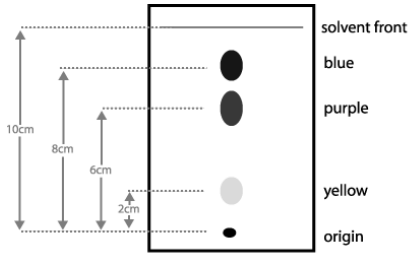
WLHS / Biology / Monson

Name_____
Date_____Per_____

LAB: Plant Pigment Chromatography



BACKGROUND:

Photosynthesis begins when light is absorbed by pigments in the plant cell. One technique for separating and identifying these pigments is **paper chromatography**. In paper chromatography, solvent moves up the paper carrying with it dissolved substances - in this case, plant pigments. The pigments are carried along at different rates because they are not equally soluble in the solvent and are attracted in different degrees to the paper.

Many green leaves contain pigment colors that are not seen until autumn because they are hidden by the chlorophyll. A few plants have leaves that are red, orange, or yellow all year long.

In this investigation, you will use paper chromatography to determine what differences exist in the plant pigments of various colors of leaves. You will also determine which leaves or which parts of leaves contain the chlorophyll necessary to carry out photosynthesis.

PRELAB QUESTIONS:

1) What are the requirements for photosynthesis to occur?_____

_____

2) Why do some trees appear green during the spring and summer, but then red / orange / yellow in the fall?_____

3) What are two factors that cause pigments to move at different rates during paper chromatography?

_____ & _____

Problem / research question: Which plant pigments can be found in different colored leaves?

Materials:

2 pieces of filter paper	coin	pencil fresh spinach leaf
1 - 150 mL beaker	ruler	red leaf such as *coleus* leaf
1 glass plate (cover for beaker)	scissors	70% isopropyl alcohol (rubbing alcohol)

Procedure:

1) Make two filter- paper rectangles that are each approximately 10 cm by 3 cm. Using a **pencil**, draw a base line 1.5 cm from the bottom of the long side of each rectangle.

2) Place a spinach leaf over the pencil line on one of the rectangles. Roll the coin over the leaf so that a horizontal green line is transferred to the pencil line. Repeat this step with the red leaf and second filter-paper rectangle.
*****NOTE: you can't have too much pigment! Make sure your line of pigment is dark!!***

3) Add just enough isopropyl alcohol to the beaker to cover the bottom. **Do NOT add more than 1 cm** to ensure that the pigment line will not be submerged when the paper is lowered into the beaker.
CAUTION: avoid inhaling the alcohol.

4) Lower each paper rectangle into the beaker containing alcohol. The solvent will begin to move up the paper and cause the pigments to move as well.

5) Cover the beaker with a glass plate. Do not disturb the beaker for approximately 15 minutes, or until the solvent is about 1 cm from the top of the paper.

6) When the solvent is about 1 cm from the top of the paper, remove the paper and mark the farthest point of the solvent's progress (front line) **with your pencil before this line evaporates.**

7) Allow the filter-paper strips to dry, and then **make a sketch of each chromatographs**. Some possible colors and the pigments they represent are:

faint yellow / orange - carotenes
yellow - xanthophyll
blue green - chlorophyll a
olive green - chlorophyll b
red - anthocyanin

(see sample chromatogram diagram on the last page for possible sequence results)

Observations: DATA TABLES

TABLE 1: SPINACH LEAF

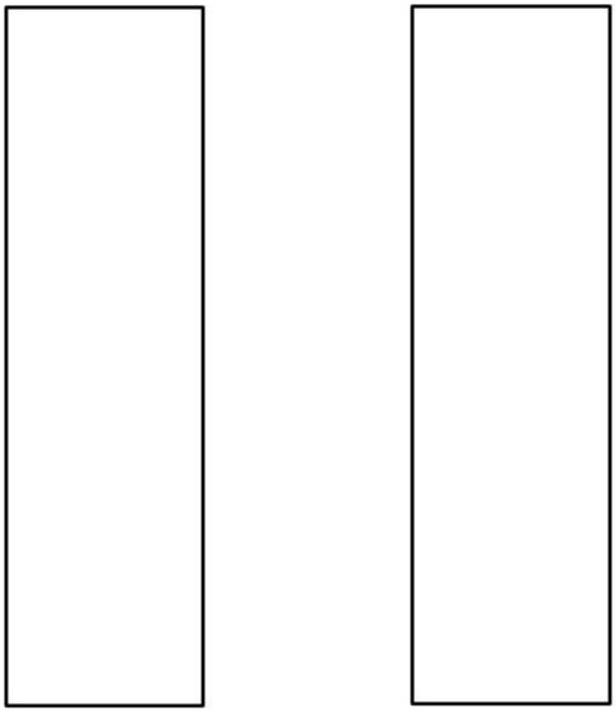
LINE / PIGMENT:	**Distance from bottom of paper (cm)**	**Color Observed:**	**Probable pigment:**
Pigment origin	1.5 cm	NA (not applicable)	NA
1 (closest to bottom)			
2			
3			
4			
5 (front line)			
Solvent front (mark with your pencil)		NA	NA

TABLE 2: RED LEAF

LINE / PIGMENT:	**Distance from bottom of paper (cm)**	**Color Observed:**	**Probable pigment:**
Pigment origin	1.5 cm	NA (not applicable)	NA
1 (closest to bottom)			
2			
3			
4			
5 (front line)			
Solvent front (mark with your pencil)		NA	NA

POST LAB QUESTIONS / ANALYSIS:

1) Sketch your two chromatograms in the image here. Label the colored lines with their distance traveled from the origin, and the likely identity of that pigment.



2) Why is chromatography useful?_____

3) Why are chlorophyll a & b green? (or, why do they appear green to us??)_____

4) Why do leaves change colors in the fall?_____

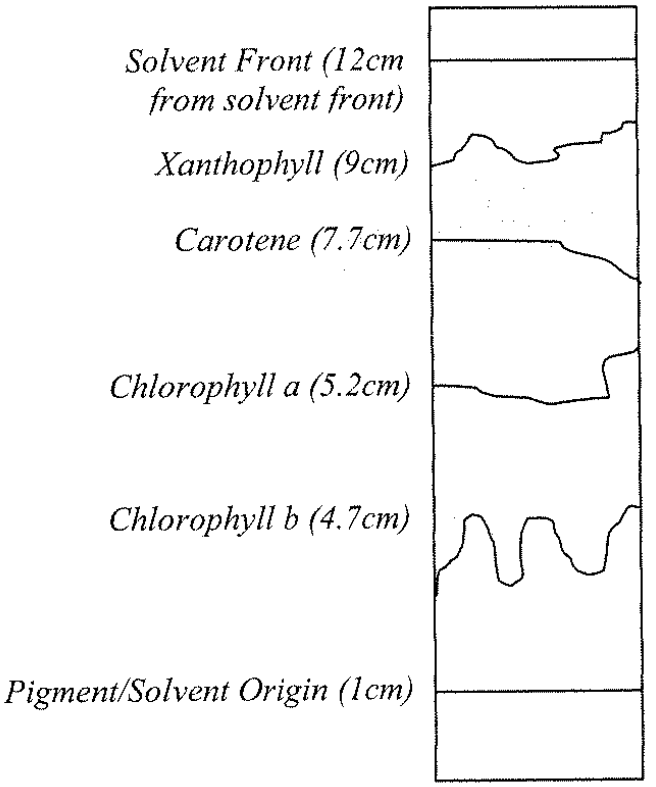
5) How could you predict the color a tree's leaves will turn in the fall?_____

6) What is the main function of the green pigments (chlorophyll a & b)?_____

7) What are some functions of the plant pigments that are NOT green?_____

(on to next page!)

8) Use the chromatogram shown here to complete the table below.



to calculate the R_f value:

R_f = distance moved by pigment from original spot / distance moved by solvent from original spot

	Chlorophyll a	Chlorophyll b	Xanthophyll	Carotene
Pigment / solvent origin				
Pigment front				
Solvent front				
R_f value				