Materials Needed Per Class for Bacteria Serial Dilutions:

600 ml sterile water
24 microcentrifuge tubes
48 15–ml Culture Tubes
25 sheets of Aerobic bacteria 3M Petrifilm®
120 Graduated Beral-Type Disposable Pipettes (Graduated to 100 µl)
24 Plastic Sandwich/Freezer Bags
8 pairs of scissors
Test Tube Racks
permanent markers
soil test core auger(s)
1 biohazard bag
(soil nutrient test kit–optional)

Materials Needed Per Class for Yeast & Mold Serial Dilutions:

360 ml sterile water
24 microcentrifuge tubes
48 15–ml Culture Tubes
25 sheets of Yeast/Mold 3M Petrifilm®
120 Graduated Beral-Type Disposable Pipettes (Graduated to 100 µl)
24 Plastic Sandwich/Freezer Bags
8 pairs of scissors
Test Tube Racks
permanent markers
soil test core auger(s)
1 biohazard bag
(soil nutrient test kit–optional)

Protocol Instructions for Performing Serial Dilutions to Estimate Bacteria, Yeast, or Mold Population Densities in the Soil

A. Making Your Soil Scoop

Materials

1 microcentrifuge tube
permanent marker
pipette
scissors
water

1. Using one clean pipette transfer 0.5 ml of water into a microcentrifuge tube.
2. Cap the tube and turn it cap-side down and mark the water line with a permanent marker

(see figure 1).

3. Pour the water out and cut the microcentrifuge tube in half along the waterline mark using scissors (see figure 1). Note: Be sure to use sharp scissors.
4. Keep the end with the cap. This is your 0.5 cc soil scoop (see figure 1). Use with any of the instructions in Procedures B or C below. Just be sure to clean and rinse it each time between soil samples.

B. Performing a Serial Dilution to Count Bacteria

Materials For 1 Bacteria Serial Dilution for 1 Soil Sample:

25 ml sterile water
2 15–ml Culture Tubes
1 sheet of Aerobic bacteria 3M Petrifilm®
5 Graduated Beral-Type Disposable Pipettes (Graduated to 100 µl)
1 Permanent marker
1 Plastic Sandwich/Freezer Bag
2 Test Tube Racks

1. Collect 1Aerobic Bacteria 3M Petrifilm® plate and cut the plate into thirds lengthwise (see Figure 2).
2. At the top of the first third of a sheet, label it with the sample # and a dilution factor of 10^{-3}. Label the second third of a sheet with the sample # and a dilution factor of 10^{-4}. Reserve remaining third of sheet for any future dilutions. Set sheets aside for later plating.
3. Using a new, clean pipette, transfer 5 ml of sterile water into a 15 ml culture tube using the measurements on the side of the tube as a guide. Label this pipette with a permanent marker "Sterile Water" and set aside for future use.
4. Use the soil scoop to fill one level scoop with soil from the sample being tested. Invert contents and empty all of the soil into the 5 ml of sterile water in the first culture tube. This is the10^{0} diluted solution. Note: You may have to open the cap and use a clean glass stirring rod to push any remaining soil out. Be sure to wash and dry scoop and glass stirring rod between each sample.
5. In the second culture tube use the "Sterile Water" pipette from step 3 to fill the tube with 4.5 ml of sterile water (use the measurements on the side of the tube to measure 4.5 ml).
6. Cap the tube containing the 10^{0} diluted solution and shake it vigorously until the soil is evenly dispersed in the water.
7. Take a second, new, unused pipette and label it "Dilution Pipette" with the permanent marker. Then use it to remove 0.5 ml of the 10^{0} diluted solution from the first tube (see figure 3) and place it into the second tube (see figure 4). The second tube is now the 10^{-1} diluted solution.
8. Now empty the contents of the tube containing the 10^{0} diluted solution, and rinse THOROUGHLY (it is not necessary at this stage to wash with soap and water).
9. Once the tube is cleaned, use the "Sterile Water" pipette to add 4.5 ml of sterile water into this culture tube. This will become the tube containing the 10^{-2} diluted solution (see figure

5).

10. Using the "Dilution Pipette" from step 7, transfer 0.5 ml of the solution from the tube containing 10^{-1} diluted solution to the cleaned 10^{-2} dilution tube (see figure 4).
11. Now clean the contents of the 10^{-1} dilution tube as per directions in step 8.
12. Use the "Sterile Water Pipette" to place 4.5 ml of sterile water into the newly cleaned culture tube. This will become the tube containing the 10^{-3} diluted solution (see figure 5).
13. Shake the contents of the 10^{-2} dilution tube and using the "Dilution Pipette," transfer 0.5 ml of solution from the tube containing the 10^{-2} diluted solution into the 10^{-3} tube (see figure 4).
14. Now empty the contents of the 10^{-2} dilution tube as per directions in step 8.
15. Into the newly cleaned culture tube, place 4.5 ml of sterile water using the "Sterile Water" pipette. This will become the tube containing the 10^{-4} diluted solution (see figure 5).
16. Cap and shake the 10^{-3} dilution tube and then using the "Dilution Pipette, " transfer 0.5 ml of the solution from the tube containing the 10^{-3} diluted solution into the 10^{-4} dilution tube (see figure 4). These two tubes are your finally dilution tubes.
17. Stop the dilution process at this point and use a clean, new pipette to collect 100 µl of the solution in the 10^{-3} tube (see figure 3). Plate this sample on the designated third of a 3M Petrifilm® plate from step 2 by lifting the cover of the sheet and distributing the 100 µl in a series of small drops of the solution down the center of the sheet until all of the solution is out of the pipette (see figure 6).
18. Now lower the cover back down over the drops and press down on the sheet with a finger to distribute the solution across the plate (see figure 7). Note: Control the spread of the solution to keep within the edges of the sheet.
19. Next, repeat steps 17 & 18 with the 10^{-4} dilution tube.
20. Empty the 10^{-3} and 10^{-4} dilution tubes and wash and clean with soap and water thoroughly. BE SURE TO RINSE **VERY** THOROUGHLY TO REMOVE ANY SOAP. These tubes may now be reused for any other samples being tested.
21. Let 3M Petrifilm® plates sit for 48-72 hours.
22. Follow instructions provided with the 3M Petrifilm® plates to identify bacteria. Determine which sheet contains the most diluted sample on which can be found between 5 and 30 colonies. Record the number of colonies and the corresponding dilution level of that sheet on which they were found. Note: A magnifying glass may aid in this process (see figure 8).
23. To determine the density of any of these microbes, use the data from the most diluted sample in which 5 or more colonies were found in the following equation:

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

C. Performing a Serial Dilution to Count Yeast and/or Mold

Materials For 1 Yeast/Mold Serial Dilution for 1 Soil Sample:

15 ml sterile water for yeast/mold protocol
2 15−ml Culture Tubes
1 sheet of Yeast/Mold bacteria 3M Petrifilm®

5 Graduated Beral-Type Disposable Pipettes (Graduated to 100 µl)
1 Permanent marker
1 Plastic Sandwich/Freezer Bag
2 Test Tube Racks

1. Collect 1Yeast/Mold 3M Petrifilm® plate and cut the plate into thirds lengthwise (see Figure 2).
2. At the top of the first third of a sheet, label it with the sample # and a dilution factor of 10^0. Label the second third of a sheet with the sample # and a dilution factor of 10^{-1}. Label the last third of the sheet with the sample # and a dilution factor of 10^{-2}. Set sheets aside to use for plating in later steps.
3. Using a new, clean pipette, transfer 5 ml of sterile water into a 15 ml culture tube using the measurements on the side of the tube as a guide. Label this pipette with a permanent marker "Sterile Water" and set aside for future use.
4. Use the soil scoop to fill one level scoop with soil from the sample being tested. Invert contents and empty all of the soil into the 5 ml of sterile water in the first culture tube. This is the10^0 diluted solution. Note: You may have to open the cap and use a clean glass stirring rod to push any remaining soil out. Be sure to wash and dry scoop and glass stirring rod between each sample.
5. In the second culture tube use the "Sterile Water" pipette from step 3 to fill the tube with 4.5 ml of sterile water (use the measurements on the side of the tube to measure 4.5 ml).
6. Cap the tube containing the 10^0 diluted solution and shake it vigorously until the soil is evenly dispersed in the water.
7. Take a new, unused pipette and transfer 0.5 ml of the 10^0 diluted solution from the first tube (see figure 3) and place it into the second tube (see Figure 4). The second tube is now the 10^{-1} diluted solution.
8. Stop the dilution process at this point and use a clean, new pipette to collect 100 µl of the solution in the 10^0 (see Figure 3). Plate this sample on the designated third of a 3M Petrifilm® plate from step 2 by lifting the cover of the sheet and distributing the 100 µl in a series of small drops of the solution down the center of the sheet until all of the solution is out of the pipette (see Figure 6).
9. Now lower the cover back down over the drops and press down on the sheet with a finger to distribute the solution across the plate (see Figure 7). Note: Control the spread of the solution to keep within the edges of the sheet.
10. Next, empty the contents of the tube containing the 10^0 diluted solution, and rinse THOROUGHLY (it is not necessary at this stage to wash with soap and water).
11. Once the tube is cleaned, use the "Sterile Water"pipette to add 4.5 ml of sterile water into this culture tube. This will become the tube containing the 10^{-2} diluted solution (see Figure 5).
12. Using the "Dilution Pipette," transfer 0.5 ml of the solution from the tube containing 10^{-1} diluted solution to the cleaned 10^{-2} dilution tube (see figures 3 & 4 again).
13. Stop the dilution process again at this point and repeat steps 8 & 9 for the tubes containing the 10^{-1} diluted solution and the tube containing the 10^{-2} diluted solution. Make certain to plate each sample on the appropriate designated third of a 3M Petrifilm® plate from step 2

14. Empty and clean the 10^{-1} and 10^{-2} dilution tubes and wash and clean with soap and water thoroughly. BE SURE TO RINSE **VERY** THOROUGHLY TO REMOVE ANY SOAP. These tubes may now be reused for any other samples being tested.
15. Let 3M Petrifilm® plates sit for 48-72 hours.
16. Follow instructions provided with the 3M Petrifilm® plates to identify yeast and/or mold colonies on the plated sheets.
17. Determine which sheet contains the most diluted sample on which can be found between 5 and 30 colonies. Record the number of colonies and the corresponding dilution level of that sheet on which they were found. Note: A magnifying glass may aid in this process (see figure 9).
18. To determine the density of any of these microbes, use the data from the most diluted sample in which 5 or more colonies were found in the following equation:

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

Materials Needed Per Class for Protozoa Extraction:

1.2 L of distilled water
24 12x12–cm^2 pieces of 1 mm^2 nylon mesh
48 9oz (266 ml) plastic cups
24 3oz plastic bathroom cup
48 100x15 mm petri dishes
48 15.24x15.24–cm^2 pieces of nylon bridal veil
24 piece of 12.5 cm qualitative filter paper
24 capillary tubes
24 Graduated Beral-Type Disposable Pipettes (Graduated to 100 µl)
compound light microscopes
balances
funnels
ring stands
rings for ring stands
Methyl green stain
microscope slides
18x18 mm^2 cover slips
100 ml graduated cylinders
rubber bands
permanent markers
soil test core augur(s)
50–ml beakers

A. Making Your Uhlig Extractor

Materials

2 9oz (266 ml) plastic cups
2 15.24 x15.24 cm^2 pieces of nylon bridal veil
scissors

1. To make a modified Uhlig extractor, taking one of the two 9oz cups (Cup A) and cut the bottom out of it, including the first indentation of the side of the cup, as instructed in diagram 1A.
2. Next, take the other 9oz cup (Cup B) and cut out ONLY its bottom, and then make "legs" for it as instructed in diagram 1B.
3. Now, turn Cup A upside down and layer both pieces of the bridal veil together over the cup opening, making sure you align the mesh so that the lines of it do not cross each other.
4. Holding the nylon square(s) in place, insert Cup A into Cup B as shown in Figure 2. You now have a modified Uhlig extractor.

B. Instructions for Extracting and Counting Soil Protozoa

Materials for Protozoa Extraction:

balance	ring stand
compound light microscope	1 ring for ring stand
1 12x12–cm^2 piece of of 1–mm^2 nylon mesh	1 capillary tube
50 ml of distilled water	Methyl green stain
funnel	1 microscope slide
1 3oz plastic bathroom cup	1 18x18 mm^2 cover slip
2 100x15 mm petri dishes	100 ml graduated cylinder
1 Graduated Beral-Type Disposable Pipettes (Graduated to 100 µl)	1 piece of 12.5 cm qualitative filter paper
1 rubber band	50-ml beaker

1. Place a 2 cm diameter x15 cm long cylinder of soil into the bottom of a clean, empty petri dish, and allow to dry completely (usually 24 hours).
2. Next, place the soil in a 3-oz plastic cup (you may wish to use a clean mortar and pestle to help crush up the dirt; just be sure to wash and dry thoroughly between each sample to avoid contamination) and cover the top with the square piece of nylon mesh, using the rubber band to secure the mesh in place (see figure 3)
3. Using the balance, sift 9-10 g of the soil through the mesh into a 2^{nd} clean petri dish (see figure 4) and be sure to record the final amount of soil in your data chart.
4. Add 20 ml of distilled water to the sifted soil, cover the petri dish with its lid and allow to sit for at least 7 hours.

5. Place 30 ml of water into a clean, empty petri dish (you may use the washed and dried one from step 1) and set the Uhlig extractor upright in the water (see figure 5).
6. Scoop the rehydrated soil into the bottom of the Uhlig extractor and allow it to sit for 24 hours.
7. Remove the Uhlig extractor from the petri dish and set aside to clean according to your teacher's instructions. **If you are performing any chemical tests in conjunction with estimating the density of the protozoa, you MUST perform the test on the soil remaining in the Uhlig extractor at the same time as you perform steps 8–13.**
8. Set up a ring stand with its ring and a funnel (see figure 6), then fold the filter paper according to your teacher's instructions and place in the funnel.
9. Pour the filtrate in the petri dish that contained the Uhlig extractor into the funnel and filter this liquid into a clean, dry beaker. This liquid now contains the protozoa you will examine under the microscope.
10. Using a capillary tube, deposit 7 μl of methyl-green stain on a clean microscope slide (1 μµl = 1 drop from the capillary tube–see Figure 7).
11. Then using the pipette, add 18 μl (the first demarcation on the pipette–see figure 8) of the filtrate from the 50–ml beaker to the stain and cover with the cover slip.
12. Examine your slide under the microscope on the 40X power and count the number of protozoa you see (they will be the blue objects) and write this number in your data chart.
13. Finally, use the following equation to determine the population density of protozoa in your soil sample:

[(# per field of view at 40X) • (total ml of water used) • 747] ÷ (grams of sifted soil) = # of protozoa per gram of soil

Sample Data Table for Serial Dilutions

Column A	Column B	Column C	Column D	Column E
Location of soil sample	# of (bacteria, yeast, or mold) colonies found on Petrifilm Plate	Petrifilm Plate's Dilution #	#of (bacteria, yeast, or mold) in Dilution Tube (column B x 10^2)	# (bacteria, yeast, or mold) in 1 cc soil sample (Column D x $10^{\text{\| dilution \# at which these colonies were found\|}}$.)

Sample Data Chart for Protozoa

Column A	Column B	Column C	Column D (optional)	Column E
Location of Soil Sample	Grams of Soil Used	# of protozoa in field of view	Average # of protozoa in field of view	# of protozoa per gram of soil = [Column D x 50 ml x 747] ÷ Column B