SOIL MICROORGANISMS **#2100 Student Instructions**

There is a whole community of organisms that are too small to be seen, living in the soil beneath your feet. These are the soil microorganisms. In this kit, you will test a sample of soil to see what types of microorganisms it contains. You will observe the growth of these microbes both with the unaided eye, and with the help of a microscope. If you are not sure how to use the microscope, check with your teacher for instructions.

Microorganisms have a variety of requirements in order to grow. Some microbes may grow very well in conditions that would kill others, and vice versa. You will be preparing three different growth media to examine four different types of microbes: the *bacteria, fungi, algae,* and protists. More types of microorganisms inhabit the soil, but these four are the only types that will grow on the growth media you will be preparing.

Growing the Microorganisms

First you will need a sample of soil to test. Your instructor may have a particular sample for you , or may have you obtain your own soil sample. In either case, you should use soil from the top few inches of the ground. This is where the largest number of microbes live.

Place a teaspoon of a soil sample in a plastic sample cup labelled with your name. Examine the soil carefully. As you do, describe it here:

Answer example: There appear to be pieces of twigs, and bits of leaf There are also two small insects and what seems to be a bit of egg shell (Responses will, of course, vary on this item).

Obtain two culture tubes from your teacher, and label them with your name. These tubes may already contain a growth media; if not, follow the directions below:

Locate the container labelled "nutrient broth." The broth is very much like beef soup, and is used to grow one of the types of microorganism found in the soil, bacteria. Add nutrient broth to one of your culture tubes until the tube is about 2/3 full. Set the tube aside for a minute.

The other tube will contain a Jello-like material called agar. In this experiment, you'll be using a special agar to grow another type of soil microbe, fungi. The agar should be poured from its container while it is still liquid, and allowed to harden in the tube at a slant. If this has not been done for you, ask your teacher for assistance in carrying out this procedure.

Add enough water to the plastic cup containing your soil sample so that the cup is about 2/3 full. Swirl the container gently to mix the soil and water. Using a clean pipette, add a drop of the soil water to the tube containing the nutrient broth. Seal the tube, and swirl it gently to mix the contents. Set the rest of the soil and water mixture aside for the moment.

Sprinkle a small amount of dry soil on the agar in the slant tube. Seal the tube. Put both of your tubes in the storage racks indicated by your teacher.

Get a small piece of a leaf, and wash it in water. Rinse the leaf in alcohol to remove any organisms from its surface, then rinse it gently in water again. Put the leaf in the cup containing the soil water (if the leaf will not fit, tear it into smaller pieces), and place the cup in a location exposed to sunlight. Make sure that the cup is labelled with your name.

Bacteria

After 24 hours, observe the tube with the nutrient broth. Is it different in appearance from the start of the experiment?

What probably grew in the broth were bacteria from the soil. Bacteria are very small organisms that have some features of both plants and animals. Most bacteria are either rod-shaped (called bacillus) or round (called coccus).

We can see these two types of bacteria better by preparing a slide, staining it, and examining it with a microscope. Get a clean glass slide, an inoculating loop, methylene blue stain, a Bunsen burner or alcohol lamp, and a microscope. Pass the tip of the loop through the flame for about 10 seconds, then allow it to cool. Carefully open the tube of broth, and dip the loop into the broth. Transfer the small drop of liquid to a microscope slide, and spread the liquid around until it forms a thin film. Reseal the tube, and pass the loop through the flame again to kill any bacteria still on the wire.

After the film of liquid has dried on the slide, pass the slide, film side up, quickly through the flame to "fix" the film to the surface. Add a drop of methylene blue stain to the film, and let the stain sit on the slide for about 30 seconds.

Rinse the stain off the slide by swirling the slide in a beaker of tap water. Change the water and rinse again. Do not rinse the slide directly under running tap water. After rinsing the slide, gently blot it dry with a paper towel. Place the slide under the microscope objective and observe with both low and high power. Look for small, darkly stained rods and dots. Draw some of the organisms you see:

Some bacteria will produce a protective transparent capsule around themselves if their environment becomes hostile. Why might bacteria in you culture begin to produce capsules?

What do you think bacteria use for food in the soil? (There is, after all, no nutrient broth in the soil!)

After 48 hours, observe the broth tube again. Are there any changes, either in its appearance, or in the microorganisms which you can see with the microscope? Make a slide to find out.

There are more of the encapsulated bacteria, probably because the environment is more hostile to them now. There may also be more of the dot-shaped bacteria.

Fungi

Observe the slant tube containing the agar. Do you observe any growth after 24 hours? If so, describe its appearance.

Use a inoculating loop to remove a small piece of the growth which you observe, flaming the loop before and after, and place the material on a clean glass slide. Add a drop of water to the material, and spread it gently with the inoculating loop. Allow the film to dry. Pass the slide through a flame quickly, film side up to fix the film to the slide. Add a drop of methylene blue stain to the slide, and let the stain sit on the slide for about 30 seconds. Gently rinse the slide by swirling it in a beaker of tap water. Do not rinse the slide directly under the running water. After the slide is rinsed, blot it dry with a piece of paper towel.

Observe the slide under high power of the microscope. Make a drawing of what you see:

If there are different types of growth present on the agar, make up a separate slide for each colony. How do the different types of growth compare?

The fuzzy growth which you probably observed is called fungus. Some fungi (mushrooms) look rather like plants, but, unlike a plant, they cannot make their own food using sunlight. They must absorb nutrients from the soil, and are usually found growing in places that have many decaying leaves, or where an animal has died. The type of fungus which is present in the soil is referred to as mold. Have you ever seen this type of growth before?

Let the tube sit for another 24 hours, and observe it again at that time. How does it compare with your first observation?

If there are any new types of growth, make slide of them. How does the new growth compare with the first one observed?

The strands or fibers of material which make up the fuzzy mat of growth are called hyphae. They grow through the soil, absorbing nutrients. The hyphae of a single fungus body can cover many square feet. Sometimes you can see the hyphae in the soil if you gently turn over a small section of damp soil. The whitish material in the soil is probably the mass of hyphae from molds.

Algae and Protozoa

After it has sat in the sun for one or two days, get the sample cup with the soil and the piece of leaf. Obtain a clean glass slide, a cover slip, and a pipette. Using the pipette, remove a small amount of the water from near the surface of the leaf. Place the liquid on a clean glass slide. Add a cover slip, and observe the slide under both low and high power of the microscope. Make drawings of any objects which you think are living:

Were any of the organisms which you saw plants? How could you tell?

The greenish organisms are called algae. Algae are tiny, simple plants, producing their own food with the help of sunlight. Algae live in water, and most people think that algae live only in ponds and lakes, but there are many algae present in the soil, living in the thin films of water around the soil particles. Where do you think you would find the largest number of them in the soil?

You may also have noticed small, animal-like creatures swimming or moving around. These are called protozoans, and they also need to live in water. They feed on algae, on each other, and on decaying material in the soil. Where do you think you would find the most protozoa in the soil?

Let the soil water/leaf mixture sit for another day. Add more water if it appears to be drying out. Observe the culture again on the following day, using the same techniques. Are there any differences in the number and types of microscopic plants and animals which you can see?

Why do you think that algae and protozoans might be important to the soil?

Note: Some algae join *symbiotically* with fungi to from an organism called a lichen. These are often found on trees or rocks, and look like a thin crusty growth. They are important in breaking down rocks to form new soil.

Questions For Discussion

1) Why are most soil microorganisms found at or near the surface of the soil?

2) Do you think that you were able to culture all of the microorganisms which live in you soil sample? Why or why not?

3) Why are bacteria and fungi important to the soil?

4) If the soil can support such a wide variety of microscopic life, what can you say about the makeup of the soil?

5) Suppose that some air pollutant or pesticide were to kill off all the soil microorganisms in a particular area, but did not effect the other plants and animals. What might be the effect over a period of time?