Microbe Probe

Overview

In this activity, students research the antimicrobial properties of copper. To test copper's effects on microbes, students use copper sulfate (which ancient alchemists called blue vitriol). Students vary the concentration of copper sulfate solution to determine its efficacy at killing bacteria.

Grade Band: 6 – 8

Topic: Antimicrobial Properties

Real World Science Topics

- Natural Selection
- Minerals

Objectives

Students will

- Hypothesize the effects of copper sulfate solution on bacterial cultures.
- Investigate the effects of solute concentration on bacterial cultures.
- Draw evidence-based conclusions about the antimicrobial properties of copper.
- Create design ideas for ways in which future technologies could use the antimicrobial properties of copper.

Next Generation Science Standards

MS-LS1-1. Conduct an investigation to provide evidence that living things are made of cells; either one cell or many different numbers and types of cells.
MS-LS1-5. Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms.
MS-ETS1-2. Evaluate competing design solutions using a systematic process to determine how well they meet the criteria and constraints of the problem.
MS-ETS1-3. Analyze data from tests to determine similarities and differences among several design solutions to identify the best characteristics of each that can be combined into a new solution to better meet the criteria for success.

Time Needed: 1-2 hours and three 10-minute observation sessions

Background Information

How long have humans known about copper's antimicrobial effects?

We have known only since the late nineteenth century that germs cause disease. However, people have benefited from copper's antimicrobial effects since ancient times. As far back as 2500 BC, Egyptian alchemists and physicians developed various medicines that contained copper. People noticed that water stored in copper vessels was healthier and purer than water stored in other ways. In the pre-antibiotic era, copper was an essential weapon in the doctor's arsenal for the fight against disease. More recently, researchers have suggested that copper plays a vital role in the function of the immune system as well as being able to cure diseases.

Key Vocabulary

Antimicrobial – Destroys or inhibits the growth of microorganisms

Materials

- Distilled water
- Agar
- Copper sulfate
- Petri dish
- Sterile cotton swabs
- 5 ml graduated polyethylene transfer pipets
- Probiotic capsule pills containing Lactobacillus acidophilus or L. plantarum
- Nutrient broth (e.g., chicken or beef stock)
- Plastic film

Equipment

- 100 ml beaker
- Bunsen burner or hot plate
- Scale
- Microwave
- Rubber gloves
- Safety goggles
- Marker pen
- Ruler with millimeters
- Breathing mask
- Incubator (optional)

Preparation

- This activity suggests using bacteria cultured from probiotic pills, which are readily available in most supermarkets or from pharmacies over the counter. Be sure to use a single species brand, such as Lactobacillus. However, results may be more reliable using a safe strain of E. coli live culture, which can be purchased from online classroom science equipment vendors.
- To save time, make up the copper sulfate solutions beforehand. You will need solutions of five different concentrations. To make the solutions, dissolve anhydrous copper sulfate (CuSO4) crystals in distilled water. Since copper sulfate dust is an irritant, use a safety breathing mask while preparing the solutions.
- Purchase petri dishes that have been pre-poured with agar. Some kits come with swabs that are used to streak the bacteria-containing liquid onto the agar.
- For each of the solutions, dissolve the following weight of copper sulfate in one liter of distilled water:
  - 0.3 g
  - 0.6 g
  - 0.8 g
  - 1.3 g
  - 1.6 g
- Since the agar will absorb the copper solution, be sure to pre-soak the plates in their respective solution, leaving them overnight to ensure the solution is distributed uniformly. Soak control plates in distilled water. Pour off any excess solution. Alternatively, consider pouring your own plates, using copper sulfate solution instead of water. You could use this as a demonstration for students.
- Ideally, students incubate their agar plates at 37°C. If this temperature is unavailable, any warm, dark location will do. However, more time may be needed for bacterial colonies to become visible.
- The dilution step carried out by students ensures that the plated culture provides colonies rather than a “lawn” of bacteria. If individual colonies are not visible, the students can experiment with different dilutions.
- If needed, demonstrate the methods for labeling plates and for streaking and isolating bacteria on an agar plate. If students don’t spread bacteria properly, individual colonies may not be visible. However, students should still see the difference in growth between the solutions of different concentration.
- Concentrations above 0.6 g may be toxic to bacteria, so the correct hypothesis is that bacterial growth will be visible in the 0.3 g, 0.6 g, and control plates. Growth will be less or even absent in the 0.8 g, 1.3 g and 1.6 g plates.

Instructional Options

- Ensure that students follow lab safety procedures at all times.
- Quantities will vary according to class size and ability. Ensure enough of each item so that student groups can test at least two dilutions and a control (four agar plates per group).
  However, depending on time available and student ability, consider assigning just one of the plates to each group, and then compare group results in the class.
- Students can provide written or visual descriptions of their observations. If appropriate, students can take photographs of their agar plates to record their observations.

Procedure

1. **Warm-Up Activity:** Engage the class by asking what will happen when "super-bugs" become resistant to all antibiotics. Clarify with students that "super-bugs" are strains of bacteria that have become resistant to several types of antibiotic drugs. This means that medicines we typically use are no longer an effective treatment.

2. Explain that medicine may revert to the pre-antibiotic era, when many diseases that are easily curable and treatable today, were then often fatal.

3. Guide students to research online the options for alternatives to antibiotics, including the antimicrobial properties of copper.

4. Provide the class with the student worksheet to begin the activity. Organize groups of 3-4 students each.

5. Ensure that students follow safety procedures and best practices for lab activities.

6. Encourage students to hypothesize on the effects of the copper sulfate solutions of different concentrations.

7. **Evaluate: Summary**
   Invite students to write up their experiment in their notebooks, explaining whether or not their observations are consistent with their hypotheses.

Extension Activity

Still working in groups, students brainstorm ideas for ways in which future technologies could use the antimicrobial properties of copper. Students present their ideas to the class. For this presentation, students choose their own medium such as poster, webpage, brochure, skit, etc.

Additional Optional Resources

http://www.antimicrobialcopper.org/
http://www.copper.org/publications/newsletters/innovations/2000/06/medicine-chest.html
http://www.sciencebuddies.org/science-fair-projects/ask-an-expert/viewtopic.php?t=15667
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC93308/
https://www.addgene.org/plasmid-protocols/streak-plate/
http://www.copper.org/education/c-facts/facts-print.html
http://teach.genetics.utah.edu/content/microbiology/plates/

Microbe Probe Student Worksheet

Topic: Investigating the antimicrobial properties of copper

In this activity, you will research how copper affects bacteria. You will use different strengths of copper sulfate solution. You will discover how the different concentrations kill bacteria. Your experiment uses safe bacteria. These bacteria are grown on agar plates. The agar plates are treated with the different concentrations. You will work in small groups. After your activity, you will present your findings to the class.

Materials

- bottle of hand sanitizer
- 100 ml distilled water
- 6 pre-poured sterile LB-agar plates each of 4 solutions with different concentrations of copper sulfate
- 6 sterile cotton swabs
- 5 ml graduated polyethylene transfer pipet
- probiotic capsule pills containing Lactobacillus acidophilus or L. plantarum
- 250 ml nutrient broth (e.g., chicken or beef stock)
- plastic film

Equipment

- 100 ml beaker
- Bunsen burner or hot plate
- Rubber gloves
- Safety goggles
- Marker pen
- Ruler with millimeters

FOLLOW YOUR TEACHER'S INSTRUCTIONS AND ALL SAFETY PROCEDURES!

Procedure

1. Research the antimicrobial properties of copper. Then hypothesize the differences between the four concentrations of copper sulfate solution. Be sure to include the control in your hypothesis.

2. Prepare your bacterial culture. Boil the nutrient broth for five minutes. Cover with plastic film and allow to cool.

3. Set aside 225 ml of the nutrient broth, keeping it covered with the plastic film. If possible, refrigerate this portion.

4. Empty the probiotic capsule into the remaining 25 ml of broth. Shake to mix well. Store the culture in a warm place for 24 hours. (The exact temperature and time don't matter.)

5. Dilute the culture. Take 1 ml of the culture and add 100 ml of the nutrient broth you set aside. Take 1 ml of this first dilution and add another 100 ml of the broth. Take 1 ml of the second dilution and add 10 ml of the broth. You will use this diluted culture.

6. Identify each of the agar plates according to the solution concentration, including the control.

7. Transfer 1ml of the diluted culture onto one of the agar plates. Use a cotton swab to spread the culture evenly across the plate. Repeat this step for each of the prepared agar plates containing solution and the control plate.

8. Leave the plates in a warm, dark location for 24 to 36 hours, ideally at 37°C.

9. When colonies become visible on any of the plates, note your observations. Count the number of colonies and measure the diameter of the colonies. Enter the data into the table.

10. Continue to record your observations for four to five days.

11. When you have completed your observations, construct an explanation that supports or refutes your hypothesis.

Sample Data Table

N = number of colonies
S = average size of colonies (mm)

	Control		0.3 g		0.6 g		0.8 g		1.3 g		1.6 g	
	N	S	N	S	N	S	N	S	N	S	N	S
Day												
1												
2												
3												
4												
5												