



Outdoors

Finding the beauty in microbial diversity

Grade level	High school
Standards	<p>HS-LS2-3. Construct and revise an explanation based on evidence for the cycling of matter and flow of energy in aerobic and anaerobic conditions.</p> <p>ESS2.E Biogeology The many dynamic and delicate feedbacks between the biosphere and other Earth systems cause a continual co-evolution of Earth's surface and the life that exists on it. (HS-ESS2-7)</p>
Learning outcomes	<p>1.) Visualize microbial life through a microscope</p> <p>2.) Sketch and describe microbial colonies grown on agar plates.</p>
Goals	<p>Field trip: Collect environmental samples that could harbor microbes. Find evidence of microbial interactions with the environment. (This is done with the “finding the beauty in microbial diversity” field trip module)</p> <p>Class day 1: Construct winogradsky columns from collected samples Observe growth of microbes on the agar plates, evaluate the difference sources of environmental samples and their effect on microbial growth</p> <p>Class day 2:</p> <ul style="list-style-type: none"> ● Examine winogradsky columns and note changes/differences in the column ● Correlate the changes in the column to different aspects of spatial organization, nutrient cycling, and environmental conditions.

	<ul style="list-style-type: none"> ● Observe the microscopic communities that grew and link that to the environmental source ● Extend the growth in the Winogradsky column to the real world, consider natural environments that are influenced by microbes
Brief description	This is a paired lesson about microbes with two classroom lessons/lab activities following a field trip where samples were collected.
Time	<p>Day 1: 1 hr 30 mins Day 2: 1 hr 30 mins</p> <p>Day 1 happens ~1-2 weeks after field trip samples were collected; Day 2 happens >1 month after Day 1.</p>
# students	20
Materials	<p><u>Day 1:</u> Students' samples collected from field trip Extra mud samples for anyone who missed the trip or to make "example" columns for the whole class Agar plates collected from field trip</p> <p>INCUBATION AGAR PLATES AFTER FIELD TRIP AT ROOM TEMPERATURE FOR TWO DAYS THEN PUT IN FRIDGE!</p> <p>Microscopes Slides & cover slips Toothpicks (for colony picking from agar plate) Winogradsky columns (tissue culture flasks) Labels for columns (sharpies) 10 mason jars for mixing material & sample (for working in pairs at station to make columns). Stirring rod in case of needing to stir Column material (assemble beforehand into falcon tubes): –diatomaceous earth –cellulose –salts –yeast extract –fresh water media (mock, made up in lab, or from Caltech campus lily ponds or similar) <i>*Detailed recipe is attached to this document</i> Bags/containers for mixing column material slurry (8 oz jars)</p>

	<p>Worksheet to record observations over time Camera (photograph setup)</p> <p><u>Day 2:</u> Microscope (if connecting to a projector, ensure there is an HDMI adapter. USB connection to computer. AmScopeAmLite Software for the microscope is available at https://amscope.com/pages/software-downloads. Select Camera > MD500) Pre-made winogradsky columns Glass slides & cover slips Toothpicks Plastic pipettes or scoops (to sample Winogradsky columns)</p>
*Location	Classroom (Developed for John Muir High School)
*Logistics	<p><u>After field trip:</u> Incubate agar plates that were collected on field trip for “Day 1” classroom lesson visit – if <2 weeks, @ 30° C; if > 2 weeks, leave at room temperature.</p> <p><u>Day 1:</u> Before visiting classroom, create falcon tubes of Winogradsky column materials by measuring out materials following detailed recipe (below, attached). Collect ~6 liters of Fresh Water Media to bring to create columns (create in lab or from pond)</p> <p><u>Day 2:</u> No prep outside of the classroom.</p>
*Caltech student needed?	<p>This was developed as part of an MBL-funded Outreach program, for collaboration between Caltech GO-Outdoors and teachers at John Muir High School.</p> <p>2 GO-Outdoors volunteers are suggested per classroom day.</p> <p>Several GO-Outdoors volunteers are suggested to help assemble column material & other logistics before the lessons.</p>
*Accessibility	<p>This lesson is based on visual observation, so having pairs to describe visual changes for any students who require vision assistance is suggested.</p> <p>The assembly requires manual dexterity and fine-motor control sufficient to open jars & manipulate/pour contents into beakers</p>

& select microbial colonies to make microscope slides, so students will work in pairs and help each other.

Lesson activities

Day 1:

~12:20 pm: Arrive at classroom (Leader 1 & Leader 2)

12:30 pm: Personal introduction/hello

Leader 1: Set up microscopes & stations

Leader 2: introduce lesson / logistics

Introduction (15 mins): (slide show?)

Explain to students that they'll be incubating their samples they got from the field trip for 1 month! They get to see changes over time, which they will record, and we will come back to see what they find!

12:45 pm: Break into 2 groups at 2 stations – (1) Microscope/agar plates (2) column assembly

Rotate after 30 minutes (1:15 pm)

Station 1: Microscope/agar plates (Leader 1)

10 students

Colonies from agar plates will be selected using toothpicks, place onto slides and cover with coverslip

Station 2: Winogradsky Column assembly (Leader 2)

10 students, work in pairs (5 pairs):

Each pair gets 2 sets of material for columns & 1 set of 2 beakers/jars for stirring things up.

Make 1 student's column, dumping dry (diatomaceous earth etc) ingredients into 1 beaker, wet (soil sample & Fresh Water media) into 2nd beaker – each student shake up to homogenize into a slurry.

Students pour into their first column & label.

Rinse these beakers

Repeat this process for second student in pair.

Rinse the beakers, and leave for the next group of students.

Leave columns in a set location, so that during clean-up, students can choose their spot where column will incubate for the next month!

1:45 pm: Wrap-up:

- Hand out observation worksheets & instructions for observations over time
- Students help clean up
- Volunteer & leader pack up

2:00 pm: leave!

Day 2:

~12:15 pm: Arrive at classroom and set up computer and microscope to projector. If they have their own microscopes for the class, set tables up with microscope, pipette tips, slides and coverslips

12:30: Class starts, brief re-introduction, have kids collect their Winogradsky columns

- Leader 1 remind the class about the field trip and how the columns were setup
- Leader 2 prepares the winogradsky sample for the projected microscope
 - Using plastic pipettes, pull small amounts of material from an interesting layer of the column - try for films or mats visible in the layers
 - sometimes difficult to get deeper layers without contamination. Try staying along the side until reaching the layer of interest
 - smear or drip the material onto the slide and cover with coverslip
 - **MAKE SURE NOT TO ADD TOO MUCH MATERIAL** - you want it to be mostly microbes and water, a thick layer or sediment will make it harder to visualize

12:45: Think-Pair-Share at tables (groups of 2-3)

- What changes have happened to the column since day 1? Refer to observations made over time if they've kept regular notes, or a blank column without added samples.
 - Color, layers, etc
 - Did the changes happen at the same time?
- What similarities/differences are there between the columns?

~1:00: have students share their responses. Write on the board common changes that happened to the columns (can draw a column diagram to write on).

- Expect a top green layer; ask the students what the differences might be between the top layer and the lower layers?
- Are there other colors? What might those other colors mean?
 - Either changes in the chemistry or presence of new pigments

1:10: Introducing the microscope - we successfully grew microbes that we couldn't see until we could, and now we can look even closer.

(Optional: have students sample from their columns and set onto their own slides - add 15 minutes to introduce how to get samples and put onto slides)

- Project up sample from the premade winogradsky column - what can we see about the microbes from this view that we couldn't before?

1:30: Discuss that if there are different communities, why have different layers formed? How do they decide where to go from the big mixture they started at?

- They breathe different things other than oxygen!
- Do we think if we sampled somewhere else we would see something different?
- If we let them be, will it stay the same? Will it eventually stabilize?
- How well do these columns represent the real world?
- Examples of relevant environmental microbes:
 - Algal blooms from runoff after rain
 - Biofuels
 - Wastewater treatment plants
 - Bioremediation (after oil spills)
 - Antibacterial compounds derived from microbes (deep sea and terrestrial) - may have seen an example on the agar plates

1:50: survey, cleanup and farewell

Optional extension activities

[Winogradsky column overview and lesson](#)

[Microbes used in LA wastewater treatment](#)

Videos:

[Life Without Oxygen](#)

[Winogradsky Column Video](#)

[Purple Sulfur Bacteria](#)

***Instructor support**

Handling of Agar plate: any direct contact with the agar material has the possibility to lead to microbial growth, so be careful to handle with gloves to avoid contamination. Keep at room temperature for ~1-2 weeks, or in an incubator (30 or 37 °C) for a few days for growth.

Winogradsky column details are in a recipe attached below – students will use a simplified recipe in their handout.

Here's what a Winogradsky column is, and how to handle it:

[Winogradsky column overview and lesson](#)

Microscope/slide creation info :<https://www.wikihow.com/Use-a-Microscope-to-Observe-Microorganisms>

Links/details about how microbial culturing works:

<https://www.coursehero.com/study-guides/boundless-microbiology/microbial-culture-methods/>

***Common misconceptions about the lesson**

Microbes (bacteria, viruses) are always bad

We can grow all microbes in the lab easily

A petri dish shows all of the microbes that are in a sample

Microbes are too small to affect the global environment

There are a lot of places that microbes can't live

If I can't see microbes then they aren't there (like clear water or in sand)

***Opportunities to engage students in planning**

If the lesson structure and outreach relationships with the classroom permit, you may wish to actively engage students in the planning or conducting of the lesson. For examples of this process, explore the lesson plans at

<https://sciencegals.org/lesson-plans/>.

***Handouts**

Recipe for Winogradsky Column handout

Worksheet to record observations over time

Preparing Winogradsky Columns

Salts: Each student will need all of the following salts in individual 1.5 mL plastic tubes. These weights are approximate +/- 20% is fine! Label the top of each with a "P", "N", "S", or "C" based on their elemental composition. Sort into five mason jars.

- 0.5 grams NH₄Cl
- 0.5 grams Na₂SO₄
- 0.5 grams KH₂PO₄
- 0.5 grams cellulose + 0.5 grams CaCO₃ + 0.2 grams Yeast Extract (same Epp tube)

20 students total + 5 extra = 25 tubes of each salt!

Winogradsky Columns:

Preparing environmental samples:

- 1.) Measure ~300mL (with mason jar) of environmental water
- 2.) Put solid environmental sample (soil, mud, etc.) into the water and stir with a spoon
- 3.) Let the solids fall to the bottom of the mason jar

Preparing Diatomaceous Earth Slurry:

- 1.) Mix 300 mL diatomaceous earth with N, P, and S salts into another mason jar
- 2.) Pour only the environmental liquid (see above) onto the diatomaceous earth (**avoid pouring out large chunks of sediment**)
- 3.) Stir into a mixture that has the consistency of a thick milkshake

Preparing column container:

- 1.) To the bottom of the container add the carbon salts and spread them around evenly on the bottom

Inoculate Winogradsky Column:

- 1.) Using a funnel, pour the diatomaceous earth slurry into the column.
- 2.) Allow the diatomaceous earth to settle

5YE Agar Plate Recipe: When opening the sleeve of empty plates, carefully cut the top off so it can be rolled up as a storage container afterwards.

*1L makes 2 sleeves of agar plates

*For 1 liter of media

- 1.) 1 L Tap water
- 2.) 5g yeast extract
- 3.) Autoclave
- 4.) Pour plates
- 5.) Put plates back in the sleeve (no need to Parafilm individually)

Finding the beauty in microbial diversity: Agar Plate Observations

Question	Answer
Where did the samples come from? Did you notice any life growing there?	
How many colonies are growing on the plate?	
Describe the colonies growing on your plate. (i.e. color, shape, size, etc)	
How many different types of colonies can you identify?	
What questions do you have about what grew on the plate?	

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Finding the beauty in microbial diversity: Winogradsky Observations

Question	Answer
Where did the samples come from?	
Draw a diagram of your column. Note layers, color changes, and any other observations you have. What do you think the layers are?	
What questions do you have about the microbes in the column?	

Finding the beauty in microbial diversity: Winogradsky Observations

Question	Answer
Where did the samples come from?	
Draw a diagram of your column. Note layers, color changes, and any other observations you have. How many layers of different microbes do you think there are?	

What questions do you have about the column?	
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Finding the beauty in microbial diversity

Topic	How much do you know now, after the lesson?				How much did you like learning about this topic?		
	Nothing	A little	Some	A lot	Dislike	Like	Love!
Some life on Earth can exist without oxygen.							
There is lots of diversity among microorganisms on Earth.							
There are ways to observe microbial life with my eyes.							

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