Lesson #5

Winogradsky in a Bottle:
Beginning a Winogradsky Column

Objectives:

- The learner will demonstrate application of measurement skills by marking a bottle for construction purposes.
- The learner will demonstrate analysis of changes in a system by recording qualitative and quantitative data and analyzing the results.
- The learner will demonstrate evaluation of a small scale system as a model for the macroscale environment around them by describing the pro's and con's of studying a small scale system.

National Science Education Standards:
UCP 1, UCP 2, SAI 1, PS 3, LS 4, ESS 1, HNS 2

Benchmarks:
2B, 3B, 4B, 4C, 4D, 4E, 5E, 11A, 11B, 11C, 11D, 12C, 12D

Materials: (per bottle)

clear plastic drink bottle (1/2 liter)
clear silicone caulk
compressed artists charcoal sticks (2)
razor blade or scalpel (teacher use only)
soil or sediment (50 ml)
pond water (from the site of the sediment if possible)
construction sand – see notes in preparation section
sand paper (medium grained)
food source (instant mashed potatoes or bullion cube, 1.5 g)
250 ml beaker (glass or plastic)
plastic spoon

Background:

Microbes are all around us, even though we rarely see them (unless we are looking through the lens of a microscope). While we go about our daily lives, many microorganisms are regulating our environment to keep it in a balance which allows us to survive. To better understand the changes which take place to allow for this balance, it is helpful to study a small portion of that environment. To do this we will be using a modification of the "Winogradsky Column". Named after Sergei Winogradsky (1856-1953), this relatively simple system will allow students to view, observe, and analyze the changes taking place in a model of aquatic sediments. Because this system is a smaller version of what is happening in the environment, students will then be able to use their findings to better understand the larger system around them.

In this activity, students will be monitoring the changes in this system not only qualitatively by making visual observations, but also quantitatively by conducting a colorimetric assay and measuring electric voltage and current.

Preparation:

Before beginning this lesson and the lessons that follow, I would like to briefly discuss grouping for these activities. I have found that groups of three or four students work well with these experiments. While the set up does take time and the group is able to prepare the bottles more quickly than individual students would, the interactions between the students make the experience more meaningful for all involved. These groups were able to discuss their particular experiment with each other and by doing so were able to bring about many ideas and questions for the group. Consider the needs of your students, the size of your class and the resources available before deciding on the appropriate groupings for your students.

Sediment - To set up the columns, you will need a sample of sediment to put into the bottom of the bottle. You may decide to collect the samples with the students or collect them on your own and bring them in for the students to use. If you do collect them on your own, be sure to spend some time talking to your students about the collection process. For directions about how to collect the sediments, please refer to lesson plan #4 "A Sedimental Journey - Taking Sediment Samples".

Sand - This activity requires sand which contains oxidized iron. This will allow the bacteria to use the iron in the oxidation and reduction reactions which students will be observing. There are a few different sources which you can use for your sand. Depending on where you live, there may be iron-rich sand in your area. This may require some investigation on your part, but it could be included as a part of the lesson. You may also find iron-rich sand at a local construction store. If the sand is rich in iron, it will have an orange rusty color. Refer to the "Guide to Good Sand" for examples.

If these options fail, you can make your own iron-rich sand. To do this you will need $FeCl_3$, distilled water, and NaOH in pellet form. These can be purchased through Fisher Scientific at 1-800-766-7000 or www.Fishersci.com . Be careful to use safety precautions including safety goggles, gloves, and an apron while preparing the sand. You will first need to make "Iron Floc", a suspension of non-crystalline FeO(OH). To do this, place 500 ml of distilled water in an Erlenmeyer flask. If you have a stir plate, add a stir bar to help with the process. Measure out 27.03 g of $FeCl_3$. Be sure to break it up if it is in small chunks as the smaller the pieces are, the easier it is to dissolve the solid. Add the $FeCl_3$ and stir. Carefully add NaOH pellets one at a time until the pH reaches 7. It is important to add these one at a time and wait for them to dissolve completely before continuing so that you do not go past pH 7. You will probably need about 12 g for this. Once pH 7 has been reached, turn off the stirrer and allow the suspension to stand for 5 hours. After 5 hours, most of the iron floc will have settled to the bottom of the flask. Carefully draw off the water with a pipette. Once you have removed most of the water, add 750 ml of distilled water and stir for about 3 min.to rinse the iron. Again, let the suspension stand for another 5 hours. You will need to follow this rinsing procedure a total of 3 times to get rid of the salt that is produced by this reaction. The water drawn off the top can be rinsed down the drain with plenty of water as it is a salt. Once the iron floc has been rinsed, mix it into about 20 lb of sand. This can be plain play sand that you get from the hardware store or even beach sand. Spread the sand out and allow it to dry completely. You now have your sand for the column. (Note – You can let the iron floc sit overnight while waiting to draw off the water for each of the washings.)

It is advised that you try setting up a sample column on your own before the lesson. This will give you an opportunity to try the technique and refine some of the procedures which may need to be adjusted to your students' needs and ability levels. This also provides a model which students can refer to during the construction process.

The students will be recording observations about their bottles for five weeks. It may be helpful to plan the organization of their notes before starting. You could have a science log that each student keeps at school and records in every day. Alternatively, you could have students keep their notes in a science log in their notebooks. Either way, it should be easily accessible for students to use in the beginning, or end of class, depending on how you structure your plans. Students will be recording other observations during this five week period, some of which you may have them include in their science logs and others which they can record directly on their lab sheets.

Note – The colorimetric assay and the voltage/current tests need only be conducted for three weeks to see noticeable changes. It is important for students to record their observations longer (five weeks) for them to be able to see all of the visual changes taking place in the bottle. You may even want to discuss why this needs to be done. Be sure to explain that some changes take place slowly, and they can be measured by scientific tests before these changes are actually visible.

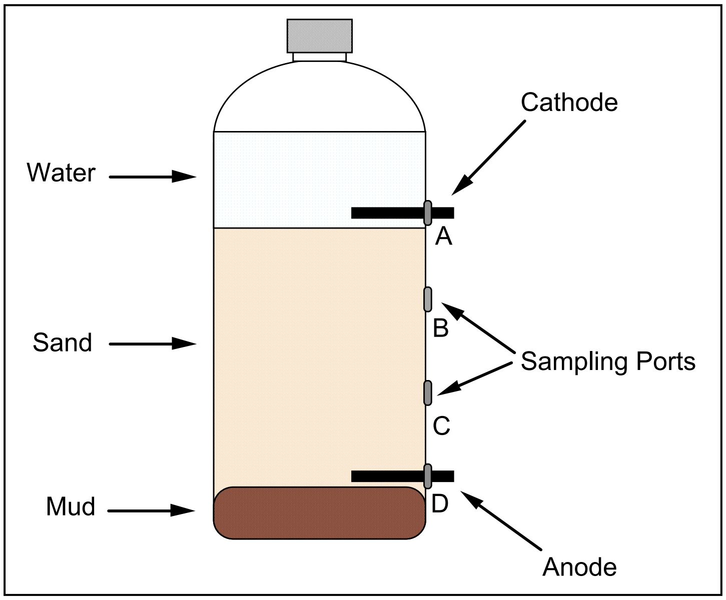
Part 1 - Preparing the Column

Warm-Up:

Ask students how they could study the environment using a small scale system. "What would be some of the challenges of studying the changes caused by microbes in the environment? How can a small scale system of the environment be used? What are some of the concerns in setting up one of these systems?" Encourage students to relate the activity occurring in a small system to the same activity occurring in a larger system.

Tell students that they will be setting up a modified Winogradsky column to study the environment around them. Explain that they will be looking at the ways that bacteria in the environment contribute to the iron cycle. Inform them that the work of these bacteria will allow them to make a battery out of the column.

Use the diagram below to help you in setting up the bottle.



Procedures:

1. Students will clear the bottle of any labels or wrappers. They should rinse out the bottle to remove the original liquid (soda, iced tea, etc.).
2. Students will need to measure the bottle to find the locations for the ports and the electrodes which will be placed in the bottle. If you will be doing one of these follow-up activities, but not the other, then you will eliminate that piece of the procedure. The ports will be used in the colorimetric assay and the electrodes will be used in the battery activity. The bottom port should be about 4 cm from the bottom of the bottle. The top port should be located about 1 cm under the part of the bottle which begins to curve in to form the neck of the bottle. Students will then need to locate the sites for two other ports. These two other ports will be placed between the top and bottom port so that all four ports are equidistant from each other. Have students mark these sites so you can check their work. (This will provide for an informal assessment of students' measuring techniques as well as their logical thinking skills in finding the locations for all four equidistant ports.)
3. Once you have approved the sites, students should use a small piece of sandpaper to rough the spot on the bottle where the silicon will be placed to form the port. This will help the silicon caulk to adhere to the bottle.
4. Students are now ready to locate the site for their electrodes. Students should place a mark about 4 cm away (horizontally) from the top and bottom port locations. These sites must be cut with a razor or scalpel. To do this, students can ask you to come over to their lab bench and cut on their mark. Making small cuts in an X pattern will work well.
5. For this activity about 3/4 of the charcoal stick needs to be inside the bottle and about 1/4 outside the bottle. Both sticks should have the same length inside of the bottle, so students should mark both of the sticks and place them in the bottle so that their lengths inside are equal. Once this is done, students should seal the edges around this hole with

the silicon caulk. Be observant to insure that students are creating a full seal around the charcoal to avoid leaks.

6. To create the ports, students will place round beads of caulk on the four spots which they marked and sanded earlier. The beads should be about the size of a nickel and about a centimeter high. This is a step where an example would be helpful for students who have a hard time conceptualizing the required size of the port. Once students have applied the caulk bead, it should rest for a few minutes.
7. Once a thin skin has dried on the silicon bead, students may use their finger to push down on it gently. This will give a smooth surface as the caulk may be standing up straight. Another option is to put some Windex on your finger and then you will be able to smooth out that piece of hanging caulk before waiting for it to set. The Windex will keep your finger from sticking to the caulk.
8. At this point, students will be eager to begin the process of filling the bottles, but it will be necessary to allow time for the caulk to fully cure. You can begin filling the bottles after the caulk has set for 48 hours.

Variations and Follow-Up Activities:

Once the bottles are completed and set aside to dry, a discussion should return to the warm-up question. Ask students "Why do you think we put four ports into the bottle? Why are the ports at four different heights in the bottle? Why did we put the electrodes in the bottle? What do we expect to see happening? What activity will we have to test for?" Use the questions as a guide to discuss predictions.

Assessments:

There are many opportunities for informal assessment during the activity. Measuring skills are assessed as students work to plan their port and electrode locations on their bottles. Logical thinking skills can be assessed during the port location process as students will need to think about how to mark the two locations between the top and bottom ports so that all four ports are equidistant from each other.

A more formal assessment is possible at the end of the activity. Some possible assessments include a paragraph written by the student explaining the procedures used in selecting port sites.

Part 2 - Adding the Sediment

Warm-up:

Hand out sediment samples to each of the groups. What is in the sediment we collected? (If you collected the sediment on your own, this would be a great time to tell students about your collection procedures and the site where the sediment was collected.) Allow students to talk to their groups members and come up with some ideas. After a few minutes, ask groups to volunteer their ideas and list them on the board. Write down all ideas and then take a few minutes to discuss what the students came up with. You can use this discussion time as an informal assessment of students' understanding of the components of sediment and the microbial populations found in sediment.

Procedures:

1. Decide how much of the sediment will be used for each group. Use 100 ml if you are using a 1 liter bottle or 50 ml for a half liter bottle. Add 1.5 grams of the food source to the sediment and mix.
2. Add the mixture to the bottle. A small spoon may be helpful to add a little bit at a time to avoid getting the sediment on the side of the bottle. Once the sediment is added, a small amount of water can be used to wash the excess sediment from the sides of the bottle.
3. Using a funnel, fill the bottle with sand to just under the top electrode.
4. Pour in pond water and let it settle for a few minutes.
5. Once the water has settled and most of the air has bubbled out, add more water until it is about 3 cm from the top of the bottle. Cap the bottle.
6. Describe and diagram the bottle in a lab notebook. It may be helpful to use color because the colors of the bottles will change as time goes on.
7. Additional measurements of these columns are included in Lessons #6 and #7. These activities will help to complete the students' questions.

Variations and Follow-Up Activities:

The activities that follow will be very helpful in extending this activity. This lesson is designed to be the basis for the activities involving the iron tests and voltage measurements. In addition, there is an activity for students to set up their own experiment.

For those who are unable to follow with those lessons, it may be appropriate to spend time discussing the ways in which color is an indicator of activity occurring within the bottle. In particular, the rust color which forms at the top of the column from the oxidation of the iron is easy to see and can be measured in qualitative terms by comparing colors on a daily basis. In this case a digital camera could record the colors seen each day. These colors could then be analyzed by the students and bacterial activity could be inferred from this data. The graphing activity that follows would also be a way to analyze the changes in the bottles as the data for sample bottles is given. In this case, no special equipment is necessary.

Assessments:

As students make observations of their bottles on a daily basis, simple questioning techniques can be utilized to provide informal assessment and check for understanding. The conclusion will provide a more concrete assessment tool. The questions presented are designed to assess students' knowledge of the activity by reflecting on their hypothesis as well as project further research which could be conducted. The conclusion is written to be completed after Lessons #6 - #8 are completed. If these lessons will not be done, then the assessment should be changed.

Constructing the Column

- Measure and mark the positions for sampling ports and electrodes.
- Cut holes for the electrodes and rough-up the spots for ports.
- Apply the caulk and let it dry.

- Mix the sediment and food source together well.
- Carefully transfer the mixture to the bottom of the bottle.

- Add the sand until it is just below the level of the top electrode.
- Fill the bottle with pond water and cap.

Finished Column

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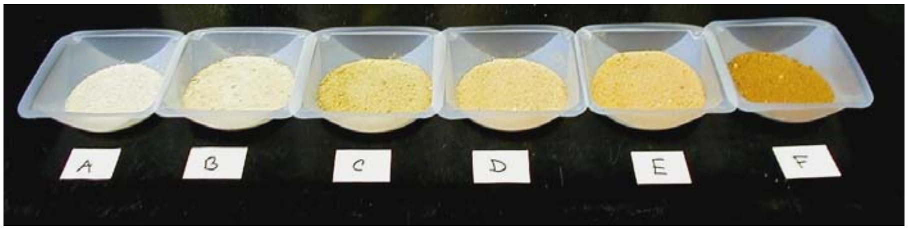
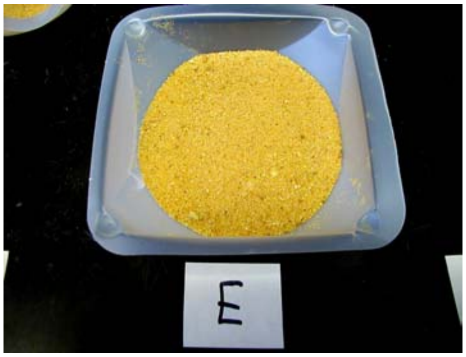
Funded by the National Science Foundation, Biocomplexity in the Environment Program, Award #0120453

Guide to Good Sand

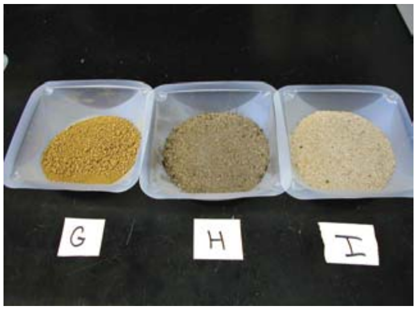
Each sand sample is labeled with a letter. Use this information to help you in choosing the right sand for your Winogradsky Bottle. You should try for sand of a quality similar to D or better, but C would probably be ok.



A. No Iron – Cleaned sand
B. No Iron – Beach sand
C. Some Iron – Beach sand
D. Moderate amount of iron – Construction sand
E. Moderate amount of iron – Iron coated sand
F. Lots or iron – Sand sample from iron rich area



Do Not use these:



G. Soil
H. Black sand
I. Cleaned black sand

Observations:

My students loved using the caulk in this lesson. We talked about how silicon caulk is also used in the home, especially for bathrooms. Many students showed an interest in jobs which would use caulk such as construction work. This provided students with some insight into jobs that they may someday be interested in.

I found that using a caulk gun instead of a squeezable tube of caulk provided both more precision for the students as well as more fun. I was able to help guide students as they placed the caulk on the bottle. This can also be more economic as the larger containers which fit into the caulk guns can be less expensive than several small tubes.

Be VERY careful to look for leaks. The students may enjoy using the caulk to seal the electrodes in place, but these seals may not always be as tight as needed. Check each of the electrodes to be sure that the caulk gives a tight seal to avoid leaks. If a leak does occur, use some extra caulk to seal it up. Caution students to be careful when handling the bottles as wear and tear on the bottles can cause leaks later in the experiment. Most importantly, store bottles with a paper towel underneath them so that if there is an undetected leak, your classroom does not become dirty.

I found that having a cleanup plan was very important in this lesson. Students filled their bottles over plastic trays so that extra dirt and sand that fell could be easily cleaned up. If you are prepared for a mess, you will be pleasantly surprised when your foresight keeps the classroom from becoming dirty. My students enjoyed the fun of mixing the food source with the sediment and then putting it into the bottle. With an easy cleanup plan, they were able to work without being concerned about making a mess.

The following are images of some of the bottles that my students made.

Name ____________________
Date ____________________
Winogradsky in a Bottle

Problem: How can the activities of bacteria in the environment be observed and measured using a small scale system?

Background:

Microbes are all around us, even though we rarely see them (unless we are looking through the lens of a microscope). While we go about our daily lives, many microorganisms are regulating our environment to keep it in a balance which allows us to survive. To better understand the changes which take place to allow for this balance, it is helpful to study a small portion of that environment. To do this we will be using a modification of the "Winogradsky Column". Named after Sergei Winogradsky (1856-1953), this relatively simple system will allow us to view, observe, and analyze the changes taking place in a small model of the mud at the bottom of a pond. Because this system is a smaller version of what is happening in the environment, we will then be able to use our findings to better understand the larger system around us.

In this system, will be studying the activity of some bacteria found in sediment. Some bacteria can live autotrophically which means that they use minerals as an energy source. It is similar to the way plants use photosynthesis, but without the light. Be sure to look for evidence of the bacteria using minerals in the bottle as an energy source, particularly iron.

Hypothesis: If____,

then ____,

because ____

____

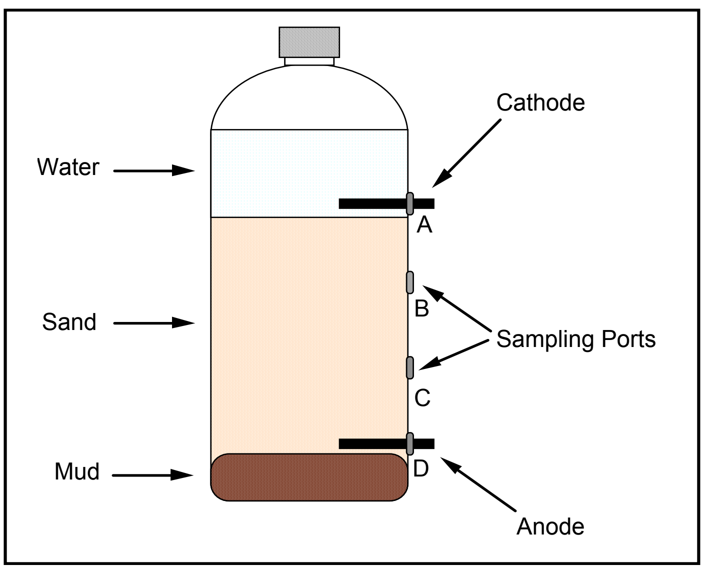
Funded by the National Science Foundation, Biocomplexity in the Environment Program, Award #0120453

Materials:

clean plastic drink bottle (1/2 liter)
clear silicone caulk
compressed artists charcoal sticks (2)
razor blade or scalpel (teacher use only)
soil or sediment
pond water (from the site of the sediment if possible)
construction sand
sand paper (medium grained)
food source (instant mashed potatoes or bullion cube)
beaker (250 ml)
plastic spoon

Procedures:

Use the following diagram to help you construct the bottle.



Part 1

1. Clean off the plastic bottle by removing the label and rinsing the bottle with water to remove any of the residual liquid from the bottle.
2. Measure 4 cm from the bottom of the bottle. Place a small mark at this spot. Find the location at the top of the bottle where it begins to curve in to form the neck. Measure 1 cm under this location. Place a small mark at this spot.

3. Measure the distance between the top and bottom mark. You will need to place two more marks in this area between the top and bottom. Using the total distance between the top and bottom marks, figure out how far apart each mark must be so that they are all the same distance away from each other. Once you have decided on the distances, mark these last two spots.
4. Ask your teacher to check your distances. You will then need to use a small piece of sandpaper to rough the plastic surface where you have marked on the bottle. This is where you will be putting a dab of silicon caulk to use as a sampling port.
5. You will now locate the spots for your electrodes. Measure 4 cm horizontally from both the top and bottom marks. Mark these spots with an "X".
6. Ask your teacher to cut a star pattern of small slits to put your electrodes through. Once your teacher has done this, you must measure the charcoal stick you will be using as electrodes. Divide this length by 4 and mark the sticks so that one side of the mark is 1/4th of the stick and the other side is 3/4ths of the stick. Be sure that the 3/4ths side is the same length for both of the sticks.
7. Insert the sticks into the two star shapes slits which have been cut by your teacher. The sticks should be placed so that 3/4ths of the stick is inside of the bottle and the other 1/4th of the stick is outside of the bottle.
8. Carefully seal the edges of the bottle where the charcoal sticks are located with a bead of silicon caulk. Be sure to make a good seal so that the bottle does not leak.
9. Place a bead about the size a nickel and about 1cm high on each of the four spots you sanded for the ports. After about 5 minutes, you can gently push on the silicon beads to smooth off the top so that it is rounded and there are no strings of caulk coming off.
10. Place your bottle in the drying area that your teacher has set up. This is where your bottle will rest while the caulk cures. Put the rest of your materials away and clean your work space.

Part 2

1. In a beaker, mix 50 ml of sediment with 1.5 grams of food source. Be sure to mix the contents well to be sure that the food source is distributed evenly through the sediment.
2. Add the mixture to the bottle. A small spoon may be helpful to add a little bit at a time to avoid getting the sediment on the side of the bottle. Once the

sediment is added, a small amount of water can be used to wash the excess sediment from the sides of the bottle.
3. Using a funnel, fill the bottle with sand to just under the top electrode.
4. Pour in pond water and let it settle for a few minutes.
5. Once the water has settled and most of the air has bubbled out, add more water until it is about 3 cm from the top of the bottle. Cap the bottle.
6. Describe and diagram the bottle in a lab notebook. It may be helpful to use color as the colors of the bottles will change as time goes on.

Results and Analysis

1. Record your findings in your lab notebook.
2. What did the bottle look like on the first day? Include a diagram.
3. After 3 days, have you seen any changes in the bottle? Describe those changes.
4. After 7 days, have you seen any changes in the bottle? Describe those changes.
5. Continue to monitor your bottles and record any changes.
6. Include these notations along with those for the colorimetric assay and voltage and current tests in your lab notebook.

Conclusions

Use the following questions to guide you in writing your conclusions to this lab. You should include information from the lab to support your conclusions. Remember to use complete sentences.

- Did your findings support your hypothesis? How do you know?
- How did the measurements obtained from the voltage/current readings and colorimetric assay help to support your findings?
- Are small scale models useful in studying the large environment? What are the pro's and con's of using these small scale models to investigate the environment.
- How would you extend this experiment to test your hypothesis further?
- Do you think you could study the effects of pollution in our environment using this model? Explain why or why not.