DRAFT Trial Chlorophyll Test Method DRAFT

This is a new version of the lichen chlorophyll method. Since lichen samples aren't easily available, lawn cuttings will be used as substitutes. Please do not label these samples as "grass", as they will often go "up in smoke". There are different kinds of chlorophyll. Since lawn cuttings and lichen may not use the same chlorophyll, the wavelenths may not be the same( they should be similar). So the wavelengths specified in the original paper maybe off.

A couple of concerns. Centrifuge tubes are difficult to obtain, right now. Be sure there is a supply. If using plastic, make sure it does not dissolve in acetone. Also, some colored centrifuge tubes have the tube color dissolve in the test soloution-not good when doing a color determination.

Purpose-To develop a new method, compatible with "Pigment Extraction from lichens with DMSO and estimation of chlorophyll degradation" by R.Ronen, Margalith Galun ,Environmental and Experimental Botany, Volume 24, Issue 3, August 1984, Pages 239-245. The paper lists extracts in both DMSO and acetone. For safety reasons (acetone is not absorbed through the skin as DMSO is) acetone was chosen. Use care with acetone. It is flammable.

Equipment-

mortar and pestle

acetone

Refrigerator/freezer

MgCO3

Acetone

Coffee grinder(alternate)

Centrifuge/hand centrifuge

Visible spectrometer with light source

Glass cuvettes

Paper towels

Centrifuge tubes

Paper towels

Method-

1.Harvest lawn a good handfull of lawn cuttings. Store them, loosely wrapped in paper towel in a refrigerator, or preferably, in a freezer, overnight.

2. All of the next work is to be done in dim light. Grind about 2 g. of lawn cuttings with acetone in the presence of MgCO3 and store in the cold for 3 hours to ensure a more complete extraction. A mortar and pestle is great for this. The home versions should work well. If the mortar and pestle is small, try digging up a stopper to cover the pestle. Alternative (ALT)-Grind in cold coffee grinder with in the presence of MgCO3, cover with 5 mL of acetone, and store in the freezer for 3 hours. Be sure to clean coffee grinder well with soap and water and elbow grease, only.

3. Centifuge extract at 2000 g. If you have access to a centifuge, great. New ones, at some of the on line stores run about $70, although quality might be an issue. Alt- read "A 3D printed hand powered centrifuge for molecular biology" on BioRxiv. And there are several veersions of the hand centrifuge available commercially. Just a word of warning, it can take a while. The run is not complete until the solution is clear. The hand centrifuges handle about 2 mL of solution.

4.Here is where the spectrometers come into play. Standardize the instument in the visible to make sure the readings will be correct. If using a Public Lab spectrometer, or its equal, using a tungsten lamp ( i.e. a real light bulb) is recommended. These are still available, just at unusual powers. Acetone and polymer cuvettes may not mix! Use glass or quartz.

5.The exact procedures vary greatly from this point on, depending on the type of instrument used(double beam, single beam,etc.). First step, calibrate the spectrometer.

6. Take the reagent acetone (not the blank), and run the spectrum. For the Public Lab instrument, for a first shot, try 700nm to 550nm with the tungsten lamp. That may not be right, but its a first try.

7. Take the acetone blank and run the spectrum. Hopefully, it will be similar to the reagent acetone. If not, there maybe method issues to resolve.

8. Take the lawn cuttings sample and run the sample. Depending on the results, further delution may be needed.

Depending on the results, the exact wavelengths and extraction conditions needed for futher testing will be determined.