Science First®

#2100 SOIL MICROORGANISMS INSTRUCTOR MATERIALS

This kit will provide up to 12 groups of two students of junior or senior high school students with an introduction to microorganisms which live in the soil. The lab procedures allow the observation of bacteria, fungus, algae, and protozoa, and will introduce proper laboratory and diagraming techniques. This kit focuses on the organisms that inhabit the soil, their role in recycling organic material, and how the microorganism population varies in different growth media and time. The observations of the above organisms require two to three days of growth, and should be observed each day to demonstrate the responses to a changing environment.

Contents:

Microscope Slides (72)
Cover Slips (100)
Inoculating Loops (5)
Plastic Cups (15 - 30 ml)
Culture Tubes (25)
Pipettes (15)
Isopropyl Alcohol (1 - 120 ml bottle)
Potato Dextrose Agar (10 grams)
Nutrient Broth (10 grams)
Methylene Blue Stain (1 - 60 ml bottle)
Labels (48)

Teacher Manual (1)
Student Instruction Master (1)

Additional Required Materials:

Microscopes (300X or higher)
400 ml Beakers (4)
Test Tube Racks (2)
Bunsen Burner or Alcohol Lamp
Green Leaves
Distilled Water

Introduction

The study of the life in the soil begins with the smallest living things, the microorganisms. Microbes are extremely important to the balance of nature, and are the base to a successful circular ecosystem. They are responsible for fixing atmospheric nitrogen into the organic nitrogen that plants require, and for breaking down organic waste materials into basic organic molecules that both plant and animals require, recycling important nutrients. All life on earth would quickly perish if the microbial life of the soil were eliminated.

The world of soil microorganisms is also important from an economic standpoint. Many agents of both plant and animal diseases that cause great agricultural loss live in the soil. Just as important, though, are the microorganisms which help man to combat diseases, such as the penicillium mold, which also live in the soil. Regenerating farm land also depends on the activity of microbes, even with the effective use of fertilizers. Fertilizer basically primes the pump for plant growth on land, but microbes must be present to keep it going.

For the student of biology, an investigation of the microscopic life in the soil provides an excellent look at a complex biological environment. The top few inches of soil support an incredible variety of life, both micro and macroscopic. This complex system, top soil, demonstrates biological interactions and the concept of balanced ecological systems.

This kit is designed to help the student appreciate the incredible variety of microscopic life found in the soil, and the importance of each. The student selectively cultures several types of microbes found in the soil, and examines the growth of these organisms over a period of several days. Both macro and microscopic examinations are used in the experiments. Performance of the microscopic examinations requires access to scopes of 300X capability or better.

It's useful to divide a discussion of soil microorganisms into sections dealing with each of the various types of microbes, the bacteria, fungi, algae, protozoa, and, if desired, viruses. General considerations are discussed later.

Bacteria

The bacteria are what most people think of as being microorganisms, and they are found in huge numbers in the soil. A gram of soil near the surface contains as many as one billion bacterial cells. Bacteria found in the soil are typically the bacillus (rod-shaped) or coccus (spherical-shaped) type. The third type of bacteria, called spirilla for their cork-screw shape usually require conditions which are less harsh than those in the soil.

Bacteria are the major agents in decomposition. Bacterial cells digest organic and mineral matter externally, secreting enzymes into the soil to break down, for example, the waste products or remains of surface organisms. This activity recycles usable nutrients, and eliminates excess organic material. As bacteria metabolize the various components that they have digested, they excrete various materials which replenish the nutrient supplies for plants and other soil organisms.

Bacteria, and some algae as well, are involved in the nitrogen cycle. Some bacteria release nitrogen as they break down the nitrogen-containing materials, while others are capable of converting atmospheric nitrogen into organic nitrogen in the form of nitrites, nitrates, and ammonium compounds. These nitrogen- fixing bacteria are an extremely important part of the ecosystem, since they provide a source of nitrogen to the plants and animals which are incapable of fixing nitrogen, yet require it in their metabolism. Some bacteria form a symbiotic

relationship with certain legumes, such as alfalfa, forming nodules on the roots of the plant and contributing nitrogen to it directly while living in a protected environment. Plants that have such a symbiotic relationships are often used in crop rotation, since they replace the essential nitrogen that non- symbiotic plants, such as corn, drain from the soil.

Bacteria from the soil may be cultured selectively by growing them on an artificial medium, such as nutrient agar. Bacteria tend to favor neutral or slightly acidic environments within the soil and in the laboratory.

Observation of the bacterial growth may be accomplished both macroscopically and microscopically. Different strains of bacteria show different patterns of growth on culture media. Solid growth media are most useful for the culturing of bacteria, because bacteria colonies, each grown from a single original bacterium, can be seen as spots or blobs of material quite close to the surface of the agar. After several days of growth, some colonies have a characteristic color, usually related to the metabolic waste-products produced by the bacteria, and will either be dull or shiny in appearance. Some bacteria may also show a wispy growth, and may, at first, be confused with fungi (see below.) Check these cultures to see if there are actual filaments above the surface of the agar.

Fungi

Fungi are far more common in the soil than might be expected. Most fungi are not apparent to the casual observer, since the major part of the fungi's growth is underground. Fungi are not plants, and do not manufacture their own food supply. All fungi, including the visible forms like mushrooms, develop a highly extended and branched network of filaments called hyphae which extend underground, or through the body of a dead plant or animal. Often the mass of hyphae, collectively called a mycelium, may extend many meters from the original growth point. The growth of hyphae through the soil helps to breakup large chunks of material in the soil, and thus improves the soil's aeration, as well as water retention.

Fungi are very important in recycling materials, and eliminating the large amounts of waste material from larger organisms. Fungi, like bacteria, accomplish most of their digestion be secreting enzymes into the soil, and then absorbing the nutrients which those enzymes have released. Fungal material itself also forms an important part of the organic nutrient supply in the soil. When they die, the hyphae are decomposed by bacteria or other fungi, or are simply broken apart by physical process.

The fungi may be divided into three principle groups: the molds such as mildew, club-fungi (basidiomycetes) such as the cup fungi, and sac fungi (ascomycetes) such as mushrooms and yeast. Any sample of soil will probably contain some hyphae. In addition to the actual thallus (body) of the fungus, a soil sample will undoubtedly contain spores of one or more fungus types. These spores may be germinated by transferring the soil sample to an appropriate growth medium. Fungi tend to prefer a more acidic environment than do bacteria. This is one reason why tomato-based foods, such as spaghetti sauce, tend to grow fungi as they spoil.

A less well known group, the slime molds (myxomycophyta) is sometimes included as a type of fungus. These acellular organisms occur as a slimy growth, as their name implies, usually on the surface of the soil or some decayed plant material.

Algae

Although algae are thought of as being aquatic, these simple plant organisms are present, and are very important to the activity of the soil. Algae are most noticeable in areas where moisture is abundant. In some cases, the soil will appear distinctly green. Even in drier areas, algae live in the thin film of water surrounding each soil particle. Because they possess green photosynthetic pigments, algae are capable of utilizing sunlight to produce usable chemical energy. In some areas, algae make a significant contribution to the store of organic nutrients present in the soil.

Many algae are capable of converting various nitrogen-containing materials into atmospheric nitrogen, or vice-versa. These algae are, of course, important to the nitrogen cycle discussed previously. Algae also serve as a source of nutrients to some of the protozoa found in the soil, and to some of the small soil arthropods and worms.

Algae are also important because of their interactions with other soil microorganisms, most notably with the fungi. A symbiotic relationship between a fungus and an algae constitutes a lichen. Lichens are classified according to both the fungus and the algae which are contained within them, and are extremely important in the breakdown of rocks. Lichens usually contain a green or blue-green algae, although some other types may be involved as well.

Protozoa

Soil water provides a medium for the activity of many protozoa. These unicellular animals, called protists, occur in a wide variety of shapes and sizes, and may be either motile or non-motile. The extent to which protozoa are found in the soil depends to a great extent on the availability of the water. Some of the common types of protists are: flagellated organisms such as the *euglena;* ciliated animals such as the *paramecium;* protists possessing extensions called pseudopods, such as the *amoeba;* and the group referred to as sporozoans, which are usually small and quite simple in their appearance. The protozoan use free organic molecules, algae, and each other for food. They, in turn, serve as food for larger organisms, and are the second step at the very base of the food chain.

Viruses

Some viruses are found in the soil, notably plant viruses and the bacteriophages. The fact that viruses require existing cellular life for their own activity has created some difficulty in the culturing of viruses in the high school lab. For this reason, viruses are not included as one of the microbes for study.

You may wish to discuss viruses briefly with the students, as part of a background on microbiology. Viruses consist of an outer protein coat with an inner core of nucleic acid, either DNA or RNA. The viruses themselves are not capable of extensive metabolic activity, so they do not play a direct role in the chemistry of the soil. Viruses act by taking over the metabolism of a live cell, and causing that cell to manufacture new virus particles. Viruses do have an influence over some types of plants and bacteria, and to this extent they may directly effect the lives of other microorganisms in the soil.

General Considerations of Soil Microbiology

Most of the microbial life in the soil occurs near the surface of the soil or close to the roots of plants. Algae must be near the surface in order to obtain the sunlight necessary for photosynthesis. The basic food sources for soil microorganism are organic waste products and algae, both of which are found near the surface of the soil. This is one of the reasons why soil which has been eroded is not easily reconverted into fertile soil: lower layers of soil do not have the populations of microorganisms needed to recycle the nutrients contained in the wastes and remains of larger organisms.

The chemistry of the soil has a significant effect on the population of microbes which it supports. A slight change in the acidity or alkalinity of the soil, or in the concentration of various minerals, may cause sharp changes in the distribution of microbes in that soil. For this reason, sensible land-use management requires an understanding of the chemical requirements of both soil microorganisms and the macroscopic organisms which live on or in the soil.

Using The Kit In The Classroom

This kit is designed to be used by junior or senior high school level earth science and biology students. In biology courses, the kit may be used either as an introduction to microbiology, or as part of a discussion of ecology and community biology. The Science Source Kit #1900, **Introduction to Microbiology,** may be more suited toward the former use. Earth science courses will find the kit useful in providing a perspective on the biological aspects of the soil.

The objectives of the kit are that the student, upon completion of the lab experiment, shall be able to:

- culture selectively and observe microscopically fungi, bacteria, protozoa, and algae from a soil sample; demonstrate appropriate laboratory technique; and demonstrate correct handling of the microscope.
- state the importance of soil microbes in breaking down organic material, and the subsequent recycling of nutrients, and to cite the nitrogen-fixing properties of bacteria and algae.
- relate the distribution and variety of soil microorganisms to the distribution of a wide variety of nutrients and water within the soil.
- draw accurate diagrams of his/her observations, and to compare and contrast experimental results over time.

No prior concepts are required to use the kit, except a basic background on the characteristics of plant and animal life. The students should be familiar with the handling and use of the microscope.

Preparing The Materials

Beside the materials provided in the kit, the students will need the following: microscopes (one per team of two students), four 400 ml beakers, 2 test tube racks, a Bunsen burner or alcohol lamp, and a handful of green leaves.

Microscopes should be of 300X capability or better. Check them before beginning the lab activities to be sure that they are in working order. Have lens tissue available during the lab to clean eyepieces and objectives.

In the interest of safety, you may want to omit flaming the inoculating loop. Sterility is not a matter of great importance in this activity, since the soil will be providing a mixed culture of microorganisms anyway. The purpose of the flaming procedure is to acquaint the students with sterile technique. As an alternative, have the students dip the loop into the bottle of alcohol, and allow the loop to dry. The inoculating loops must be assembled before doing the activity: Take a precut piece of wire (3") lengths, make a small loop, about 1/4 inch in diameter, at the end of a length of wire, and insert the straight end of the wire into the wooden block.

The nutrient broth and agar are made from the dehydrated media prior to the lab activities. To prepare each, follow the directions below.

Nutrient Broth

Empty the contents of the vial labelled "nutrient broth" into a beaker. Add 350 ml of distilled water. Heat with frequent agitation and boil for one minute. Pour about 2 inches of broth into each culture tube, and set them in a test tube rack. Prepare the nutrient broth as near to the time of use as possible. If you need to make up the broth in advance, store it in a refrigerator prior to use.

Potato Dextrose Agar

Empty the contents of the vial labelled "potato dextrose agar" into a beaker. Add 350 ml of distilled water. Heat with frequent agitation and boil until the medium is completely dissolved. Prepare the agar as near to the time of use as possible. Pour about 2 inches of agar into the sterile culture tubes (total of 12) and allow the agar to harden for about 20 minutes at a slant by placing all twelve tubes in a test tube rack, and tilt the rack against a book or some other object.

The leaves are needed to provide excess organic material for soil algae and protozoa. The washing with alcohol is something of a ritual, but it suggests to the student the need for controlling the experiment. The leaves may be of any type, but try to avoid waxy leaves or succulents.

Guide To The Student Materials

The introduction says that the soil contains a wide variety of life forms, and that the kit will focus on the microscopic life in the soil. If needed, review the use of the microscope at this time. It is useful to have the students collect their own soil samples. Have them keep a record of where they obtained the soils, what the conditions of temperature, light, and humidity were, etc. If you wish, a class data table may be prepared to summarize the findings:

Soil Samples			**Results**			
Team #	Location	Condition	Fungi	Bacteria	Protozoa	Algae
1						
2						
3						
•						
•						
•						

Caution the students to avoid collecting soil from areas where animals have been kept, as there may be disease organisms present in these soils.

The two tubes of growth media, nutrient broth and potato dextrose agar, will culture bacteria and fungi, respectively. Potato dextrose agar is more acidic than nutrient broth or agar, and it favors the growth of the fungi. Letting the agar set at an angle will provide a larger surface area for growth. Have separate racks available for the broth tubes and the agar slants. These do not need to be incubated, but incubation will speed up the rate of growth. Watch students using the inoculating procedures. Be sure that they do not gouge the agar with the loop.

Encourage the students to make observations of the tubes before preparing slides. The broth tube should be cloudy after 24 hours, showing that growth has taken place. The students may also notice that the broth has a sour smell to it, indicating that some sort of chemical activity has occurred. The agar slant may show some growth of fungus after 1 day, but will probably show much more growth after 48 hours. Most of the fungal growth will appear as a mat of hyphae, similar in appearance to bread mold. Encourage the students to describe examples of fungal growth which they have encountered before, such as bread mold or mildew.

The microscopic examination of the bacteria depends on making the slide correctly: point out that the thinner the film of broth on the slide, the easier it will be to observe growth. Caution the students not to spill the methylene blue, as it will stain skin and clothing.

The bacteria appear as darkly stained dots and/or rods. If you wish, name the types of bacteria according to shape, and have the students label the different types. Some of the bacteria may appear to be a bit larger, and look like clear plastic rods and spheres. These are encapsulated bacteria. As the environment in the broth tube becomes hostile (the food supply drops, and the level of waste products increases) bacteria secrete a protective coating, primarily of mucopolysaccharides, which allows them to survive in a state of "suspended" activity. This provides the opportunity for an interesting discussion of the dynamics of a closed biological community.

The fungi will stain fairly darkly with the methylene blue. The student may be able to discern the nuclei scattered along the length of the hyphae. If you wish, introduce the terms hyphae and mycelium to the students. If available, show the students a sample of damp soil with fungal growth on it. Fungi are widely distributed in most types of soils.

The soil sample cup, containing soil, water, and the leaf fragments, should show some growth of algae and protists after 24 hours. The protozoa will be of different types. You may want to have the students identify some of them using a classification key found in text or reference books. Point out that the plants (algae) may usually be distinguished by the presence of green photosynthetic pigments.

You may want to have the students maintain the cultures for longer than 48 hours to study population dynamics. If so, be sure to have them add water to the soil sample cups to prevent them from drying out.

Topics For Further Discussion

Following completion of the initial set of activities in this kit, the instructor may wish to pursue some, or all, of the following topics:

1) Discuss the economic importance of some of the soil microorganisms. Consider such areas as the culturing of antibiotic fungal strains (ex. Penicillium), contamination of food and water (ex. E. coli), agricultural diseases, and soil fertility (ex. nitrogen fixing bacteria).

2) Discuss the chemical composition of the soil, and its influence on the distribution of soil microorganisms. Have the students prepare samples of soil in cans or boxes, two of each soil sample, and have them add various materials to one, such as fertilizers, insecticides, oil, etc., the other one being a control. Have the students culture the soil microorganisms after a period of time, and compare the results with those obtained from the control soil sample. The instructor may also wish to consider The Science Source Kit #2000, **Chemical Composition of the Soil.**

3) Investigate the interaction of microorganisms in a closed community. The students may wish to observe the broth tubes for an extended period of time. The types and number of bacteria found in the broth will change as time goes on. Discuss the factors in a closed system which cause the changes observed.

4) Have the students collect literature on the soil management through microorganisms. The US Department of Agriculture is an excellent source of this information. You may have field stations of the Department in your area; representatives are often quite willing to present information to school groups.

SOIL MICROORGANISMS **#2100 Student Instructions (Annotated)**

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?

The broth is cloudy, and there is sediment on the bottom of the tube.

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?

The bacteria are using up the food, and producing waste materials which might be harmful. This makes the environment hostile to them; to survive, they must protect themselves.

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

Bits of dead or decaying plants and animals, and waste products from plants and animals might serve as food.

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.

There are fuzzy white growths on the surface of the agar. They appear to be made up of extremely small filaments.

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?

Some have straighter filaments than others. One is yellowish in color, the other is white. They all have the same sort of branched filament structure when seen under the microscope.

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?

When cheese, bread, or tomato soup go bad, they get the same type of growth.

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

There is more growth on the surface of the agar. The surface is nearly covered with growth. There is also a greenish tint to part of the material.

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

There may be small dark objects on the ends of some of the fibers. They are thicker than the filaments.

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 plants were probably the green objects in the culture. Plants usually have the green color to carry out photosynthesis.

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?

The algae would tend to be near the surface in order to get more light. They would also tend to live in the film of water surrounding soil particles, especially in damp areas.

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?

The protozoa, like the algae, would tend to live in the films and pockets of water between the soil particles near the surface, since they would have to live near their food sources, such as the algae, or decaying plant or animal materials.

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?

There are more of the microorganisms present. Some of the ones seen on the first day are not as numerous. There may be more of the smaller oval-shaped protozoa.

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

The algae can add organic materials to the soil. They may also be involved in producing necessary materials for other soil microorganisms. The protozoa probably help to breakdown materials in the soil, and thus help to recycle nutrients. The protozoa may also be food for larger soil animals.

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?

The organic matter on which the organisms feed is usually found at or near the surface. Those organisms which require oxygen will have a better supply of it nearer to the surface of the soil, and those requiring sunlight must live near the surface.

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

Different organisms require different environmental conditions to grow successfully. We provided only three different set-ups for the microbes to grow. We could have seen a different variety of microbes if the growth media, light, temperature, etc., conditions had been different.

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

Bacteria and fungi are important for breaking down waste materials and the bodies of dead plants and animals in the soil. They recycle the nutrients and materials from these dead plants and animals, and prevent the loss of necessary compounds from the soil. They also help to "clean up " the soil by breaking down excess materials. (Some bacteria are also involved in nitrogen fixation.)

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

The soil must contain a wide variety of materials in order to support the wide variety of life. As mentioned in question 2, each organism requires a particular set of conditions in order to grow successfully. Since it can maintain the large number of microbes which it does, the soil must have the materials needed by each organism.

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?

Gradually organic matter would build up, since the microbes would not be present to break it down. This would disrupt the water and air properties of the soil, and would effect the plant life in the area. Nutrients would be tied up in the undecayed organic materials, and the soil would eventually become unsuited for growing plants. (A similar situation can develop if the top-soil is eroded away)

SAFETY INSTRUCTIONS: IMPORTANT

NOTICE TO TEACHERS REGARDING LABORATORY REAGENTS

Perhaps the best general rule regarding the safe handling of laboratory chemicals is to treat all of them as being potentially dangerous. This means that none of them should be taken internally, and that any external contact should be washed thoroughly. In fact, most of the chemicals provided in The Science Source kits are diluted enough that they are not hazardous. The following lists indicate appropriate antidotes for the hazardous chemicals. Check this list for the chemicals provided in the kit:

I. **Concentrated Acids & Bases** - **Do not** induce vomiting, dilute with water, then milk or egg white, call a physician immediately.

1. 25 % Acetic Acid
2. 3M Hydrochloric Acid
3. Concentrated Sulfuric Acid

II **Dilute Acids & Bases** - **Dilute** with water, then milk.

1. 1 M, 0.5M, 0.1 M Hydrochloric Acid
2. Oxalic Acid
3. Sodium Hydroxide
4. Ammonium Hydroxide

III **Miscellaneous Chemicals** - Dilute immediately with water. Induce vomiting with warm salt water, or warm baking soda solution.

1. Ammonium Chloride
2. Ammonium Oxalate
3. Barium Chloride
4. Biuret Reagent
5. Bromthymol Blue
6. Calcium Chloride
7. Ethanol (Denatured Alcohol)
8. Ferric Ammonium Sulfate
9. Hydrogen Peroxide
10. Janus Green B
11. Lead Nitrate
12. Lugol's Solution
13. Magnesium Reagent (Titan Yellow, Alcohol)
14. Mercuric Nitrate
15. Methylene Blue
16. Ninhydrin
17. Phosphorus Reagent (Ammonium Molybdate, Nitric Acid)
18. Potassium Ferricyanide
19. Potassium Permanganate
20. Silver Nitrate
21. Sodium Carbonate
22. Sodium Thiosulfate
23. Sudan IV

IV. **Organic Solvents** - **Do not** induce vomiting. Dilute with water and milk. Call a physician immediately.

Isopropyl Alcohol

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MATERIAL SAFETY DATA SHEET

MSDS No.: IX0230
Revision Date: August 29, 2013
Approved by: James A. Bertsch

MSDS No.: IX0230

Section 1 Chemical Product and Company Information

Product	**ISOPROPYL ALCOHOL, 70% SOLUTION**
Synonyms	Isopropanol, Water Solution

CHEMTREC 24 Hour Emergency Phone Number (800) 424-9300

Section 2 Hazards Identification

Emergency Overview

WARNING! FLAMMABLE!
HARMFUL IF SWALLOWED. CAUSES EYE IRRITATION.
Avoid contact with skin and eyes. Avoid repeated or prolonged inhalation of vapors. Use with adequate ventilation. Keep away from heat, sparks and open flame. Store in a cool place. Wash thoroughly after handling. Target organs: Central nervous system, liver, kidneys.

0 = Minimal
1 = Slight
2 = Moderate
3 = Serious
4 = Severe

Health	1
Fire	3
Reactivity	1
Contact	2

HMIS *

Section 3 Composition / Information on Ingredients

Chemical Name	CAS #	%	TLV Units
Isopropyl alcohol	67-63-0	70%	TWA: 400 ppm; STEL: 500 ppm
Water	7732-18-5	30%	N/A
			(ACGIH 2001)

Section 4 First Aid Measures

INGESTION: Call physician or Poison Control Center immediately. Induce vomiting only if advised by appropriate medical personnel. Never give anything by mouth to an unconscious person.

INHALATION: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

EYE CONTACT: Check for and remove contact lenses. Flush thoroughly with water for at least 15 minutes, lifting upper and lower eyelids occasionally. Get immediate medical attention.

SKIN CONTACT: Remove contaminated clothing. Flush thoroughly with mild soap and water. If irritation occurs, get medical attention.

Section 5 Fire Fighting Measures

General information: In fire conditions, wear a NIOSH/MSHA-approved self-contained breathing apparatus and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion. Use water spray to keep fire-exposed containers cool. Fires involving a small amount of combustibles may be smothered by dry chemical. In fire conditions, water may evaporate from this solution which may cause hazardous decomposition products to be formed as dust or fume. Vapors are heavier than air and may travel along the ground or may be moved by ventilation and ignited by pilot lights, other flames, sparks, heater, smoking, electric motors or ignition sources at locations distant from material handling point. CAUTION! Flame may not be visible in daylight.

Extinguishing Media: Carbon dioxide, dry chemical, water spray, alcohol foam.

Flash Point: 21.7°C (71°F) TCC

Autoignition temperature: 399°C (750°F) ASTM-E659-78 (Pure IPA)

Explosion Limits: Lower: 2% **Upper:** 12% (Pure IPA)

NFPA

0 = Minimal
1 = Slight
2 = Moderate
3 = Serious
4 = Severe

Section 6 Accidental Release Measures

Use proper personal protective equipment as indicated in Section 8. Remove all sources of ignition. Provide adequate ventilation. Recover for use if not contaminated. Absorb with inert dry material, sweep or vacuum up and place in a suitable container for proper disposal. Wash spill area with soap and water. Avoid runoff into storm sewers and ditches which lead to waterways.

(2008 EMERGENCY RESPONSE GUIDEBOOK, (PHH50-ERG2008), GUIDE PAGE NO. 129)

Section 7 Handling & Storage — FLAMMABLE STORAGE CODE RED

Read label on container before using. Do not wear contact lenses when working with chemicals. Keep container tightly closed. For laboratory use only. Not for drug, food or household use. Keep out of reach of children.
Handling: Use with adequate ventilation. Avoid contact with eyes, skin and clothing. Avoid ingestion. Do not inhale vapors, spray or mist. Wash thoroughly after handling. Remove and wash clothing before reuse.
Storage: Store in a cool, dry, well-ventilated area away from incompatible substances. Keep away from ignition sources.

Section 8 Exposure Controls / Personal Protection

Engineering controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower and fire extinguishing material. Personnel should wear safety glasses, goggles, or faceshield, lab coat or apron, appropriate protective gloves. Use adequate ventilation to keep airborne concentrations low.

Respiratory protection: Use a chemical fume hood and/or wear a NIOSH/MSHA-approved respirator.

Section 9 Physical & Chemical Properties

Physical state: Liquid.
Appearance: Clear, colorless.
Odor: Aromatic odor.
pH: N/A
Vapor pressure (mm Hg): 33 mm @ 20°C (Pure IPA)
Vapor Density (Air = 1): 2.1 (Pure IPA)
Evaporation rate (Butyl acetate = 1): > 1
Viscosity: N/A
Boiling point: ~ 85-100°C (185-212°F)
Freezing / Melting point: ~ -50°C (-58°F)
Decomposition temperature: N/A
Solubility: Complete.
Specific gravity (H_2O = 1): 0.8
Percent volatile (%): 100%
Molecular formula: Mixture.
Molecular weight: Mixture.

Section 10 Stability & Reactivity

Chemical stability: Stable
Hazardous polymerization: Will not occur.
Conditions to avoid: Excessive temperatures, heat, sparks, open flame and other sources of ignition.

Incompatibilities with other materials: Strong oxidizing materials, caustics, aluminums, metals, nitroform, oleum, chlorinated compounds can react vigorously with this alcohol.

Hazardous decomposition products: Oxides of carbon.

Section 11 Toxicological Information

Effects of overexposure: INGESTION: 100 ml can be fatal. Aspiration hazard. EYES: Liquid may cause irritation. SKIN: Prolonged or repeated contact may cause irritation and drying, cracking and defatting of the skin. INHALATION: Exposure to high concentrations (>400 ppm) may cause eyes, nose and throat irritation and excessively high concentrations may cause narcosis (drowsiness, sleepiness). Target organs: Central nervous system, liver, kidneys.

ORL-RAT LD50: 5045 mg/kg
IHL-RAT LC50: N/A
SKN-RBT LD50: 12800 g/kg

Section 12 Ecological Information

Data not yet available.

Section 13 Disposal Considerations

These disposal guidelines are intended for the disposal of catalog-size quantities only. Federal regulations may apply to empty container. State and/or local regulations may be different. Dispose of in accordance with all local, state and federal regulations or contract with a licensed chemical disposal agency.

Section 14 Transport Information

UN/NA number: UN1219
Shipping name: Isopropanol solution
Hazard class: 3
Packing group: II
Exceptions: Ltd Qty ≤ 1 Lt.

Section 15 Regulatory Information

Isopropyl alcohol: TSCA-listed, EINECS-listed (200-661-7), RCRA code D001

Section 16 Additional Information

The information contained herein is furnished without warranty of any kind. Employers should use this information only as a supplement to other information gathered by them and must make independent determinations of suitability and completeness of information from all sources to assure proper use of these materials and the safety and health of employees. * Hazardous Materials Industrial Standards.

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MSDS No.: MM0446
Effective Date: October 11, 2006

SECTION I NAME

Product	Methylene Blue, 0.004M Alcohol Solution
Chemical Synonyms	N/A
Formula	Mixture.
Unit Size	up to 3.785 Lt.
C.A.S. No.	Mixture.

24 HOUR EMERGENCY ASSISTANCE

CHEMTREC 800-424-9300
Day 585-226-6177

NFPA: 3 (top), 2 (left), 0 (right)

HMIS *	
Health	1
Fire	3
Reactivity	0

HAZARD RATING

MINIMAL	SLIGHT	MODERATE	SERIOUS	SEVERE
0	1	2	3	4

SECTION II INGREDIENTS OF MIXTURES

Principal Component(s)	%	TLV Units
Ethyl alcohol, 70%, denatured**: (CAS No. 64-17-5)	99.85%	TWA: 1000 ppm; 1880 mg/m^3
Methylene blue chloride: (CAS No. 61-73-4)	.145%	None established.

WARNING! FLAMMABLE. HARMFUL IF SWALLOWED.

SECTION III PHYSICAL DATA

Melting Point (°F)	-114°C (-173°F) *	Specific Gravity (H_2O = 1)	0.7919 - 0.7955 @ 60/60°F *
Boiling Point (°F)	74-80°C (165.2-176°F) *	Percent Volatile by Volume (%)	100%
Vapor Pressure (mm Hg)	Ca 50 @ 20°C *	Evaporation Rate (Butyl acetate =1)	Ca. 2 *
Vapor Density (Air=1)	Ca 1.5 *		
Solubility in Water	Complete.		
Appearance & Odor	Clear, light blue, mobile liquid; mild characteristic odor.		

SECTION IV FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used)	(21°C) 70°F TCC.	Flammable Limits in Air % by Volume Pure Ethyl Alc.	Lower 4.0% (V)	Upper 20.0% (V)
Extinguisher Media	Water spray, carbon dioxide; dry chemical; alcohol-type, or universal-type foams.			

SPECIAL FIREFIGHTING PROCEDURES

Wear a NIOSH/MSHA-approved self-contained breathing apparatus and protective clothing. Water spray may be used to keep fire exposed containers cool.

Autoignition Temperature: 400°C (752°F) *

(2004 EMERGENCY RESPONSE GUIDEBOOK, RSPA P 5800.9, GUIDE PAGE NO. 127)

UNUSUAL FIRE AND EXPLOSION HAZARDS

Vapors formed from this product may travel or be moved by air currents and ignited by pilot lights, other flames, smoking, sparks, heaters, electrical equipment, static discharge, or other ignition sources at location distant from handling point. **CAUTION:** Flame may not be visible in daylight. Fire or excessive heat may produce hazardous decomposition products; can react vigorously with oxidizing materials.

* Pure Ethanol
** Denaturants:
Methyl alcohol: (CAS No. 67-56-1), Methyl isobutyl kelone: (CAS No. 108-10-1), Isopropyl alcohol: (CAS No. 67-63-0), Water: CAS No. (7732-18-5)

D.O.T. Ethanol, 3, UN1170, PG II

Approved by U.S. Department of Labor "essentially similar" to form OSHA-20

SECTION V HEALTH HAZARD DATA MM0446

Threshold Limited Value
None established for this mixture by ACGIH 2001. See Section II.

Effects of Overexposure
Ingestion causes dizziness, drowsiness, decreased reaction, euphoria, nausea, vomiting, staggering gait, and coma. Inhalation may cause dizziness, drowsiness, nausea and vomiting, inability to concentrate and irritation of the throat. Contact with skin causes irritation and defatting on prolonged contact. Contact with eyes may cause blindness. Target organs: Eyes, central nervous system, liver, kidneys.

Emergency and First Aid Procedures
INGESTION: Call physician or Poison Control Center immediately. Induce vomiting only if advised by appropriate medical personnel. Never give anything by mouth to an unconscious person. **EYES:** Check for and remove contact lenses. Flush thoroughly with water for at least 15 minutes, lifting upper and lower eyelids occasionally. Get immediate medical attention. **SKIN:** Remove contaminated clothing. Flush thoroughly with mild soap and water. If irritation occurs, get medical attention. **INHALATION:** Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

SECTION VI REACTIVITY DATA

Stability			Conditions to Avoid
	Unstable		Heat, fire, ignition source.
	Stable	X	

Incompatibility (Materials to Avoid)
Can react with strong oxidizers, inorganic acids and halogens.

Hazardous Decomposition Products
Thermal decomposition or burning can produce carbon monoxide and/or carbon dioxide.

Hazardous Polymerization		Conditions to Avoid
May Occur	Will Not Occur	
	X	Not applicable.

SECTION VII SPILL OR LEAK PROCEDURES

Steps to be taken in case material is released or spilled
Remove all sources of ignition, provide adequate ventilation. For small spills, dilute with water and flush to sewer with copious amounts of water or absorb on vermiculite, paper, earth or other absorbent. Burn in an approved incinerator or open pit away from buildings and people.

Waste Disposal Method
Discharge, treatment, or disposal may be subject to Federal, State or Local laws. These disposal guidelines are intended for the disposal of catalog-size quantities only.

Dispose of in an approved incinerator or contract with a licensed waste disposal service.

SECTION VIII SPECIAL PROTECTION INFORMATION

Respiration Protection (Specify Type)
For normal laboratory use at room temperatures none should be needed with adequate room ventilation. If required work in fume hood. Do not use in confined area.

Ventilation				
	Local Exhaust	Recommended.	Special	No.
	Mechanical (General)	Recommended.	Other	Adequate to maintain below exposure limits.

Protective Gloves	Rubber.	Eye Protection	Chemical safety glasses.

Other Protective Equipment
Smock, apron, eye wash station, goggles, fire extinguisher, proper gloves.

SECTION IX SPECIAL PRECAUTIONS

Precautions to be Taken in Handling & Storing
Keep container tightly closed when not in use.
Store in a cool, dry, well-ventilated area, away from any fire hazard. Use with adequate ventilation. Do not take internally.

Other Precautions
Read label on container before using. Do not wear contact lenses when working with chemicals. For laboratory use only. Not for drug, food or household use. Keep out of reach of children.

Wash thoroughly after handling. Remove and wash contaminated clothing.

Revision No. 1	Date 10/11/06	Approved James A. Bertsch	Chemical Safety Coordinator JAB

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MATERIAL SAFETY DATA SHEET

MSDS No.: EE0069
Revision Date: September 17, 2013
Approved by: James A. Bertsch

MSDS No.: EE0069

Section 1 Chemical Product and Company Information

Product	**ETHYL ALCOHOL, DENATURED, 70% SOLUTION**
Synonyms	Ethanol, 70% Aqueous Solution

CHEMTREC 24 Hour Emergency Phone Number (800) 424-9300

Section 2 Hazards Identification

Emergency Overview

DANGER! FLAMMABLE!
HARMFUL IF SWALLOWED.
Keep away from heat, sparks, flame and all other ignition sources. Avoid breathing vapor. Use with adequate ventilation. Do not get in eyes, on skin or on clothing.
Target organs: Eyes, central nervous system, liver, kidneys.

0 = Minimal
1 = Slight
2 = Moderate
3 = Serious
4 = Severe

Health	1
Fire	3
Reactivity	0
Contact	2

HMIS *

Section 3 Composition / Information on Ingredients

Chemical Name	CAS #	%	TLV Units (ACGIH 2001)
Ethyl alcohol, denatured*	64-17-5	70%	TWA: 1000 ppm
Water	7732-18-5	30%	None established.
*Denaturants:			
Methyl isobutyl ketone	108-10-1		TWA: 205 mg/m^3 STEL: 307 mg/m^3
Isopropyl alcohol	67-63-0		TWA: 400 ppm STEL: 500 ppm
Methyl alcohol	67-56-1		PEL-TWA: 200 ppm STEL: 250 ppm

Section 4 First Aid Measures

INGESTION: Call physician or Poison Control Center immediately. Induce vomiting only if advised by appropriate medical personnel. Never give anything by mouth to an unconscious person.

INHALATION: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

EYE CONTACT: Check for and remove contact lenses. Flush thoroughly with water for at least 15 minutes, lifting upper and lower eyelids occasionally. Get immediate medical attention.

SKIN CONTACT: Remove contaminated clothing. Flush thoroughly with mild soap and water. If irritation occurs, get medical attention.

Section 5 Fire Fighting Measures

General information: In fire conditions, wear a NIOSH/MSHA-approved self-contained breathing apparatus and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion. Use water spray to keep fire-exposed containers cool. Fires involving a small amount of combustibles may be smothered by dry chemical. Vapors formed from this product may travel or be moved by air currents and ignited by pilot lights, other flames, smoking, sparks, heaters, electrical equipment, static discharge or other ignition sources at location distant from handling source. CAUTION! Flame may not be visible in daylight.

Extinguishing Media: Carbon dioxide, dry chemical, water spray, alcohol foam.
Flash Point: 21°C (70°F) TCC
Autoignition temperature: N/A
Explosion Limits: Lower: 3.3% **Upper:** 19.0%

NFPA

0 = Minimal
1 = Slight
2 = Moderate
3 = Serious
4 = Severe

Section 6 Accidental Release Measures

Use proper personal protective equipment as indicated in Section 8. Remove all sources of ignition. Provide adequate ventilation. Recover for use if not contaminated. Absorb with inert dry material, sweep or vacuum up and place in a suitable container for proper disposal. Wash spill area with soap and water. Avoid runoff into storm sewers and ditches which lead to waterways.

(2008 EMERGENCY RESPONSE GUIDEBOOK, (PHH50-ERG2008), GUIDE PAGE NO. 127)

Section 7 Handling & Storage — FLAMMABLE STORAGE CODE RED

Read label on container before using. Do not wear contact lenses when working with chemicals. Keep container tightly closed. For laboratory use only. Not for drug, food or household use. Keep out of reach of children.
Handling: Use with adequate ventilation. Avoid contact with eyes, skin and clothing. Avoid ingestion. Do not inhale vapors, spray or mist. Wash thoroughly after handling. Remove and wash clothing before reuse.
Storage: Store in a cool, dry, well-ventilated area away from incompatible substances. Keep away from ignition sources.

Section 8 Exposure Controls / Personal Protection

Engineering controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower and fire extinguishing material. Personnel should wear safety glasses, goggles, or faceshield, lab coat or apron, appropriate protective gloves. Use adequate ventilation to keep airborne concentrations low.

Respiratory protection: Use a chemical fume hood and/or wear a NIOSH/MSHA-approved respirator.

Section 9 Physical & Chemical Properties

Physical state: Liquid. ****(Pure Ethanol)**
Appearance: Clear, colorless.
Odor: Mild, characteristic odor.
pH: N/A
Vapor pressure (mm Hg): 44.6 mm @ 20°C (68°F)**
Vapor Density (Air = 1): 1.59**
Evaporation rate (Butyl acetate = 1): 4.1**
Viscosity: N/A
Boiling point: 75-80°C (173-174°F)**
Freezing / Melting point: -114°C (-173°F)**
Decomposition temperature: N/A
Solubility: Complete.
Specific gravity (H_2O = 1): 0.794 @ 60°F**
Percent volatile (%): 100%
Molecular formula: Mixture.
Molecular weight: Mixture.

Section 10 Stability & Reactivity

Chemical stability: Stable
Hazardous polymerization: Will not occur.
Conditions to avoid: Excessive temperatures, heat, sparks, open flame and other sources of ignition.

Incompatibilities with other materials: Contact with acetyl chloride and a wide range of oxidizing agents may react violently. Vapors may form flammable mixtures with air.

Hazardous decomposition products: Oxides of carbon.

Section 11 Toxicological Information

Effects of overexposure: INGESTION: Can cause central nervous system depression, nausea, vomiting, diarrhea. INHALATION: May cause headache, drowsiness, loss of appetite, inability to concentrate and irritation of the throat. EYES: Liquid or vapor may cause irritation. SKIN: May cause irritation and defatting of skin on prolonged contact. OTHER: Individual responses to Methyl alcohol vary, ingestion of less than 30 ml has been fatal to humans. In general a few ounces may cause blindness and death, as little as 4 ml may be toxic if ingested.

ORL-RAT LD50: N/A
IHL-RAT LC50: N/A
SKN-RBT LD50: N/A

Section 12 Ecological Information

Data not yet available.

Section 13 Disposal Considerations

These disposal guidelines are intended for the disposal of catalog-size quantities only. Federal regulations may apply to empty container. State and/or local regulations may be different. Dispose of in accordance with all local, state and federal regulations or contract with a licensed chemical disposal agency.

Section 14 Transport Information

UN/NA number: UN1170
Shipping name: Ethanol
Hazard class: 3
Packing group: II
Exceptions: Ltd Qty ≤ 1 Lt.

Section 15 Regulatory Information

For pure Ethanol: TSCA-listed, EINECS-listed (200-578-6), RCRA code D001

Section 16 Additional Information

The information contained herein is furnished without warranty of any kind. Employers should use this information only as a supplement to other information gathered by them and must make independent determinations of suitability and completeness of information from all sources to assure proper use of these materials and the safety and health of employees. * Hazardous Materials Industrial Standards.