Soil Ecology Protocols

Materials

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

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 6, 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 6, 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 6, 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 4th and 5th 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 5th (10-5) & 6th (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 (Density)

1. Place 15 cm sample of soil into the bottom of a clean, empty petri dish; and allow to dry completely.
2. Using a 1 mm^2 nylon screen or mesh, sift 9-10 g of the soil into a 2nd clean petri dish.
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 2nd 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 2nd 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.

D. Protozoa Extraction (Diversity)

Use steps 1 thru 6 for the density protocol to extract the protozoa from the soil. Then replace steps 6-9 above with the following procedure:

1. Using a capillary tube, deposit 5 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 second filtrate (from step 5 above) to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.

2. Examine under a light microscope at 100X for 9 different fields of view, looking for various protozoa living in the soil.

3. Record the number of the following types of protozoa in each field of view: Mastigophora, Sarcodina, Sporozoa, Ciliophora, and Amoebas. Protozoa will appear translucent and slightly bluer than their surroundings. Make sure to adjust the light so that the maximum number of protozoa are visible, and if needed, use a counter.

    a. **Mastigiophora** are flagellates. They are often smaller than other types of protozoa, with a body that is pointed at one end where the flagellum, or tail, is attached. They are usually tear-drop shaped and have no visible internal organelles at this magnification.

    b. **Sporozoa** can have many shapes, but their defining characteristic are the many organelles that are visible as darker blobs and flecks inside their bodies. These are relatively rare in soil; do not be surprised if none are observed.

    c. **Ciliophora** are often large in comparison to other types of protozoa, with oblong bodies that are pointed at both ends. Their tiny cilia will give the sides of their bodies a fuzzy appearance. Also, organelles are not readily visible in these at this magnification.

    d. **Shelled Amoeba** have spherical bodies with numerous spines protruding in all directions. Their circular shape and thick, highly visible shells & spines make them stand out.

    e. **Unshelled Amoeba** are amorphous, with none of the defining characteristics of the above protozoa. They can appear as light misty blue shapes, and may vary greatly in size. Sometimes a dark nucleus is visible within the amoeba, but the outer membrane will appear quite amorphous at best.