Paper Chromatography of Leaf Pigments

Purpose

In this exercise, you will be analyzing leaf pigments (i.e., the primary photosynthetic organ of most land plants) to determine which are present in a leaf.

Scientific Question

You will answer the question: "Do green leaves also contain other pigments that are not apparent to the human eye?"

Background

You are undoubtedly aware from personal experience that a small amount of fluid on the edge of a paper towel (or homework paper!) will spread and may eventually soak the paper. This occurs because the paper has tiny pores (spaces) between its fibers, and the fluid is drawn up into the spaces by a process called **capillary motion** or, more simply, "wicking." As a biological reference, this is also how plants pull up from their roots to their leaves.

Paper chromatography is a method that makes use of capillary motion to separate and identify different substances, like leaf pigments, in a mixture. It works because the pigments dissolve in fluid (alcohol) and are then carried along with the alcohol as it moves through the paper. However, filter paper is designed so that fluid can move through it, but particles (the pigments) will become trapped. Since particles can be large or small, they can only move through pores in the paper that are at least their size. This result is that smaller, lighter particles move through the filter paper more easily than larger, heavier particles, which will get stuck. Thus, smaller particles will move further in a given amount of time while the larger particles are left behind. As a result, light and heavy (small and large) pigments become separated on the filter paper and are then visible to the naked eye.

Coffee filters make excellent substitutes for high-grade filter paper!

If you want to see this effect (and make sure your chromatography works), you can follow the instructions below, but instead of rubbing a leaf over the line, draw a line with a black Sharpie marker over your pencil line. Is black ink black? (NOTE: This is not part of the actual assignment but is a cool experiment if you have extra filter paper.)

Photosynthetic Pigments

Leaves are the primary site of photosynthesis in algae and land plants. As you have learned in this course, photosynthesis's "photo" step involves converting energy from sunlight into chemical forms of energy. This chemical energy is ultimately used to "fix" carbon dioxide into sugars during photosynthesis's "synthesis" step.

Both algae and land plants contain **chlorophyll** molecules, the green pigments characteristic of leaves, to absorb and reflect much of the light energy required for photosynthesis. In addition to chlorophyll, accessory pigments also absorb and reflect light energy in photosynthetic organisms. The accessory pigments of algae include **phycobilins**, which are typically purple.

Accessory photosynthetic pigments in land plants include **carotenoids**. Carotenoids include **carotenes**, ranging from red to orange, and **xanthophylls**, which are yellow. Non-photosynthetic pigments (typically acting as antioxidants) include **anthocyanins**, appearing as red to blue to purple.

There are also red/yellow pigments called **betalains**, which appear to be involved in an anti-fungal activity (not photosynthesis).

Hypothesis

Formulate a hypothesis that:

1. addresses the question as to whether plants contain pigments other than those that are green in color, and
2. that can be answered experimentally with paper chromatography.

Null Hypothesis

How would you phrase the above hypothesis as a **null** (= no difference)?

Materials and Methods

- Isopropyl alcohol (i.e., "rubbing alcohol")
- Green leaf
- Filter paper (coffee filter)
- Coin
- Tall glass or clear plastic cup or jar. (Opaque cups can work as well, but it is harder to see your set-up & progress, so you'll need to be careful.)
- Ruler
- Scissors
- Pencil
- Tape
- Digital Camera/cell phone camera etc.
- Pencil (do not use a pen!)

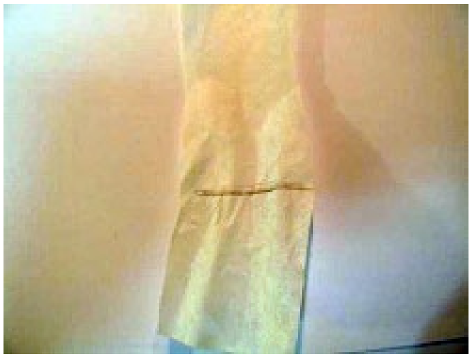
Step 1

Cut a strip from your coffee-filter paper that is ~ 2 cm longer than the height of your glass, and that is thin enough to fit into your glass. This strip should be at least 10 cm. long – if that's taller than your glass, you'll need to find a bigger glass!



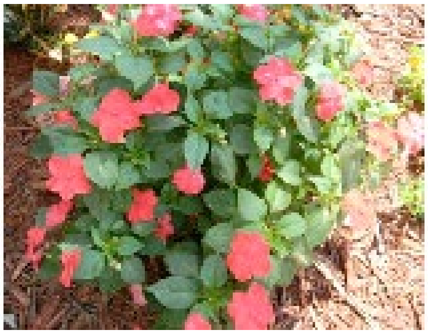
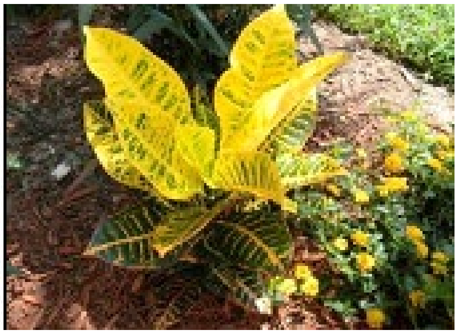

Step 2

Use a ruler to measure and lightly draw a pencil line ~ 2 cm above the **bottom** of the paper strip.



Step 3

Pluck some leaves from a green plant (non-woody) growing near your home. Most houseplants work very well, as does fresh spinach or grass – essentially, you want something that you can get a good amount of moisture/pigment from. Leaves that are dry, thick, or have a heavy waxy coating (like live oak leaves) generally don't work well. You can try several different plants if you want.

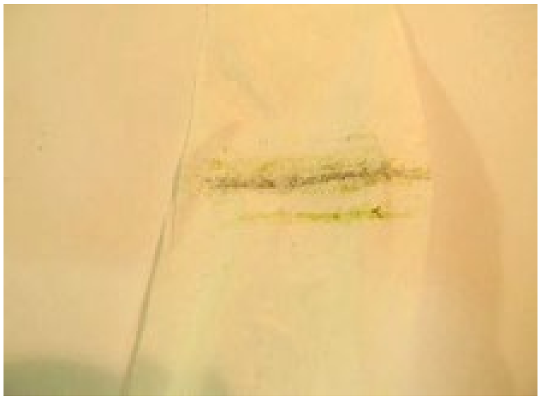




Step 4

Wrap the leaf around a coin with the top side of the leaf facing **outward**.


Step 5

Repeatedly rub the leaf along the pencil line on the paper strip until you make a dark green line (or roll the bumpy edge of the quarter along with the leaf, pushing down hard). **Try not to rub the leaf above or below the line** (in the picture below I did rub below the line… try not to do that!!). Don't rub too hard, or you'll tear the leaf and the paper.

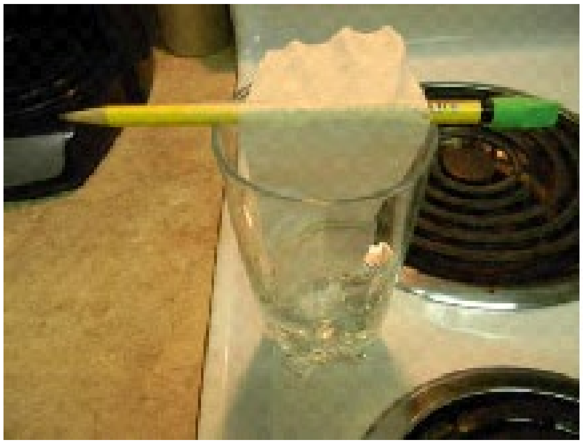


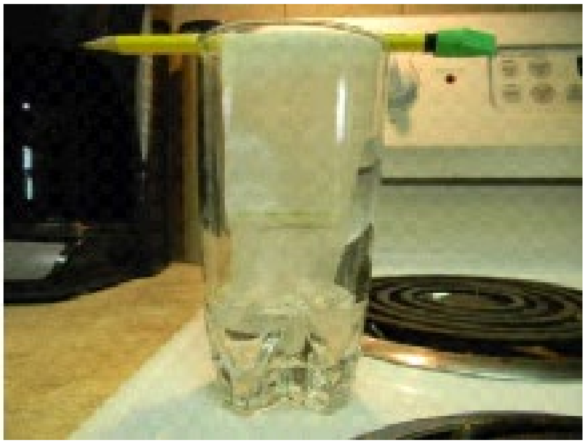
Step 6

Write your name (in pencil) along the bottom edge of your filter paper. Place filter paper with a green line on a white sheet of paper and take a photograph (as shown here).

Step 7

Tape the **top** of the paper strip to a pencil so that the end of the strip with the green line hangs down at the **bottom**. The easy way to do this is to place the paper in the cup, lay a pencil across the top, and then tape the paper to the pencil. The pencil should be able to sit across the top of the beaker with the **bottom** of the paper strip almost touching the bottom of the beaker/substitute. **If you must trim the paper, cut at the top. Do not cut the bottom of the strip with the green line.**



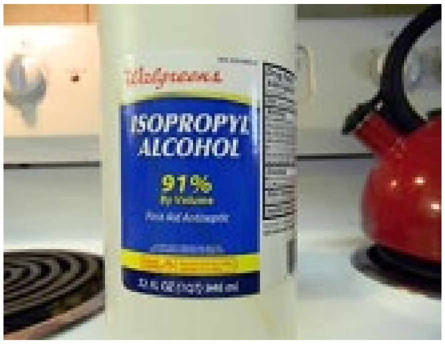


Step 8

Remove the pencil/paper strip from the beaker.

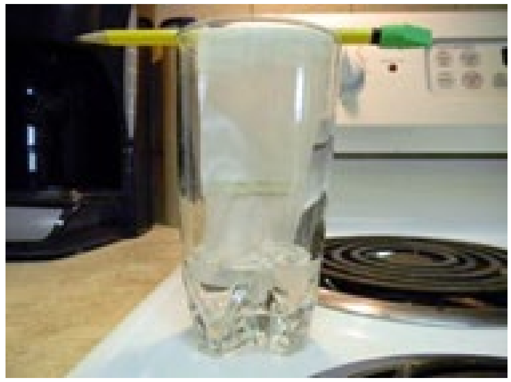
Step 9

Carefully add isopropyl alcohol to the beaker until it reaches a depth of ~1 cm.



Step 10

Lay the pencil back across the top of the beaker with the paper strip extending into the alcohol. **Make sure that the level of the alcohol is touching the bottom of the paper but is still below the green line on your paper strip. If it appears that the alcohol will cover the green line, pour out some alcohol before you get the green line wet.**

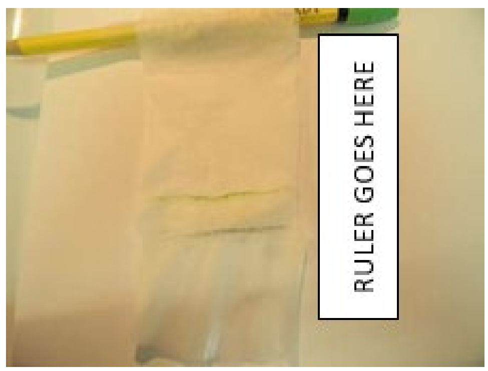


Step 11

Observe as the alcohol gets absorbed and travels up the paper. Wait until the alcohol has traveled **at least 5-6 cm** past the pencil line but has not yet reached the tape/top of the paper. This should take 20-30 minutes. Do not touch your experiment during this time.

Step 12

Remove filter paper from the glass. Draw a line in pencil that marks the furthest distance the alcohol traveled (so you can measure once it's dried.) Once it's dried, place filter paper on a white sheet of paper **with your ruler to measure the distance from the starting line to the ending line** and take another photograph.



Step 13

Use your ruler to measure the total distance of how far the alcohol traveled from the starting pencil line to the ending pencil line.

Measure how far each pigment band traveled from the starting line (e.g., a pigment that stayed on the starting line would have traveled 0 cm.) Calculate what percentage of the total possible distance each pigment traveled.