Soil Ecology Protocols

Materials

10 ml serological pipettes
15 ml culture tubes with caps
a 1-cc scoop
sterile water
Gram staining kit
microscope slides (both flat & depression)
cover slips
sterile cotton swabs
nutrient agar plates
methyl green stain
distilled water
(soil nutrient test kit–optional)
empty petri dishes
balance
disposable dropper or pipettes
P200 micro-pipette with tips
light microscope
soil core samplers
plastic bags
Nylon mesh
Nytex mesh
Uhlig Extractors

A. Serial Dilutions for Bacteria

1. Use a clean, new transfer pipette to add 10 ml to a 15 ml culture tube. Label the tube "10^0.".
2. Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube "10^{-1}."
3. Repeat step 2 three more times to three additional 15 ml culture tubes, only label them "10^{-2}," "10^{-3}", and "10^{-4}" respectively.
4. Place 1 cc of your soil sample into the "10^0"culture tube.
5. Cap the tube and shake vigorously.
6. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^0" tube and place into the "10^{-1}" tube.
7. Cap and shake vigorously.
8. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the "10^{-1}" tube and place into the "10^{-2}" tube.
9. Cap and shake vigorously.
10. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the "10^{-2}" tube and place into the "10^{-3}" tube.
11. Cap and shake vigorously.
12. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the "10^{-3}" tube and place into the "10^{-4}" tube.
13. You should now have a total of five culture tubes.
14. Plate 100 µl samples from the 4^{th} and 5^{th} tubes (dilutions 10^{-3} & 10^{-4}) onto their own separate, labeled petri plates containing nutrient agar (NOTE: on your first sample, plate ALL 5 dilutions to determine which two dilution values will give you the best data; dilutions 10^{-3} & 10^{-4} are only the most probable ones).
15. Allow to grow for 48 to 72 hours.
16. Examine each of the plates for individual bacteria colonies and choose the plate

with the fewest colonies (but at least 5) to make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula:

Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$.

17. If there are not individual colonies but still a "lawn" at the 10^{-4} dilution, repeat the dilution adding a 5^{th} (10^{-5}) & 6^{th} (10^{-6}) dilutions, etc. as necessary until individual colonies are observed.

B. Serial Dilutions for Fungi

Follow the same steps as those in a bacterial dilution, except stop at the 10^{-2} dilution and plate all three samples (10^0, 10^{-1}, & 10^{-2}) on the appropriate media.

C. Protozoa Extraction

1. Place 15 cm sample of soil sample into the bottom of a clean, empty petri dish; and allow to dry completely.
2. Sift 9-10 g of the soil into a 2^{nd} clean petri dish using a 1 mm^2 nylon screen or mesh.
3. Add 20 ml of distilled water to saturate the soil
4. Cover the petri dish with its lid and allow to sit for 7 hours.
5. Place the soil sample in a modified Uhlig extractor containing 30 ml of distilled water for 24 hours.
6. Remove the filtrate and filter a 2^{nd} time using 12.5 cm qualitative filter paper.
7. Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide (1 µl = 1 drop from the capillary tube). Then using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of the 2^{nd} filtrate from step 6 to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.
8. Examine under a light microscope at 40X (for quantitative) or 100X (for qualitative) observations of the various protozoa living in the soil.
9. Use the following equation to determine the population density of protozoa in the soil sample:
   [(# per field of view at 40X) • (total ml of water used) • 747] ÷ (grams of sifted soil) = # of protozoa per gram of soil.

Soil Ecology Problems Handout

As part of the **Baltimore Ecosystem Study/Community Research Partnership Program**, our class is going to examine how the school's management of the campus is affecting one of the most critical components of any ecosystem: the soil. Specifically, we will look at the micro-organisms that inhabit the soil and determine the ecological "health" of our lawns, playing fields, and woodlands.

Below are a list of possible problems and research questions that your team can choose to pursue for your final project of the year. Your job is:

a. to pick one and narrow it down to your specific question;
b. learn the various research techniques for working with micro-organisms;
c. design an experiment or testable solution to your question and test your hypothesis.

You may use any of the materials in the classroom or outside of it, but be sure to keep a list of what you use and ask the teacher's permission. You may use any sources of information your team needs (web pages, encyclopedias, newspapers, textbooks, scientists, teachers - anything), and if your need help contacting someone, your teacher will help. You will be graded on the creativity of your project and on your ability to use the scientific method. The success or failure of your experiment or test will NOT affect your grade, but your ability to interpret the results of your experiment and to share what you did will.

Good luck!

1. What is the population density of micro-organisms in the soil in various areas of the campus?

2. What is the population density of micro-organisms at different soil depths?

3. What is the level of biodiversity of micro-organisms in the soil in various areas of the campus?

4. What is the level of biodiversity of micro-organisms at different soil depths?

5. What is the relationship between the soil chemistry and any of the above:
   a. pop. density vs. location
   b. pop. density vs. depth
   c. biodiversity vs. location
   d. biodiversity vs. depth

6. What kinds of micro-organisms inhabit the soil at RPCS?

7. Does soil chemistry influence who lives where?