



Identifying Organic Matter in Soil

Introduction

Carbon (C) and hydrogen (H) are elements found in all organic compounds. Many organic compounds also contain additional elements such as oxygen (O), nitrogen (N), and phosphorus (P). Bacteria and fungi break down organic matter into smaller organic molecules and inorganic matter, which is absorbed by plants through their roots. The presence of organic matter in soil improves soil structure and increases water availability, directly affecting plant development. The availability of organic materials and water in soil contributes to soil fertility.

*Organic matter in soil consists of material originating from living organisms, remains of living organisms, plant residues, and animal excretions.

In this experiment, you will use potassium permanganate solution (KMnO_4), to identify the presence of organic matter in different types of soil. The purple color of the KMnO_4 solution changes in the presence of organic matter. The more organic matter present in the soil, the lighter the purple color becomes – until it becomes colorless.

Information about the presence of organic matter in soil allows for a deeper understanding of soil conditions and helps in making decisions to improve soil quality.

- What do you think could be done to improve soil quality?

Materials and Equipment

- Permeant marker
- Test tube rack with:
 - Two test tubes containing 2 grams each of different soil types
 - Three stoppered test tubes containing 15 ml each of KMnO_4 solution



Procedure

- Using the permeant marker, label the four test tubes containing KMnO_4 solution with the numbers 1-4.
- Transfer the soil from the soil-containing test tubes to test tubes 2 and 3.
- Transfer the Ethanol to test tube 4.
- Stopper test all the tubes.
- Complete the missing information in Table 1, column 2.
- Hold the test tubes with your thumb on the stopper and shake the test tubes for two minutes.
- Return test tubes to the rack and wait for the soil to settle in test tubes 2 and 3.
- Complete the missing information in Table 1, column 3.
- Complete the missing information in Table 1, column 4.

Table 1

	1	2	3	4
Test Tube	Contents	Initial Color	Color After Two Minutes	Color Change (Yes/No)
1	KMnO_4 solution			
2	KMnO_4 solution with soil type _____			
3	KMnO_4 solution with soil type _____			
4	KMnO_4 solution with Ethanol			

Questions

- Determine which soil type among those you tested contained more organic matter. Explain your determination.
- Based on the introduction, explain the importance of bacteria and fungi in soil.
- Based on the introduction, explain why the presence of organic matter in soil can affect soil properties and the development of plants growing in it.
- What is the importance of test tube 4 in the test you conducted?
- If we were to test soil samples from a forest and from a desert, where would you expect to find a higher amount of organic matter? Explain why.



**Faculty of
Education**
Bar-Ilan University



The Center for
Development and Support
for High School Biology Labs



Ministry of Education,
State of Israel
Supervision of Biology Teaching
Supervision of Science Teaching

6. A student tested soil samples from a forest and from a field where corn is grown intensively (high plant density per unit area) and found that the amount of organic matter in the forest soil sample was relatively higher than in the field sample. Suggest possible reasons for these results.



Identifying Organic Matter in Soil
Teacher and Lab Technician Guide

Target Audience: Grades 7-9

Duration: 45 minutes

Objectives

1. To demonstrate testing for the presence of organic matter in soil.
2. To understand the relationship between organic matter, bacteria, fungi, and plants in soil.
3. To develop skills in observation, comparison, drawing conclusions, and understanding the importance of controlled variables in experiments.

Central Activity

Students will identify the presence of organic matter in different soil types using a reaction between organic matter in soil and potassium permanganate (KMnO_4) solution.

Curriculum Connection (grades 7-9)

- ****Matter and Energy Transfer in Ecological Systems (Extension)****
- Carbon cycle in nature
- ****Mineral Nutrition****
- Importance of minerals for plants
- Process of mineral absorption from soil

Key Concepts for Teaching the Experiment

Organic matter, Soil microorganisms, Carbon cycle

Materials and Equipment Table (per pair of students)

Materials and Equipment	Quantity
Stoppered test tubes containing 15 ml of KMnO_4 solution (0.0005M)	4
Test tubes containing 2g each of different soil types, appropriately labeled	2
Test tubes containing 10 ml of Ethanol 99%	1
Test tube rack	1
Glass-marking pen	1
Timer	1



*To prepare 1 liter of KMnO_4 solution at 0.0005M concentration:

- Dissolve 0.08 g KMnO_4 in 1 liter of distilled water, OR
- Dissolve 0.8 g KMnO_4 in 1 liter of distilled water and dilute 100ml of the prepared solution 10-fold

Information and Notes for Teachers and Lab Technicians

- It is recommended to use sandy soil, which has low organic matter content, and soil rich in organic matter (such as potting mix – available at nurseries) where we expect to find a high amount of organic matter.
- Emphasize safety precautions when working with KMnO_4 solution. KMnO_4 is a strong oxidizer and can cause stains. At the concentration used in this test, protective equipment is not required, but gloves are recommended.

Teacher Information

- A preparatory lesson should be conducted before the activity covering:
 - The importance of soil microorganisms as decomposers and their impact on plant development, carbon cycle, and food chain
 - The importance of organic matter in maintaining soil structure and composition

Importance of Organic Matter in Maintaining Soil Structure and Composition

Organic matter in soil is crucial for maintaining soil properties. It improves soil structure by strengthening aggregate stability and improving pore size distribution, which enhances water movement and oxygen penetration. Additional benefits come from soil microorganisms, which secrete organic materials, primarily polysaccharides, that further strengthen bonds between soil particles. Together, these factors help maintain soil structure and contribute to long-term plant development.

Soil Bacteria

Soil bacteria participate in important processes such as carbon and nitrogen cycling, including nitrogen fixation from the air and converting it to a form available to plants. Some bacteria form symbiotic relationships with plant roots, improving water and mineral absorption, thus contributing to optimal growth conditions. Moreover, certain bacteria help suppress soil pathogens through resource competition or by producing antimicrobial substances, thereby improving plant health.

Answers to Student Questions



1. Organic matter was identified in the potting mix soil. This can be concluded from the lighter color (less purple) of the solution after adding the soil. The more organic matter in the soil, the lighter the color of the KMnO_4 solution becomes.
2. Bacteria and fungi break down organic matter into smaller organic molecules and inorganic matter, which is absorbed by plants through their roots. The presence of organic matter in soil improves soil structure and increases water availability, directly affecting plant development. The availability of organic materials and water in soil contributes to soil fertility.
3. Test tube 4, containing only KMnO_4 solution, serves as a control. It verifies that any color change is caused by reaction with specific soil components and not due to spontaneous decolorization.
4. In the forest soil sample, a higher amount of organic matter would be expected compared to desert soil. This is due to the accumulation of organic matter from the wide variety and density of plants, animals, enhanced microbial activity. Forests maintain continuous accumulation of plant and animal matter. In contrast, desert soil has minimal organic matter due to very low density of plants and animals, resulting in low microbial activity of soil bacteria.
5. In forests, there is a wide variety of plants and animals whose remains decompose slowly by microorganisms, creating organic matter. In the corn field, plant diversity is limited, which restricts soil microorganism diversity. Additionally, intensive cultivation leads to high utilization of inorganic materials in the soil. Most of the plant mass is harvested and removed from the field, so less organic matter returns to the soil. Furthermore, frequent plowing in the corn field breaks down organic matter faster than in the forest.



Testing Cellular Respiration of Living Organisms in Soil

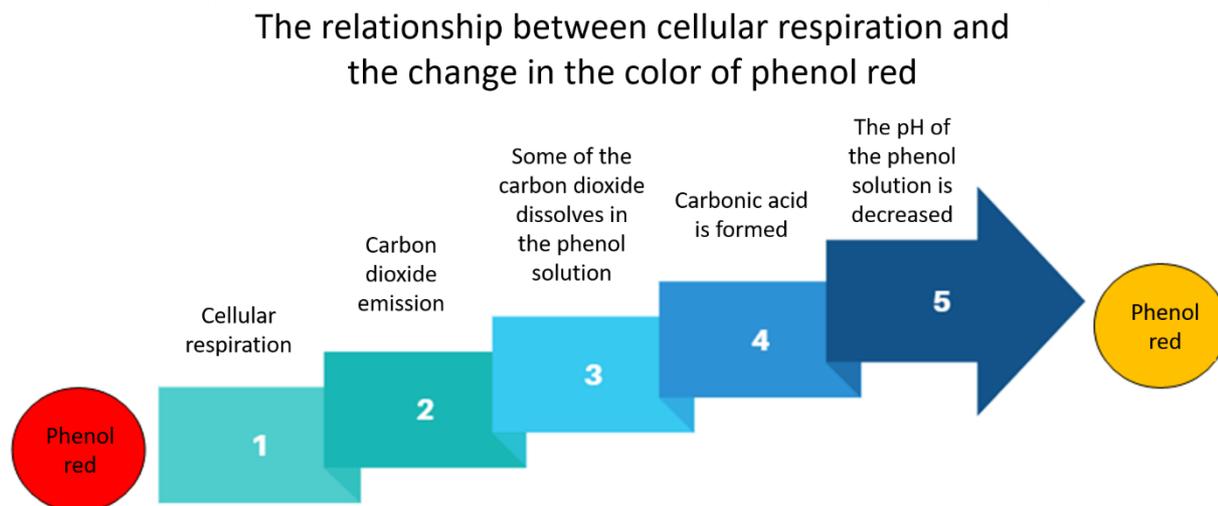
Introduction

Bacteria and fungi break down organic matter into smaller organic molecules and inorganic materials, with some breakdown products being absorbed into their cells. One of these absorbed materials is glucose. Inside the cells, glucose is broken down through cellular respiration, and the energy released in this process is used for specific cellular processes. The extent of organic matter decomposition in soil affects soil properties and consequently influences plant development. One of the products of cellular respiration is carbon dioxide (CO_2). In an aqueous environment, some CO_2 molecules react with water molecules to form carbonic acid, which causes a decrease in the pH of the solution.

To identify changes in solution acidity, a pH indicator can be used. Phenol red is an indicator that appears red in neutral-basic environments and changes to orange-yellow in acidic environments (see Figure 1).

As soil organisms' activity increases, the rate of cellular respiration increases, resulting in higher CO_2 emission to the environment and increased soil acidity.

Figure 1



In this activity, you will examine the cellular respiration process of soil bacteria.



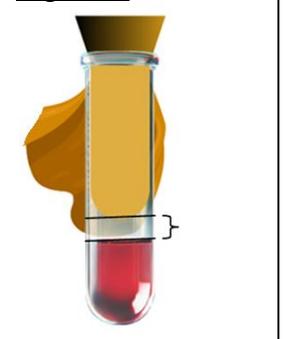
Materials and Equipment

- Container with 2 large stoppered test tubes and 1 small stoppered test tube, each containing 10 ml of water
- Test tube rack with one tube containing 10 g sterilized soil and another tube containing 10 g non-sterilized soil, appropriately labeled
- Dropper bottle with phenol red solution
- Two beakers each containing 50 ml starch suspension
- 2 nylon stockings
- Stirring rod
- Two empty plastic containers
- Permeant marker

Procedure

1. Using the permeant marker, label the two large water-containing test tubes as 1 and 2, and the small water-containing test tube as 3.
2. Add one drop of phenol red to each of the three labeled test tubes.
3. Transfer the ***sterilized*** soil from its tube into one of the nylon stockings and place the stocking in an empty plastic container.
4. Hold the stocking inside the plastic container and widen its open end.
5. Using the stirring rod, stir the starch suspension and pour it all ***slowly and in a thin stream*** into the stocking opening while kneading the soil in the stocking to thoroughly wet the soil.
6. Squeeze the stocking to remove excess suspension so that when you lift the stocking above the plastic container, the suspension does not drip.
7. Transfer the stocking with the soil into the large test tube labeled 1.
8. Use the thick end of a pencil or pen to push the stocking into the test tube so that the open end remains outside the tube and the bottom end does ***not touch*** the liquid at the bottom of the tube (see Figure 2 for proper placement of soil-filled stocking in the test tube).
9. Stopper the test tube and transfer it to the container.
10. Repeat steps 3-9 with the non-sterilized soil. Use a clean plastic container and fresh starch suspension to wet the soil.
11. Photograph the test tubes.

Figure 2.





Results

This section will be completed several days after conducting the test

12. Observe the solution colors in both large test tubes, compare the solution color to the phenol red solution color in the small test tube containing only phenol red solution.

13. Complete the missing information in Table 1.

Table 1: Effect of Soil Type on Cellular Respiration of Bacteria and Fungi in Soil

Test Tube	Solution Color Before Adding Soil Stocking	Solution Color After ___ Days	Solution Color Change at Bottom (Changed/Unchanged)
1 - Sterilized soil			
2 - Non-sterilized soil			

Questions

1. In which of the two test tubes containing soil was a more significant change observed compared to the solution color in the small tube? What can be concluded from this?

2. a. Explain what sterilized soil is.

b. Explain why sterilized soil was used in one of the test tubes?

3. Based on the test results, explain how the cellular respiration process of bacteria affects the pH of the solution at the bottom of the test tube?

4. What is the reason for adding starch solution in the experiment? Why do you think we used it? (Hint: starch is a polysaccharide composed of many glucose molecules).

5. Why was it important to use a test tube containing only phenol red solution?

6. Gardeners often spread compost (natural fertilizer created from the decomposition of organic materials such as food scraps, leaves, plant trimmings, and other organic waste by bacteria and fungi) in gardens.

Explain how adding compost contributes to plant development in the garden.

7. How can information about cellular respiration processes in soil help in evaluating soil fertility?



Testing Cellular Respiration of Living Organisms in Soil

Teacher and Lab Technician Guide

Target Audience: Grades 7-9

Duration: Experiment setup - double lesson. Results summary - one lesson.

Objectives

1. Demonstrate the cellular respiration process of living organisms as measured by phenol red indicator color change.
2. Compare activity between living organisms in sterilized and non-sterilized soil
3. Demonstrate the effect of cellular respiration on environmental pH levels.

Central Activity

Students will examine the cellular respiration process of living organisms in sterilized and non-sterilized soil, measured through color changes in the phenol red indicator.

Curriculum Connection (grades 7-9)

- The Cell: Structure and Function

- Energy production in cellular respiration

- Organism-Environment Interactions

- Decomposers as part of the food web
- Impact of biotic factors on abiotic factors
- Example: Impact of soil-dwelling organisms on soil structure and composition

Key Concepts for Teaching the Experiment

Cellular respiration, organic matter, glucose, carbon dioxide, pH, carbonic acid, indicator, starch, organic matter, soil fertility, carbon cycle.

Materials and Equipment Table (per pair of students)

Materials and Equipment	Quantity
Large test tubes (approx. 50 mL) with stoppers, containing 10 mL water each (see lab technician notes)	2
Small test tube (approx. 25 mL) with stopper, containing 10 mL water (see lab technician notes)	1
Test tube rack	1



Test tube rack suitable for large tubes or container for standing large tubes	1
Thin test tube containing 10g sterilized soil (potting mix)	1
Thin test tube containing 10g non-sterilized soil (potting mix)	1
Approx. 10 mL phenol red solution in dropper bottle (see preparation instructions)	1
Beaker containing 50 mL of 2% starch suspension	2
Nylon stockings (approx. 10 cm length)	2
Spoon	1
Empty plastic containers (approx. 100 mL)	2
Glass-marking pen	1

Information and Notes for Lab Technicians

1. Use water (tap water/distilled water/mineral water) with pH as close as possible to 7. Add a drop of phenol red indicator to the water to verify that the indicator color doesn't change in reaction with the water.
2. Preparing sterilized soil: Use an autoclave for soil sterilization. If an autoclave is not available, an oven can be used. Heat the soil to 160°C for two hours. Cool the soil before use.
3. To prepare 0.1% phenol red solution, dissolve 0.1g phenol red in 100 mL water with pH as close as possible to 7. Transfer about 10 mL phenol red solution to a dropper bottle labeled "phenol red". The solution can be prepared 3-4 days before testing.

Information and Notes for Teachers

1. This activity is a continuation of the "Testing for Organic Matter in Soil" activity, where organic matter presence is tested and soil rich in organic matter is selected for testing cellular respiration of living organisms within it.
2. The purpose of stage 2 testing is to verify that some of the organic matter found in the soil during stage 1 testing originates from living organisms performing cellular respiration.
3. The cellular respiration process is measured through color changes in phenol red solution occurring due to carbon dioxide release into the test tube during cellular respiration. Some of the released carbon dioxide dissolves in the phenol red solution, forming carbonic acid, which causes a decrease in solution pH and consequently changes the indicator color from red-pink to yellow-orange.
4. Usually, indicator color change can be observed within an hour of introducing soil to the test tubes. However, since cellular respiration rate is affected by various factors (such as temperature, soil



type, type and quantity of microorganisms in soil, etc.), color change might occur after several hours, so it's recommended to observe test results in an additional lesson several days later.

5. If needed, test tubes with soil can be left without concern for result deterioration for a week or more.

Answers to Student Questions

1. A more significant change was observed in tube 2 containing non-sterilized soil - from red to yellow-orange. This suggests that non-sterilized soil has more microorganism activity performing cellular respiration, producing more CO₂ and consequently lowering solution acidity.

2. a. Sterilized soil is soil that has undergone a process that killed all living microorganisms in it.

b. We used sterilized soil as a control - to compare between soil with living microorganisms and soil without living microorganisms, thus proving that color change is caused by microorganism activity.

3. The cellular respiration process of microorganisms causes a decrease in solution pH (makes it more acidic). This occurs because cellular respiration releases CO₂, which reacts with water to form carbonic acid, causing solution pH to decrease.

4. Starch is a polysaccharide composed of glucose molecules. We added it as a food source for microorganisms to perform cellular respiration at a faster rate. The microorganisms break down starch into glucose and use it in cellular respiration for energy production.

5. The tube with phenol red alone serves as a control - it shows us the original indicator color under normal conditions. This allows us to compare the changes in color in the other two tubes.

6. Compost contributes to plant development in several ways:

- Provides organic materials that decompose through microorganisms and release nutrients for plants

- Increases beneficial microorganism population in soil

- Improves soil structure and moisture retention capacity

- Provides continuous nutrition to plants through organic matter decomposition

7. Higher cellular respiration activity in soil indicates greater presence of microorganisms. High microbial activity indicates healthy and fertile soil, as microorganisms break down organic matter and release nutrients available to plants.



Effect of Soil Organisms on Seed Germination and Growth

Introduction

Soil is a complex system containing an enormous variety of living organisms, which are one of the factors affecting germination and growth processes. Some of these organisms, bacteria and fungi, assist in breaking down organic materials in the soil into smaller organic molecules and inorganic materials that are absorbed through plant roots. Some bacteria even produce substances that enhance root growth.

Excessive use of pesticides (substances used to control pests) harms soil organisms, leading to decreased soil quality and plant health.

In this activity, you will examine the effect of soil organisms on seed germination and growth processes of different seed types, comparing non-sterilized potting mix versus sterilized potting mix (which has undergone a process that eliminates all living organisms).

Materials and Equipment

- 2 growing containers
- Sterilized potting mix
- Non-sterilized potting mix
- Various types of seeds
- Container with tap water
- Ruler
- Measuring tool (measuring cup or cylinder)
- Wooden skewer
- Labels/stickers
- Marking pen

Procedure

1. Fill both containers with equal amounts of potting mix. Add non-sterilized mix to one container and sterilized mix to the other.
2. Using the marking pen, mark a line on the wooden skewer 2 cm from the tip (use the ruler).
3. Insert the skewer into the potting mix up to the marked line to create a hole.

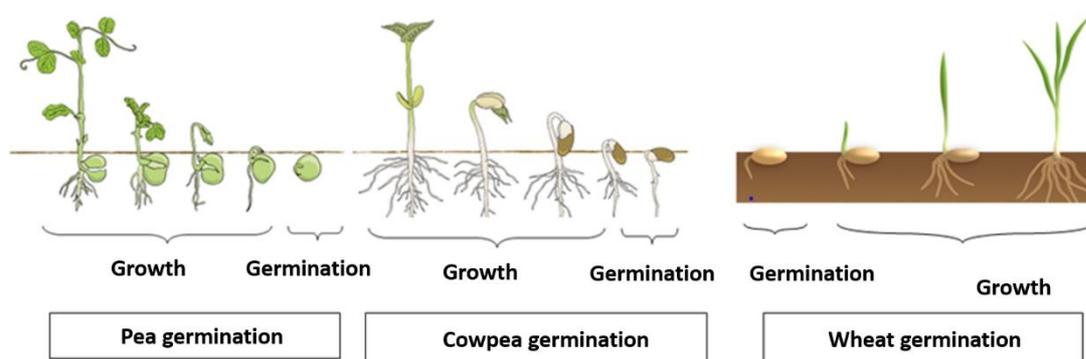


- Repeat step 3 to create 4 additional holes at equal distances from each other.
- Place one seed in each hole in both containers and cover the holes with potting mix.
- Using the measuring cylinder, water each container with enough water to thoroughly moisten the mix, using equal amounts of water in each container.
- Write your name, seed type, and potting mix condition (sterilized/non-sterilized) on labels/stickers and attach them to both containers.
- Place the containers in a well-lit location (natural light).

****The following steps will be performed two days after planting: ****

- After two days, count the germinated seeds in both containers (see Figure 1). Complete the missing information in Table 1.
- Continue watering the seeds according to the moisture level of the potting mix.

Figure 1 - Stages of Germination and Growth



<https://commons.wikimedia.org/wiki/User:MAKY.OREL>

****The following step will be performed one week and two weeks after planting: ****

- After one week, measure the shoot length of the seedlings (carefully, without damaging them) in both containers. Calculate the average shoot length for each container and complete the missing information in Table 1.
- Repeat step 11 two weeks after planting.

Germination is the process where a seed begins to develop into a plant. Initially, water from the seed's environment enters the seed, causing an increase in enzyme activity (special proteins that accelerate chemical processes) in the seed. The enzymes accelerate the breakdown of stored food



(storage materials) in the seed. Glucose produced from breaking down the storage material starch is used to generate energy needed for germination.

The germination process ends at the radicle (initial root) emergence stage. The process of plumule (initial shoot) emergence is called growth.

Summarize the growth process results in the table:



Table 1. Seed Type: _____ (Fill in)

	Container with Non-sterilized Potting Mix					Container with Sterilized Potting Mix				
Number of germinated seeds after two days										
Shoot lengths after one week (cm)					Average shoot length:					Average shoot length:
Shoot lengths after two weeks (cm)					Average shoot length:					Average shoot length:

Questions

1. Describe the germination in both containers.
2. Explain the effect of presence/absence of microorganisms in the potting mix on the germination and growth process.
3. Explain why you think it was important to include a container with non-sterilized potting mix in the experiment.
4. Explain why you think it was important to water the containers with equal amounts of water, add equal amounts of soil, and place the containers in the same conditions.
5. Hypothesize what might be the reasons for differences in seedling growth between the two containers.
6. Propose an experiment that could test your hypothesis.
7. Based on the experiment results and the "Introduction" section, what do you think could be the effect of excessive pesticide use on agricultural crops? Explain your answer.



Effect of Soil Organisms on Seed Germination and Growth
Teacher and Lab Technician Guide

Target Audience: Grades 7-9

Duration: About 45 minutes. Subsequently requires measurements after two days, one week, and two weeks.

Time required for each measurement: about 10-15 minutes.

Experiment Objective

To demonstrate the effect of presence and absence of soil organisms on germination and growth processes in different types of seeds.

Central Activity

During the experiment, students plant seeds in both sterilized and non-sterilized potting mix and monitor seedling growth in both containers.

Curriculum Connection (8th grade)

Plant Development: Plant Life Cycle - From Seed to Seed (Extension)

- Germination under different environmental conditions
- Plant development - Conditions required for plant development (water, temperature, light)

Key Concepts for Teaching the Experiment

Germination, growth, factors affecting germination and growth processes

Materials and Equipment (per pair of students)

Materials and Equipment	Quantity
Empty container approximately 10 cm in diameter suitable for planting (e.g., 20 mL disposable containers that can be reused after washing)	2
Potting mix	Amount according to container size
Sterilized potting mix	Amount according to container size
Tap water	Amount sufficient for thorough soil moistening (about 50 mL per container)



Labels/stickers	For marking seed type on growing containers
Seeds (Cowpea/Wheat/Green lentils/Black lentils or other seed types)	10 seeds

Information and Notes for Teachers

- Guide students regarding seed watering: Pour water from above in a thin, gentle stream to avoid creating holes in the soil that might expose the seeds.
- After initial planting and watering, maintain moist but not waterlogged potting mix and water seeds/seedlings as needed. Water quantity depends on season and container location in the room.
- If students cannot water the seeds after initial watering, containers can be placed in tall-sided vessels that allow for shoot elongation and covered with plastic wrap. Pierce the plastic to allow gas exchange.
- Guide students to measure shoot length carefully without damaging the shoots.
- Germination and growth are two important stages in plant development. The germination stage is relatively short and characterized by seed swelling, increased enzymatic activity, and radicle emergence. Energy for germination comes from seed storage materials. Growth is an ongoing process that begins after germination, during which the plant grows and develops using glucose produced through photosynthesis for energy and creating additional organic materials.
- In this activity, seeds are covered with potting mix, so the germination stage cannot be observed directly, only the growth stage when shoots emerge above the mix.

Answers to Student Questions

1. Most seeds germinated in the container with non-sterilized soil. Student should specify the number of germinated seeds in each container.
2. According to the introduction, soil microorganisms (bacteria and fungi) affect germination and growth in several ways:
 - Help break down organic materials into smaller molecules and inorganic materials that plants can absorb
 - Some bacteria produce substances that enhance root growth
3. The container with non-sterilized mix serves as a control group in the experiment. It allows comparison of the effect of microorganism presence versus absence (in sterilized mix) on germination and growth.



4. It's important to maintain constant factors (water amount, soil amount, environmental conditions) so that the only difference between treatments is the presence/absence of microorganisms. This is an important scientific principle - isolating a single variable to draw conclusions about its effect.
5. Growth differences between containers may result from:
- Microorganisms' ability to break down organic materials into nutrients available to plants
 - Production of root growth-promoting substances by bacteria
 - Improvement of soil structure by microorganisms
6. Possible experiment to test the hypothesis:
- Grow plants in three groups:
 1. Regular soil with microorganisms
 2. Sterilized soil
 3. Sterilized soil + added inorganic nutrients needed by plants (e.g., fertilizer addition)
 - If growth in group 3 is similar to group 1, this would support the hypothesis that the difference is due to nutrient availability.
7. Based on the file information, excessive pesticide use could:
- Harm beneficial soil microorganism populations
 - Reduce nutrient availability to plants
 - Impair root growth
 - Lead to decreased agricultural yield and plant health

This would damage soil fertility and long-term agricultural crop success.