Investigating Leaf Pigments

Purpose

To explore what pigments exist in leaves and their importance

Overview

Working individually or in groups, students will conduct an experiment using paper chromatography to separate pigments present in leaves.

Student Outcomes

Students will learn stated key concepts and be able to apply process skills in understanding chromatography and understanding that pigments other than chlorophyll are present in leaves.

Science Concepts

Physical Sciences

- Chemical reactions take place in every part of the environment.

Life Sciences

- Energy flows through the ecosystems in one direction (photosynthesis-herbivores-carnivores-decomposers).
- Living systems require a continuous input of energy to maintain their chemical and physical organizations.

Scientific Inquiry Abilities

- Observing and inferring
- Hypothesizing and predicting
- Collecting data
- Analyzing and interpreting results
- Communicating results and conclusions
- Design and develop scientific investigations.
- Use appropriate tools and techniques.
- Develop explanations and predictions using evidence.
- Use appropriate mathematics to analyze data.
- Communicate results and explanations.

Time

One class period for gear up and to set up experiment

One more class period for observations

Level

Middle, Secondary

Materials and Tools

- White coffee paper filters, paper towels, filter paper or chromatography paper cut to 2 cm wide by 15 cm long
- Non-Crayola brand felt-tipped pens with black, water soluble ink
- Glass (baby food/juice jars) or plastic containers (8-10 oz. cups)
- Water at room temperature
- Hot water from faucet
- Rubbing alcohol (70% isopropyl alcohol or 99% isopropyl alcohol)
- Pencils, popsicle sticks, or drinking straws
- Scissors
- Ruler
- Tape
- Green leaves
- Leaves that have changed color from green
- Covers for jars, aluminum foil, or plastic wrap
- Shallow pan or tray
- GLOBE Science Log
- Hand lens (optional)
- Mortar and pestle for grinding leaves (optional)

Preparation

None

Prerequisites

ADULT SUPERVISION IS REQUIRED when using isopropyl alcohol. Please read all instructions completely before starting.

Observe all safety precautions.

Background

Chromatography is one of the procedures commonly used in many fields of science and industry to separate and identify substances within a mixture. A *chromatogram* is the separation pattern produced by each different mixture. The mixture is placed onto a medium such as paper or chalk, which absorbs water, alcohol or other solvents. As water or alcohol moves up by *capillary action* on the paper, molecules or substances dissolved in the mixture will travel at different speeds. The water or other solvent moves up the paper because capillary action is stronger than gravity and capillary action depends on *cohesion* and *adhesion*. Cohesion is the mutual attraction of water molecules and adhesion is the attraction of water molecules to other kinds of molecules, in this case paper. Various colors will appear at certain distances from their starting point because molecules of these colors have different sizes, shapes and solubilities. Molecules that dissolve better in the solvent will move along the paper more easily and quickly and will travel the greatest distance. Other molecules are not able to move as quickly and are left behind. By using paper chromatography, the different colors that make up black ink, and pigments in leaves can be separated and made visible.

Pigments are colorful compounds that absorb light. Pigment structure and amount determine variations in color. The pigment chlorophyll in leaves helps make photosynthesis happen by absorbing from sunlight the energy needed for putting together carbon dioxide and water to form glucose or food. Chlorophyll gives plants their green color and may hide the other pigments present in leaves. Chlorophyll absorbs all colors of visible light except green, which it reflects to be detected by our eyes. If all colors or wavelengths of visible light are absorbed and none are reflected, the pigment appears black to our eyes. Conversely, if all wavelengths are reflected, the pigment appears white to our eyes.

In autumn, changes occur in deciduous plant leaves before they finally fall from the branch. Chlorophyll breaks down and leaves change color when water and sap stop flowing into the leaves. As the green fades, orange color from the pigment *carotene*, and yellow color from the pigment *xanthophyll* appear. These pigments are also found in foods like carrots, bananas and egg yolks. Carotene and xanthophyll are secondary pigments that support the photosynthetic process by passing their absorbed light energy to chlorophyll. Further chemical changes in the leaves stimulate production of *anthocyanin* pigments giving bright red and purple colors. These are also commonly found in plants such as beets, red apples, and purple grapes, and flowers like violets and hyacinths. In the leaves, these pigments are formed in the autumn from trapped glucose. Different mixtures of chlorophyll and other pigments in the leaf give a wide range of autumn colors. Brown colors come from *tannin*, a bitter waste product. It is important to remember that the key photosynthetic pigment is chlorophyll because the light energy it absorbs is directly used for photosynthesis while the other pigments have to pass the light energy they absorbed, to chlorophyll.

The following explorations can be conducted by individual students or teams of students.

What To Do And How To Do It

Getting Ready

1. Write some words with a black (water soluble, non-Crayola) felt tipped pen on a piece of white paper. Ask students to make observations. How many colors do they see?
2. Ask students if they know what pigments are and what they do.
3. Ask students if they think there may be other colors hidden in the black ink and why they think so. If they seem at a loss for an answer, you could ask what colors do they think they need to make the ink in the black pen. Have they ever tried mixing different colors of paint? What did they observe? Discuss.
4. Ask students how they think they can find out if pigments other than black exist in the black ink.
5. Ask students what they think would happen if a dot of black ink were placed on a strip of coffee filter paper or paper towel and then the tip of the strip was placed in water?

Exploration 1

Introductory Learning Activity for Middle/ Secondary School Students

(This activity may also be used for Primary School students)

Separation of Colors in Black Ink

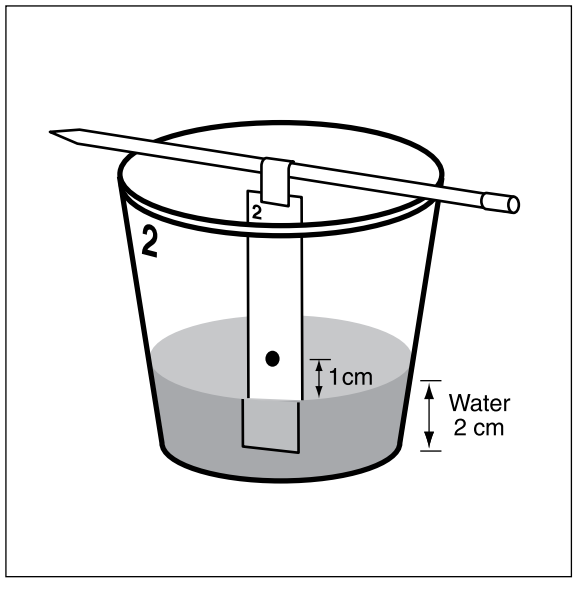
Have the students do the following steps or if you are trying to save some time, you may want to do steps 1-4 ahead for the students and use only one type of pen with water soluble black ink.

1. Cut several coffee filters into strips (2 cm wide by 15 cm long), one strip per pen.
2. Tape a number to each pen.
3. Using one of the test pens, place a dot of ink near the bottom (about $1^1/_2$ cm- 2 cm from the end) of each strip. Use a pencil to label the test strips at the top, with the number of the pen used to make a dot.
4. Put water (about 2 cm depth) in a glass or plastic container also labeled with the number of pen used to make ink dot. See Figure EA-P5-1.
5. Tape the end of the strip to a pencil or popsicle stick. Adjust the length of the filter paper by rotating the pencil so that the ink dot is 1 cm above the water level when the paper strip is put in the water by laying the pencil/stick across the top of each container. (If the ink dot is put below the water level, the ink will leach into the water instead of traveling up the strip)
6. Allow the water to travel about three quarters of the way up the strip and watch what happens to the ink dot.
7. If the ink you are using does not spread out, with adult supervision, re-test using rubbing alcohol instead of water as the solvent.
8. Repeat this process for each strip marked with a different pen and compare your results.
9. Let the strips dry and tape them to a sheet of paper as a record of different pen types.
10. Examine the strips with a hand lens. Measure how far the color(s) have travelled from the ink dot (for older students).

Generalize

- Ask students for their observations.
- Ask students for ideas about why other colors were not visible initially. Ask them for ideas about what might be happening (Why did some colors travel higher up the strip while others didn't? What do the results tell them about the makeup of the ink in the pen/pens?)
- If different pens are used, ask them what the similarities or differences in color patterns produced from different pens are and why they think this might be.
- Why it is that when all the colors are combined as in the pen or marker ink, it appeared black to our eyes? (Do not include this question if you think it is inappropriate for the grade level.)

Figure EA-P5-1: Paper Chromatography Setup



Formative Assessment for Introductory Exploration.

Science Log Entry

Have students write and draw about:

- Their observations (What happened to the ink dot when water traveled up the paper strips?).
- Why they think what they observed occurred (why some colors traveled higher up the strip while others didn't).
- How the patterns of color separation are different on strips marked with different pens and why this might be.
- Why it is that when all the colors are combined as in the pen or marker ink, it appeared black to our eyes. (Do not include this question if you think it is inappropriate for the grade level)

Skills of Science Checklist

Use the checklist during the lesson to document students' skill abilities in the processes of science.

Getting Ready for Color/Pigment Separation in Leaves

1. Ask students why they think leaves are green. (See if they come up with the idea of chlorophyll and how it absorbs all colors or wavelengths of visible light except green which it reflects)
2. Ask students what they think autumn leaves and ripening bananas might have in common.
3. Ask students whether they think other pigments besides chlorophyll exist in leaves. Why do they think so? How can they demonstrate this?

Exploration 2

Separate Colors in Green Leaves Using Chromatography

Green leaves may be collected from vegetation that dramatically changes color during senescence. For example, sugar maple leaves turn brilliant yellow in the autumn while white oak leaves turn dull brown. Thus sugar maple leaves would be a better choice than white oak for the experiment. And birch leaves would be better than alder leaves. However, students may not know which leaves change color dramatically in the fall. They may learn just as much if they choose green leaves from any vegetation. Have student teams do the following steps.

1. Collect 2-3 large fresh green leaves. Note location of plant and if you know it, also the plant genus.
2. Tear or cut up the green leaves into as small as possible pieces. Place the leaf fragments in a glass or plastic container.
3. Label the container with a number or name of leaf if known and location of the plant.
4. Add enough rubbing alcohol to cover the leaf fragments. Using a plastic spoon, carefully but vigorously stir the leaves in the alcohol (leaves can also be ground with a mortar and pestle if available).
   Safety Note: Isopropyl rubbing alcohol can be harmful if mishandled or misused. Read and carefully follow all warnings on the alcohol bottle. Supervise students closely.
5. Cover the container very loosely with lids or plastic wrap or aluminum foil. Place the containers carefully into a shallow tray containing 1 inch of hot tap water.
   Safety Note: Hot water above 66˚ C can quickly cause severe burns.
6. Keep the jars/containers in the tray of water for at least a half-hour, longer if needed, until the alcohol has become colored (the darker the better). Twirl each jar gently about every five minutes. Replace the hot water in the water tray if it cools off. Covered containers or jars may be kept overnight and contents used for chromatography afterwards.
7. Cut a long thin strip (2 cm wide by 15 cm) of coffee filter paper or chromatography paper for each of the containers and label it with a leaf name or code.
8. Remove containers from the water tray and uncover. Tape a strip of filter paper or chromatography paper to a pencil. Lay the pencil across the top of each glass/plastic container. Adjust the length of the filter paper by rotating the pencil so that the end of the paper strip

just touches the alcohol. The alcohol will travel up the paper, bringing the colors with it.

9. After 30-90 minutes (or longer) or after alcohol has traveled three quarters of the way up the strip, the colors will travel different distances up the paper. Different shades of green, and possibly some yellow, orange or red, depending on the type of leaf, may be seen on the chromatogram. Remove the paper strip and put on top of a paper towel to dry, then tape to a piece of plain white paper.
10. Examine the strips with a hand lens. Measure the distance(s) that the color(s) has/have traveled up the strip of paper. Save for the paper strip to compare results from the next experiment (Exploration 3).

Generalize

1. Ask students to write and draw their observations, and to share and discuss their findings with the whole group.
2. Ask them what they think the reason is for the way some colors traveled higher up the strip while others didn't? (Molecules of colors with bigger size and more adhesion, would travel shorter distances than those which are smaller in size and less adhesion).
3. Ask students what they think can be inferred regarding what pigment molecules are represented on the strip? (The different pigment molecules present in the leaves are represented by the colors shown on the strip: green for the chlorophyll pigment, yellow for the xanthophyll, orange for carotene).
4. Ask students what can be inferred if green is the dominant color present on the strip (Chlorophyll is the main photosynthetic pigment which gives leaves the green color. Other pigments may dominate when chlorophyll levels decrease.)
5. Ask students what accessory photosynthetic pigments are. If they can't remember, ask them how they think leaves are similar to bananas and egg yolks? (They contain carotene and xanthophyll which give them the orange/yellow color.) If appropriate for the grade level, ask them why some pigments are designated as accessory? (Chlorophyll is the key photosynthetic pigment because it directly transfers the light energy it absorbs for photosynthesis. Xanthophyll and carotene, examples of accessory pigments, must pass the energy they absorb from sunlight to chlorophyll and not directly to the photosynthetic pathway.)

Formative Assessment

Science Log Entry

Have students write and draw in their science logs about:

- What their observations are. (What happened when the alcohol/ground-up-leaves mixture traveled up the filter paper?)
- Why they think what they observed occurred.
- Ask students to infer what pigment molecules might be present in the leaves based on the colors on the strip.
- What accessory pigments in leaves are and why they think these pigments are important.

Skills of Science Assessment Checklist

During the lesson/exploration, use the checklist to document students' skill abilities in the processes of science.

Exploration 3

Separate Colors in Fall Leaves Using Chromatography

- Repeat steps 1 through 8 from Exploration 2, this time using leaves that have changed color.
- You may have to wait much longer in steps 4 and 7.
- Compare strips with those obtained from Exploration 2. Write and draw their observations and comparisons, share and discuss with the whole group.

Investigating Leaf Pigments
Skills of Science Assessment Checklist

Criteria	Student Names							
Correctly follows instructions and steps in procedure to set up experiment and gather information								
Observes carefully								
Records data and explanations for observations, experiments, in science log								
Identifies similarities and differences in patterns of color separation								
Infers reasonable causes for variations in data obtained								
Verbal communication of understandings during generalization discussions, and brainstorming								

Generalize

1. Ask students what they observed when the alcohol/ground-up-leaves mixture traveled up the filter paper. Have them compare the processed strips or chromatograms to those from Exploration 2. Ask students to write and draw their observations, similarities and differences with results from Exploration 2, share and discuss with the whole group.
2. Ask them what they think the reason is for the way some colors travelled higher up the strip while others didn't? (Larger, more adhesive molecules travel shorter distances than smaller, less adhesive molecules if solubilities are the same).
3. Ask students what they think can be inferred regarding what pigment molecules are represented on the strip? (The different pigment molecules present in the leaves are represented by the colors shown on the strip: green for the chlorophyll pigment, yellow for the xanthophyll, orange for carotene and bright red or purple for anthocyanins).
4. Ask them what can be inferred from the presence of the dominant color present on the strip. (Chlorophyll is the main photosynthetic pigment usually present in high quantities in green leaves. Other pigments may dominate when chlorophyll levels decrease.)
5. Ask them if they know what accessory photosynthetic pigments are. (Chlorophyll is a photosynthetic pigment. Xanthophyll and carotene are examples of accessory pigments designated as accessory because they cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll.

Formative Assessment

Science Log Entry

Have students write and draw their observations in their science logs about:

- Their observations. (What happened when the alcohol/ground-up-leaves mixture traveled up the filter paper?)
- Why they think what they observed occurred.
- Ask students to infer what pigment molecules might be present in the leaves based on the colors on the strip.
- What accessory pigments are and why they think these pigments are important .
- What the similarities and differences are in the patterns of color separation on strips with alcohol extracts of green leaves to strips with extracts from leaves that have changed color.

Skills of Science Assessment Checklist

During the lesson/exploration, use the checklist to document students' skill abilities in the processes of science.

Final Assessment

Science Log Entry

Have students: 1) discuss their understandings of chromatography and its importance, 2) discuss their understandings of pigments and their importance including their importance to leaves, and, 3) how their observations are similar or different from chromatography of green leaves extract with that from leaves that have turned color (non-green leaves)

Performance Task

Have students conduct their own inquiry on materials not previously explored such as different color pens, mixtures of food color or cake décor color pastes, colored candies, and, different kinds of natural and non-toxic chemical dyes by predicting what will happen, performing chromatography, reporting their results and analysis.

Use the rubric below to score the final GLOBE Science Log entry and the performance task.

Criteria	Developing	Proficient	Exemplary
Discussion of chromatography and its importance	Discussion shows lack of thorough understanding of chromatography and its value	Discussion shows thorough understanding of chromatography and its importance	Discussion shows thorough understanding of chromatography and its importance and show ability to apply to new situations
Discussion of pigments and their importance (including to leaves)	Discussion shows lack of thorough understanding of pigments and their importance	Discussion shows thorough understanding of pigments and their importance in leaf photosynthesis	Discussion shows thorough understanding of pigments and their importance in leaf photosynthesis, and show ability to connect to plant chemistryin general