Chromatography and Spectrophotometry Lab

PURPOSE: To extract pigments from a spinach leaf and analyze them separately for transmission and absorption of light at wavelengths from 380 to 720 nm.

GROUPS: Each lab station will prepare one chromatograph and analyze two pigments.

MATERIALS:

Spinach leaf
Chromatography paper
Scissors
Solvent (9:1, petroleum ether: acetone)
Chromatography tube w/ stopper
Spectrophotometer
Spectrophotometer tubes (perfectly clean)

PROCEDURES:

Day one: Chromatography - practice the placement of pigment on the chromatography paper with short pieces of paper.

Cut a strip of chromatography paper 2-3 cm longer than the tube, and cut a point on the end.
Roll a line of pigment (plant juice) 1 cm above the bevel.
Set it in solvent midway up the bevel.
Allow 10-15 min. for chromatography to happen.
Remove from tube, let dry and give it to Miss. Kirk.

Day two: Carefully separate the four pigment bands with scissors.
Collect all of each pigment in small, labeled beakers-DON'T MIX THEM UP.
Catch up on theory, and practice spectrophotometry with colored water.

Day three: Spectrophotometry

Add solvent to beakers, swirl for 4-5 min. to remove pigments.
Each station will analyze one or two pigments, using a blank to reset the machine between each reading.

Day four: Analysis

Combine data for all groups.
Subtract transmission from 100% to get absorption
Graph results.
Transpose color spectrum onto wavelength scale

Notes:

Roll quarter 4 times, allowing a few minutes for drying between each roll.
Spectrophotometry tubes must be absolutely clean.
Tubes are marked. Always position the tube so the marks line up.
Never touch the tubes except at the top.

VARIABLES:

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