As a result of having only a third grade education, Sonya Carson never learned how to read and could not help Ben and his older brother, Curtis, with their schoolwork. When Carson was only eight, his parents divorced, and Mrs. Carson was left to raise Benjamin and Curtis on her own. She worked at two, sometimes three, jobs at a time to provide for her boys. Later, Ben Carson experienced difficulty in school eventually falling to the bottom of his class. He became the object of name-calling and subsequently developed a violent, uncontrollable temper. Determined to turn her son's life around, Carson's mother limited his television-watching and refused to let him go outside to play until he had finished his homework each day. She required him to read two library books a week and to give her written reports on his reading, even though, with her own poor education, she could barely read what he had written. Carson soon amazed his instructors and classmates with his improvement. "It was at that moment that I realized I wasn't stupid," he recalled later. Carson continued to amaze his classmates with his new-found knowledge and within a year he was at the top of his class. After determining that he wanted to be a psychiatrist, Carson graduated with honours from high school and attended Yale University, where he earned a degree in Psychology. From Yale, he went to the Medical School of the University of Michigan, where his interest shifted from psychiatry to neurosurgery. His excellent hand-eye coordination and three-dimensional reasoning skills made him a superior surgeon. After medical school, he became a neurosurgery resident at the Johns Hopkins Hospital in Baltimore, Maryland. At the age of 33, he became director of Paediatric Neurosurgery.

In September 1987 Carson performed at DSA a procedure to separate a pair of seven-month-old German conjoined twins, who were joined at the head. Carson was the lead surgeon on the team which performed the complex procedure. In 1997 Carson and his team went to South Africa to separate Joseph and Luka Banda, infant boys from Zambia. Both boys survived, and neither one suffered severe brain damage. The Bandas were the first set of twins joined at the tops of their heads to be successfully surgically separated. The operation lasted 28 hours, from 10:15 to 4:46). In 2003, Carson was a member of the surgical team that worked to separate conjoined adult siblings Ladan and Laleh Bijani. Neither survived the surgery. When asked why he had performed such a risky surgery, Carson stated that they conveyed to him they would rather die than stay conjoined. Dr. Carson also pioneered the hemispherectomy which is the surgical removal of the affected half of the brain in cases of Rasmussen's encephalitis or syndrome in children

BACKGROUNG INFORMATION

PHOTOSYNTHESIS

$$\text{Sunlight} + 6\,CO_2 + 6\,H_2O \xrightarrow{\text{chlorophyll}} C_6H_{12}O_6 + 6\,O_2$$

[Sunlight + carbon dioxide + water → glucose + oxygen

Plants use light to survive and go through the process of photosynthesis. Light is actually energy, electromagnetic energy to be exact. When that energy gets to a green plant, all sorts of reactions can take place to store energy in the form of sugar molecules.

Plant do not use all of the light. It actually uses only certain colors to make photosynthesis happen. Plants mostly absorb red and blue wavelengths.

Photosynthesis happens in the chloroplast. Within this cell organelle is the chlorophyll that captures the light from the Sun. The chloroplasts are working night and day with different jobs. The molecules are moved and converted in the area called the stroma. Chlorophyll is the magic compound that can grab that sunlight and start the whole process. Chlorophyll is actually quite a varied compound. There are four (4) types: a, b, c, and d. Chlorophyll can also be found in many microorganisms and even some prokaryotic cells. However, as far as plants are concerned, the chlorophyll is found in the chloroplasts.

The other molecules involved are water (H2O), carbon dioxide (CO2), oxygen (O2) and glucose (C6H12O6). Carbon dioxide and water combine with light to create oxygen and glucose.

The whole process doesn't happen all at one time. The process of photosynthesis is divided into two main parts. The first part is called the light dependent reaction. This reaction happens when the light energy is captured and pushed into a chemical called ATP. The second part of the process happens when the ATP is used to make glucose (the Calvin Cycle). That second part is called the light independent reaction.

Chromatography is a technique used to separate pigments in a specimen based on their differences in structure and/or composition. The mobile phase, or the solvent, climbs up the chromatography paper (the mobile phase) using capillarity. As the solvent moves past the point of origin it will carry some of the pigment. The liquid moves over the stationary phase, this of course would be the paper.

In this activity, students should see a variety of colours. In the alcohol solution, they should find two shades of green, a blue-green chlorophyll *a*, a yellow-green chlorophyll *b,* a faint yellow band of xanthophylls, and a thin orange band of carotenes. In the water solution, blue, red, or brown pigments may appear.

Activity 1: Chromatography – extracting plant pigments

Purpose: To understand how paper chromatography is used to study plant pigments.

Suggested format: students can do this in groups of two.

Item	**Quantity (for 10 students)**
Fresh spinach leaves	20 leaves
Medicine dropper	6
Chromatography paper	12 strips
Transparent beaker or cup	12
Rubbing alcohol	60 mL
Pencil	6
Paper clips	12
Ruler	5
Scissors	5
Mortar and Pestle	1

In order to be familiar with procedure, mentor should endeavour to perform experiment at least once before actual day of presentation.

Procedure:

Caution: be careful when handling the chromatography paper as oil from our skin can alter results. That said, only lift paper by its sides and be careful not to make contact with the front side of the paper.

1. Draw a faint line using **pencil** across the narrow part of the chromatography paper, approximately 2 cm from the bottom.

2. Grind and squeeze the spinach or iceberg leaves with the mortar and pestle until a liquid is obtained. Place a drop of plant extract onto the pencil line using an eyedropper. Once paper is dry, add a second drop to the same spot. Before you continue the spot should look very dark.

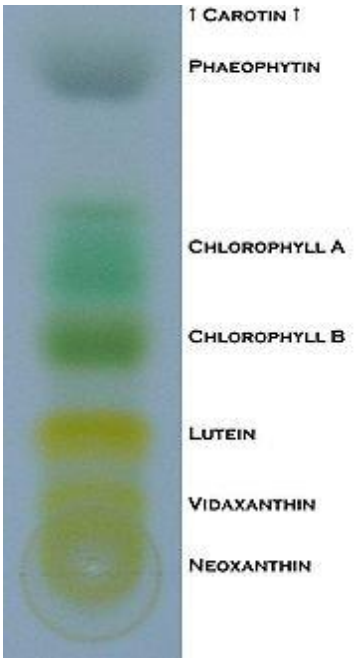
   OR

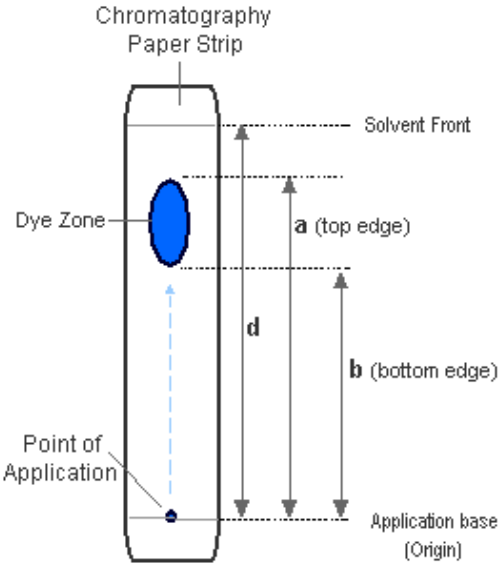
   Lay the leaf over the chromatography paper. Pressing hard, roll the edge of a coin over the leaf, such that the coin follows the path of the line already drawn on the paper as shown below. Repeat until line is very dark.

3. Repeat these steps for a second strip of chromatography paper.
4. To test precision of the experiment, prepare 6 strips in total.
5. Place 15mL of alcohol in one cup, and 15 mL of water in the other cup.
6. Attach the top of the strips of paper to a pencil or coffee stir stick. This can be done by making a loop with the top of the paper and fastening it with a paper clip.
7. Now lay the pencil across the top of the cup so that it suspends the chromatography paper above the liquid. About one centimetre of the bottom of the paper should enter the solvent. **Make sure the solvent does not surpass the point of origin!**
8. Allow 15-30 minutes for the solvents to rise through the strips.

**At this point in the activity there is down time. Mentor can choose to: conduct Activity 1 here, clean up any mess in the classroom with the students' help, or explain the plant pigments table found above.

9. Remove the strips when the colours have stopped spreading or just before the solvent reaches the top. **Make sure you remove the paper before the solvent reaches the top of the strip!**
10. Lay strips down, face up. Mark the line where solvent stopped.
11. Allow strips to dry
12. Before the pigments fade, mark (with a pencil) the top of each colour you can identify.





RF top = RF(a) = (a) / d
RF bottom = RF(b) = (b) / d
RF center = RF(c) = [(a+b)/2] / d

Activity 2: Chlorophyll absorbs light

Source: sites.bio.indiana.edu/~nsflegume/download/**Photosynthesis**%20**Activity**.doc

Purpose: To understand what chlorophyll is, and how it absorbs light energy

Suggested format: students should work in pairs.

Item	**Quantity (for mentor and 10 students)**
Fresh spinach	10 leaves
100% ethanol	50mL
150mL glass beaker	1
Hot plate	1
Forceps	1 pair
Paper towels	10 sheets
Transfer pipette	1
Small glass vial (25mL bottle)	1
Blue LED Flashlight	1
Black plastic garbage bags	2
Viewing box with red filter	1
DCMU (1×10^{-4} M in 10% ethanol)	30mL
Cotton swabs	5
Black plastic sheet ~2in. x 4in. (cut from garbage bags)	5
Gloves	1 pair
Copy paper box (for portable dark room)	1
Scissors	1 pair

Procedure:

Prep before activity (about 15 minutes):

1. Create a "portable dark room" by placing an empty copy paper box inside a black plastic garbage bag. Place the viewing box with the red filter inside this "dark room" so that the red filter is on top.
2. Cut small plastic strips (~2 inches by 4 inches; **1 per group**) out of the remaining garbage bag.
3. Rinse leaves (about 2 leaves per student group) with water and pat dry.

TO BE DONE BY MENTOR:

1. Cut several leaves into small pieces (1 x 1 inches; 2 per student group).
2. Pour about 50 mL of alcohol (100% ethanol) into a 150 ml beaker**.** Place the beaker on a hot plate and bring alcohol to boil.
3. Submerge one leaf piece (keep the other one for part two below) per student group in boiling alcohol for 5 min, or until leaves are pale green. Pull out the leaf pieces with a forceps (tweezers) and blot dry with a paper towel.
4. Take the beaker off the hot plate and turn off the hot plate.

5. Use a transfer pipette to fill a small glass vial 1/2 way with the green solution left in the beaker.

Each Student Group:

Record your observations in the following table:

Sample	**Color and Brightness**
extracted chlorophyll	
untreated leaf	
ethanol-treated leaf	

Part 1:

Place the vial with the green chlorophyll solution **inside the viewing box** inside the portable dark room. Use the blue L.E.D. mini-flashlight to **shine blue light** on the liquid in the bottle. Look at the bottle through the top of the viewing box. What do you observe?

The red filter blocks the blue light from reaching your eye, thus the vial should appear dark, unless the blue light is converted to red light.

Part 2:

Next you will repeat the above steps to observe your two leaf pieces (one treated with ethanol and the other not). Which leaf do you predict will be brighter? Why?

Place one untreated leaf and one ethanol-treated leaf on the black plastic sheet. Place inside the viewing box, and shine the blue L.E.D. flashlight on the leaves. Record observations.

Useful links:

- http://www.livescience.com/51720- photosynthesis.html
  Contains additional information on photosynthesis
- http://www.pbs.org/wgbh/nova/nature/photosynthesis.html
  A GREAT online activity that outlines the process of photosynthesis visually in steps. Explains the chemistry in a very simple and straightforward way.
- http://www.yesmag.ca/projects/paper_chroma.html
  This activity is very similar to the lesson but instead uses a marker. If time allows this could be done in addition to separating plant pigments. The method is similar and provides pictures in its description.
- http://www.reachoutmichigan.org/funexperiments/quick/csustan/mrsketch.htm
  Provides images and a written description to calculate the R_f value in paper chromatography.
- http://www.sciencebuddies.org/science-fair-projects/project_ideas/Chem_p010.shtml
  This explains atoms and its components, how charge is created and how bonds are formed. It also gives a list of terms useful in a paper chromatography lab. The questions posed can be used by the mentors if they need to further engage the students.
- http://www.marz-kreations.com/Chemistry/Chromatography/Dyes/RF-Values.html
  This is the source of the image used in the lesson plan showing a sample pigment on chromatography paper.
- https://www.boundless.com/physics/textbooks/boundless-physicstextbook/introduction-to-quantum-physics-28/applications-of-quantum-mechanics- 183/fluorescence-and-phosphorescence-676-4913/
  This page has a short description of the reaction of molecules that produce fluorescence