Building Winogradsky Columns

Winogradsky columns are a method for essentially taking a snapshot of a sediment environment and studying the microbial communities that live there. Winogradsky columns can be used to teach students about microbial communities, nutrient cycles, metabolic niches, methods in microbiology and other interesting concepts. This protocol includes information on how to build Winogradsky columns, different variables that can be explored, tips for the classroom, and a field guide for collecting interesting sediment samples.

MATERIALS

Item	Amount (for 1 gallon of sediment mixture)	Purpose
Sediment sample	1 quart (or as little as one cup)	The sediment (or mud) sample is a snapshot of the environment under investigation. It is the inoculum for the community of microbes that will grow in the Winogradsky column. Therefore, the sample site is important to which microbes will grow in the column.
Sand	3 quarts	Adding sand to a mud sample creates a more gradual gradient of the spread of microbial populations throughout the column. This allows for better visualization of the different layers of microbes. (If the sediment sample is already of sand, additional sand need not be added.)
Water (from same source as sediment sample) + dechlorinated water (leave tap water open to air for several days for dechlorination). *Note that some municipalities use chloramines which cannot be removed in this way. They will only inhibit, not prevent growth.	1-2 quarts	Water collected from the source of the mud will act as an additional inoculum of microbes and also help to recreate the original environment. Dechlorinated tap water will also sustain the microbial growth, but the other ingredients become more important.

MATERIALS (contd.)		
Item	**Amount (for 1 gallon of sediment mixture)**	**Purpose**
Dried leaves or shredded paper	1/2 cup	Dried leaves or shredded paper are the source of cellulose, or carbon.
Plaster of Paris (Calcium Sulfate dehydrate i.e. CaSO4·2H2O and other binders). Alternate: Epsom salt (magnesium sulfate i.e. MgSO4) can also be used.	1 tbs	Plaster of Paris is the source of sulfur in the column.
liquid fertilizer (N-P-K: 4-12-4)	3 tbs	The liquid fertilizer is primarily a source of phosphorous. When choosing one, select the fertilizer that has the highest ratio of Phosphorous. Without nitrogen, nitrogen-fixing microorganisms may be selected for as a part of the community.
Antacids (CaCO3, and/or NaHCO3, KHCO3 and inert binders)	20 tablets dissolved in hot water	Antacid tables are a source of calcium carbonate which is a buffering agent and a source of CO_2 for autotrophs.
Non-fat dry milk	1 tbs	A good source of protein, as well as calcium. It is important to get non-fat as the fermentation of fats could cause unpleasant odors. It is an optional addition.
Multivitamin (crushed)	1/4 – 1/2 tablet	The multivitamin adds trace metals to the Winogradsky column. It is an optional addition.
Quart jars	4	Containers for building and growing the Winogradsky columns. Glass or plastic jars will work but glass is preferable as it is much less permeable to O_2, a toxic gas to many microorganisms.
Permanent marker		To label each Winogradsky column
Measuring cups		To measure out ingredients
Shovel		For collecting sediment samples
Buckets		For collecting sediment samples and mixing ingredients

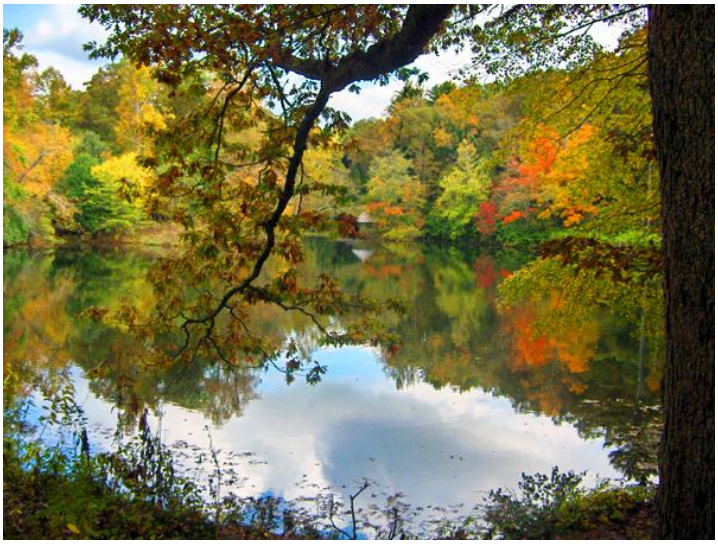
PROCEDURE

About this protocol

This protocol is designed with classrooms, teachers, and students in mind. The materials were chosen based on their low cost and easy availability. Many of the materials included in this protocol help to increase the biomass and rates of microbial growth in the Winogradsky column. Other materials can also be used to support microbial growth as long as antimicrobials are avoided (e.g. some paper towels contain bleach). Additionally, with a sediment sample from a good environment, the inoculum may be rich enough in microbes to add only water and sand.

STEP A: Collecting sediment

Sediment is the inoculum of microbes for a Winogradsky column and can be collected from just about anywhere where there is sediment and water (i.e. lake, stream, ocean, pond etc.). Though individual microbial cells can only be seen under a microscope, in some instances, large colonies of microbes produce field marks that are visible to the naked eye. The brief field guide section of the protocol describes some of these field marks and the types of microbes they indicate. However, remember that microbes are nearly everywhere, and any sample of sediment will eventually likely yield conspicuous microbial growth in a Winogradsky column.



(CC-BY-SA 3.0) Mountain Vacation Resorts

Late-summer or early fall are good times of year to collect sediment samples. Samples from a plant-matter or carbon-rich environment, particularly with signs of anoxia (refer to field guide section) are more likely to produce Winogradsky columns with diverse microbial communities.

When collecting sediment, also collect water from the same source. The water can also be an inoculum for microbes and will be added to the Winogradsky columns to help preserve the components of the natural environment from which the sediment was collected.

It is a good idea to take a picture of the location from which the sediment is collected and write a brief description about the area to keep on record.

Storing sediment samples

If you need to store the sediment samples for some time before constructing the Winogradsky columns, store them in a cool, dark place. Immerse the samples in water and cover them loosely so they do not dry out. DO NOT seal the columns completely! Gas can build up in the column and if tightly, sealed, can explode. Try to collect sediment within a week of when the columns will be assembled.

Safety concerns

Though minimal, there are some safety concerns regarding the assembling and handling of Winogradsky columns. In most cases, if a person can be outside around soil, they should be able to be around and work with the columns. The vast majority of microbes in the environment are non-pathogenic and unable to grown in the human body, which is a very different environment. Also, many of the organisms that grow in the columns (especially below the top bright green layer) are killed by exposure to oxygen which is poisonous to them.

However, it is advisable to always exercise caution when gathering samples and handling the columns. To maintain safe protocol, follow these simple cautionary steps:

- To limit growth of fungi and release of spores:
    - keep the mixture in the columns moist with a layer of water on top
    - make certain there is little to no organic material on top of the mixture
- Do not breathe in directly over an uncovered column.
- Wear gloves when handling the sediment mixture and columns.

STEP B: Constructing the Winogradsky Columns

Winogradsky columns are a tool for investigating the microbial community that is naturally present in the collected sediment sample. Therefore, when assembling a column, keep in mind that the goal is to recreate the environment from which the sediment sample was obtained in order to perpetuate the microbial diversity already present in the sample. For example, freshwater pond mud will differ from saltwater ocean sand in its level of sulfur and salt, and therefore, its pH. This recipe is designed for freshwater sources such as ponds and lakes but the quantities of the ingredients can be modified to accommodate sediment samples from any source type. For example, to match the environment of an ocean beach, aim for a lower pH when building the Winogradsky column by adding more plaster of paris (the source of sulfur in the column) and salt to match the source, or by using seawater with naturally occurring sulfide. The following instructions are based on the recipe for freshwater sources. Use seawater or aquarium salt water instead of tap water for marine samples, and omit the sulfur source, as large amounts of sulfate will already be present in the marine sample.

1. Label the jars with the following information:
    - Source of sediment
    - Date
    - Student's/Group's Name
    - Variables (optional)
2. Mix together the following materials:
    - 2 cups of the dechlorinated water heated till warm
    - Dissolve antacid tablets
    - Stir in plaster of paris (avoid clumping)
    - Stir in non-fat dry milk
    - Stir in liquid fertilizer
3. To the mixture in step 2, add sand

You now have the base mixture to which can be added the sediment sample. This is a good time to test the pH of the mixture with pH paper to see if it is approximately the same as that of the sediment sample. For samples collected from freshwater sources, a pH 7-8 is desirable. After the sand and nutrient mixture has been thoroughly mixed, measure its pH using litmus paper. If the mixture is slightly acidic, add small amounts of baking soda to raise the pH. If the mixture is slightly more basic, add small amounts of vinegar to lower the pH. If no pH paper is available, adding the full amount of sediment and using more natural water will likely yield an acceptable pH.

For the next steps, if you are working with only one sediment sample, everything can be mixed together in one bucket. However, if you are using sediment samples from different locations, divide up the base mixture from Step 3 and follow the remaining steps once for each different sample.

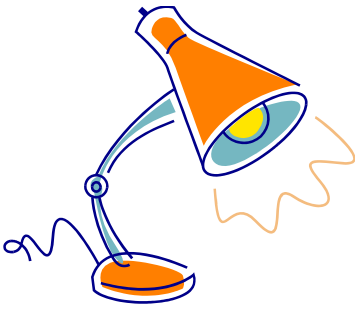
4. Mix the sediment sample into the sand mixture from Step 3. (Make sure any large sticks, rocks, roots, etc. are removed from the sediment first.) Mix everything together thoroughly.
    - Note: If you are making columns using sediment samples from multiple sources, first divide the sand mixture into separate containers and then mix in the different sediment samples. The approximate ratio of sand mixture : sediment sample should be 3 : 1.
5. Mix in water (first add in water collected near the sediment and then de-chlorinated tap water as necessary) until the mixture has a thick, sludge-like (similar to dense cake batter) consistency.
6. Prepare the columns
    - Line the bottom of each jar with the cellulose source (dried leaves or shredded paper). Wet it down to avoid air bubbles.
    - Spoon the mixture from Step 5 into each appropriately labeled jar. Add the mixture carefully and slowly and pat it down as you go to avoid air bubbles in the jar. Fill the jar up to about 2cm below the neck.
    - Add a layer of water (collected from the sediment source or de-chlorinated tap water) on top of the mixture up to about 1.5cm below the rim of the jar.
    - Place the lid on each jar (make sure the labels match the contents of the jars!). **DO NOT** tighten the lids! (Build up of gas can cause an explosion if the column is tightly covered.)
    - (Optional) Place a 1 inch strip of black construction paper or aluminum foil around the bottom of the jar to provide a non-phototropic environment at the bottom.
    - Place the columns in a well-lit area. To speed up the growth of microbes, set up a light to shine on the columns. If testing effects of light sources, the columns can be placed in different areas of varying light or under lamps with colored light bulbs.
    - Store the columns in a tray or plastic bowl to catch any overflow that may occur.

STEP C: Maintaining the columns
Once constructed, the Winogradsky columns can be kept for months to years. The microbial communities can slowly change over time and making them interesting to continually observe. To maintain the columns and prevent them from drying out, periodically add dechlorinated water so there is a layer of water on top of the sediment mixture.

VARIATIONS AND VARIABLES

A number of different activities can be done using the Winogradsky columns in the classroom. The columns can simply be allowed to mature in a well-lit area and students can observe the growth and diversity of microbes in their sample. Alternatively, students can manipulate variables to test microbial growth in different conditions. Below are some possibilities of ways students can investigate questions such as “Which microbial populations will grow better?”, “How will the community structure be affected?” etc.

Amounts of nutrients:
Students can create columns with differing amounts of a single ingredient such as, sulfur, salt, cellulose, or phosphorous. For example, a column with a high sulfur concentration will likely support growth of purple sulfur bacteria more so than a column low in sulfur. Similarly, a column with high salt concentration will select for halophiles, and if there is no microbial growth in the column, it is likely that halophiles were not present in the initial inoculum, the sediment sample.



Different lighting:
Students can place their columns under different types of lighting. Microbial growth in a column sitting in a sunny windowsill will progress differently than in a column placed in a dimly-lit room. Students can also test the effects of different colored lighting: a column under a red light versus a column under a blue light as compared to a column in natural light. Another experiment can be to wrap all or part of the column in black construction paper or aluminum foil to test for what occurs in the absence of phototrophy (and therefore limited oxygen and carbon fixation).

Different sample sites:
Different sources of sediment can yield very different microbial growth. Even different areas of the same lake or pond may show differences in the microbial communities they host. With some knowledge of the sample site, students can begin to explain the connections between the source of the sediment and the microbial species and relative abundance that appear in the columns.

Columns in the Classroom

Winogradsky columns take about 4-8 weeks to develop visible microbial growth. The columns can be prepared by the teacher prior to their use in the classroom or students can take part in constructing the columns. If students are building the columns, this can be done as a class activity. The class can take a trip together to a nearby body of water to collect sediment or students can be asked to bring in samples from near their homes. They will need to be given some instructions on how to collect and store the sediment. When assembling the columns in the classroom, it may be helpful to prepare ahead of time the sand and nutrient mixture from steps 2 & 3 in the procedure above. The students will then simply have to mix in their sediment sample and prepare their columns. (However, a stock mixture will not be possible if students are testing different concentrations of nutrients.) It is recommended that students wear gloves when building their columns. Also, cover the working area in disposable, plastic table covers or garbage bags for easy clean up.

Safety

Soil samples used to build Winogradsky columns should generally only contain non-pathogenic microbes. However, it is always best to practice safe science. As such, students and teachers should be careful to not directly touch, consume, or inhale contents of the Winogradsky columns. When handling the Winogradsky columns, make sure lids are tightened and secure. However (and this is of equal importance), make certain not to store the columns with lids tightened. **Columns must be stored with the lids loose. Gases produced by microorganisms can build up quickly and must be allowed to escape to avoid a build-up of pressure that may lead to column explosion.**

A Brief Microbial Field Guide

Although individual microbial cells are not visible with the naked eye, microbial populations can display visible field marks that can be used to observe and identify them on a macro level. This brief field guide lists just some of the types of microbes that are more commonly found in most water and sediment environments and the field marks that distinguish them.

Sulfide oxidizers:
Sulfide oxidizers are a group of bacteria that eat hydrogen sulfide and breathe oxygen. They live near black sediments rich in hydrogen sulfide. A field mark of sulfide oxidizers is the white scum and fuzz they often produce. They require oxygen and therefore, will not be too deep in the sediment, but may be hidden below the green photosynthetic bacteria and algae. The ideal location for these microbes is where the layers of oxygen rich sediment and anaerobic, hydrogen-sulfide rich sediment meet, such as marine estuaries.

Cyanobacteria:
Cyanobacteria are green photosynthetic bacteria that can be found in nearly all environments with light and water including freshwater, marine, and terrestrial habitats. Cyanobacteria are often in competition with the eukaryotic green algae (who are often a brighter emerald-green color), and the two are often heavily mixed. In freshwater habitats cyanobacteria are often army-green, but can also appear as brown/black patches, gooey reddish-orange floating masses, yellow-brown films floating on the water or attached to vegetation.

Photo by James Henriksen

Cyanobacteria and Iron-Oxidizers

Green Sulfurs:
Green sulfurs can be seen in anoxic environments that receive light (photic zones) and have high sulfur and low oxygen levels. They can be seen as a thin layer of green beneath the purple bacteria and above a black sediment rich in sulfide.

Halophiles:
Halophiles, as the name suggests, is a group of archaea that thrive in environments with high salt concentrations. They are often aerobic. They can be seen as pink, red, or orange coloring on salt crystals that form in evaporated saline water, salt lakes, salterns, salt crusts, or commercial salt sites.

Iron-oxidizers:
These microbes produce a rust red color, a field mark that appears most often over black sediments. The color is a sign of iron reducers solubilizing solid iron from minerals in the anoxic parts of the sediment.

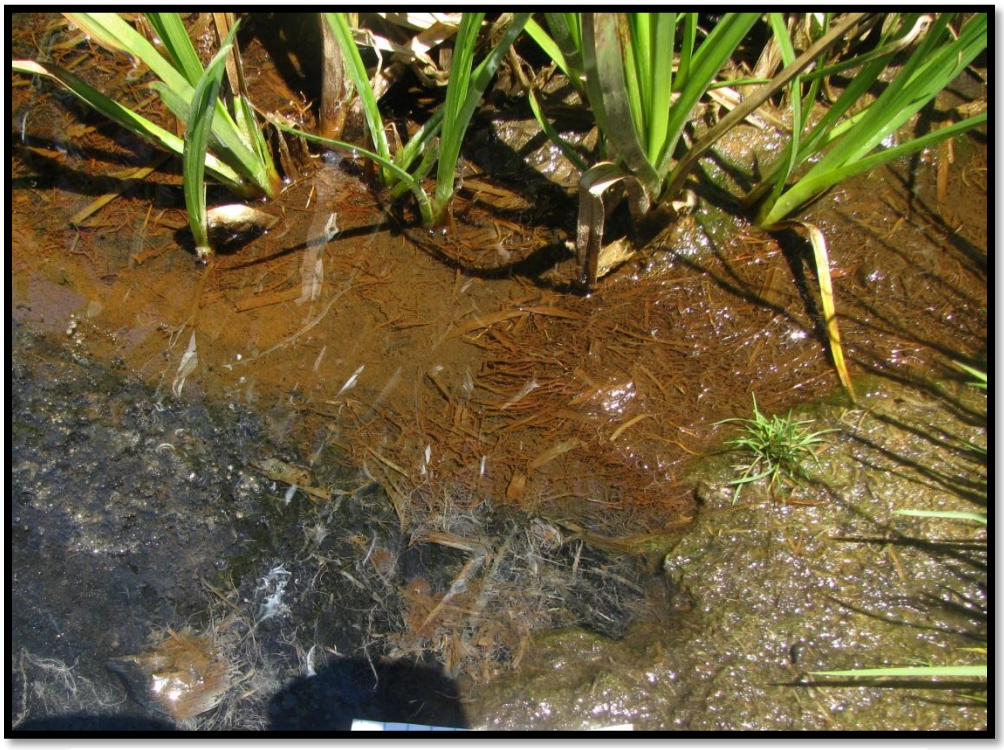


Photo by James Henriksen

Cyanobacteria, Iron-Oxidizers, Sulfur-oxidizers

Methanogens:
Methanogens are archaea that take in hydrogen, acetate, or a few one-carbon compounds and carbon dioxide and produce the gas methane. They can be found in anoxic environments such as below the surface of stagnant, swampy water. If the sediment is stirred and bubbles of gas come up to the surface, this is a sign of the methane produced by methanogens. The gas does not necessarily smell like sulfur and can be flammable. Sediment rich in methanogens is often a deep black color.

Purple Nonsulfurs:
The purple nonsulfurs can be found in anaerobic environments in the photic zone (the layer of the sediment that sunlight can reach) where they can carry out photosynthesis. Some representatives of this group are very metabolically flexible. These bacteria can be found in marine, fresh, hypersaline, or thermal waters, and are common in temporary puddles in hardwood forests.

Purple Sulfurs:
Purple sulfurs use hydrogen sulfide, light and carbon dioxide to make sugars. Therefore, they thrive in environments that are within the photic zone and are rich in sulfide compounds. These bacteria can be found as a purple-pink layer beneath the green cyanobacteria and in contact with sediments that are sulfide-rich, anaerobic, and black in color.

Photo by James Henriksen

Purple Sulfurs

Sulfate reducers:
Sulfate reducers eat hydrogen and sugars and breathe sulfate. They produce hydrogen sulfide which is the stinky smell in rotten eggs. They form a black layer of sediment below the photic zone especially in marine environments where sulfate from seawater is plentiful.

Fermenters:
Fermenters grow without oxygen to produce hydrogen, CO_2, and alcohols like ethanol. They are found in the most anaerobic environments including deep in sediment and human guts. Fermenters make the bubbles of CO_2 in bread, cheese and beer.

Biofilm formers:
Organisms that eat sugars, carbohydrates, and amino acids and breathe oxygen are called aerobic heterotrophs. Many microbes that are aerobic heterotrophs grow in thick gooey layers called biofilms. Biofilms can cause a big problem industrially and in medicine on medical devices because they form a protective layer around a microbial community and prevent antimicrobials from killing them.

Bioluminescence:
Like fireflies, bioluminescent bacteria like *Vibrio fischeri* can make molecules that are bioluminesce (or glow) when they react with oxygen in the environment. If you have ever swum in the warm ocean at night, the spectacular bioluminescent display comes from bioluminescent microbes.

Note: The information in the field guide portion of the protocol was drawn heavily from the book “A Field Guide to Bacteria” by Betsey Dexter Dyer (2003).