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The Winogradsky Column & Biofilms: Models for Teaching Nutrient Cycling & Succession in an Ecosystem

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Models for Teaching Nutrient Cycling & Succession in an Ecosystem

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Wetlands are among the most productive ecosystems in the world. The high productivity of wetlands is due to their ability to capture large amounts of the sun's energy and store it as chemical energy, as well as to their efficient recycling of what is produced (Niering 1985).

The characteristic hydric soils in wetlands reflect the effect of water when present on soil or substrate for extended periods. According to Mitsch and Gosselink (1993) hydric soils that are semipermanently or permanently flooded develop gray or sometimes greenish or blue-gray color as a result of a process known as gleying. This process is the result of the chemical reduction of iron. Another characteristic of soils that are seasonally flooded is mottle formation. Orange/reddish or dark reddish-brown/black mottled spots seen throughout an otherwise gray soil matrix suggest soils with spots of iron and manganese oxides in an otherwise reduced environment. The development of gleys and mottles is mediated by microbiological processes and the rate at which they are formed depends on the presence of sustained anaerobic conditions, appropriate soil temperature (5° C is considered optimum), and the presence of organic matter as a substrate for microbial activity. These chemical transformations in wetland soils illustrate the interaction of nutrients and microbiological activities in ecosystems.

One problem faced by biology teachers in teaching nutrient cycling is the lack of suitable exercises that can be conducted in the classroom. We feel that the use of a Winogradsky column and biofilms can help junior high and high school students develop a clearer understanding of these chemical transformations by presenting a vertical model and the microorganisms participating in the process of succession.

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The Winogradsky column, named for the Russian microbiologist, Sergei Winogradsky, is a miniature ecosystem that illustrates microbial succession of several groups of microbes. Winogradsky devised the column in the 1880s to study soil microorganisms (Madigan et al. 1997). The column is prepared by enriching a sediment sample (soil or mud) from a lake, pond, stream or seashore with a nutrient substrate and allowing it to incubate at room temperature for several weeks. To study microbial succession, several glass microscope slides are partially inserted, vertically, into the top of the soil or mud sample. In time, the glass microscope slides serve as a surface to which bacteria, and later algae, protozoans and invertebrates attach and form biofilms. Observing these biofilms over several intervals of time illustrates microbial succession.

We used this laboratory activity with 20 junior high science and high school biology teachers at a summer workshop on wetland ecology. During the following academic year, the teachers constructed Winogradsky columns and studied biofilms at their respective schools. At the annual evaluation of the project, teachers reported on how they involved their students in investigating the changes that occurred as their Winogradsky columns developed. The exercise was conducted using an inquiry-based approach with students working in cooperative learning groups. Based on earlier activities involving the characteristics of wetland soil, e.g. color, odor and texture, and microbe habitats (Kesselheim & Slattery 1995), students were asked to predict what might happen to the column sediments after several weeks. Nutrient cycling and biological succession are generally presented in textbooks as abstract theoretical concepts that students often find "difficult" and "boring". This exercise helps students to conceptualize and interpret the changes going on under aerobic, as well as under low oxygen conditions, and to identify chemical changes that might affect the activities of the microorganisms in the soil. Changing areas of pigmentation and the kinds of microbes that appear and disappear with changing oxygen and hydrogen sulfide levels are recorded in the students' laboratory journals.

The objectives of this exercise include: setting up a Winogradsky column and biofilm slides, interpreting the chemical transformations that occur in the column as a result of color changes in the soil, identifying common microorganisms, and determining the microbial composition of the column over specified intervals of time.

Materials

- One clear or transparent (plastic or glass) container with straight sides of about one liter capacity, with a diameter of at least 5 cm and a height of at least 17 cm (We used a tall, cylindrically shaped, empty plastic water bottle cut off below the neck.)
- Wetland soil or mud from a lake, pond, stream or seashore, enough to fill the container about ⅔ full
- Plastic wrap or aluminum foil to serve as a lid for the column
- Scoop for handling mud or soil
- 5 g $CaCO_3$ (calcium carbonate)
- 5 g $CaSO_4$ (calcium sulfate)

- 10 g finely shredded newspaper or other cellulose source such as sawdust, leaves, shredded woodchips, grass clippings
- Balance to weigh the chemicals
- 8 to 10 glass microscope slides per column for the biofilms
- Permanent marker to label slides
- Coverslips
- (Optional) A simple stain e.g. methylene blue stain (Morholt & Brandwein 1986)
- (Optional) Gram staining reagents: crystal violet, Gram's iodine, ethyl alcohol, safranin (Morholt & Brandwein 1986)
- Slide holder or forceps (We used clothespins as holders.)
- Squeeze bottle containing water
- Bunsen burner
- Microscope

Procedure

A. Construction of the Winogradsky Column

(modified from *Microbiology Techniques* by Kelly and Post 1991)

This laboratory activity should be planned on a long-term basis of about two to three months (or longer) for optimum column development.

1. Estimate an amount of soil or mud to fill the container ⅔ full. Remove any rocks, twigs, or any objects. Mix the soil or mud with 5 g $CaSO_4$, 5 g $CaCO_3$ and 10 g of a cellulose source (shredded newspaper, grass clippings, leaves, etc.). The cellulose serves as the source of organic carbon.
2. Place soil or mud in the container and add sufficient water to cover the soil 3 to 4-cm deep. Stir the mixture to eliminate air bubbles.
3. Place 8 to 10 clean glass microscope slides in a vertical position on top of the soil, leaning them against the side of the container. At least 70% of the length of the slide should be submerged in the water.
4. Mark the water level of the column and cover the top with plastic wrap or aluminum foil to reduce evaporation. You may need to replace water, to the original level, over a period of time.
5. Incubate the column at room temperature for several weeks in a north window, if possible, so as to receive adequate, but not excessive, sunlight.

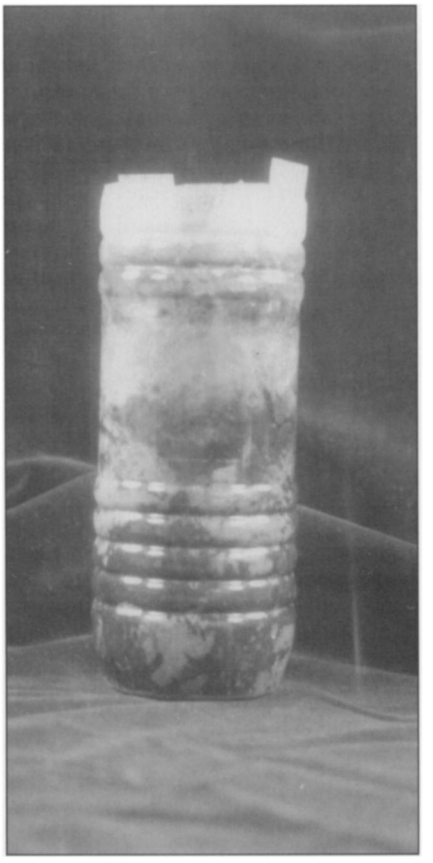

Figure 1. (a) Placement of glass slides in the Winogradsky column.

Figure 1. (b) Cover added to reduce evaporation from the column.

B. Observations of the Winogradsky Column

Students are encouraged to make daily observations during the first week of the setup. Subsequently, weekly observations are sufficient.

1. Note the appearance of the column and draw any changes in color patterns or growth that occur in the soil and water over the incubation period. Some bacteria will "bloom" and then disappear as others colonize. Record any differences that appear in the column and compare columns among students.
2. Remove the foil cover. Describe and record any changes in odors in the column. Have students compare odors of their column.
3. Students are asked to visualize the oxygen and hydrogen sulfide gradients that are formed in the column, going from top to bottom, and from bottom to top, respectively, and to diagram the changes that occur.

C. Observing the Biofilms

1. Slides should be removed on Day 2 to observe the formation of the biofilm and the succession that is taking place. Because organisms will colonize both sides of the glass slides, one side should be wiped clean.
2. Examine slides daily for at least 5 days, then at weekly intervals for several weeks (or even months), using a wet mount, under low and high power magnifications (Morholt & Brandwein 1986). Once the observations have been made, the slides may be marked with the observation date and returned to the column to be observed again at a later date. Over time, microbes will continue to adhere to the glass slides. Additional slides may be added to the column to "restock" the supply. Notations written with a permanent marker may be used to mark the date as slides are added to the column.
3. As an optional activity, slides may be stained with simple and/or differential stains (such as the Gram stain) and examined under high power and oil immersion lenses. These stained slides should not be returned to the column.

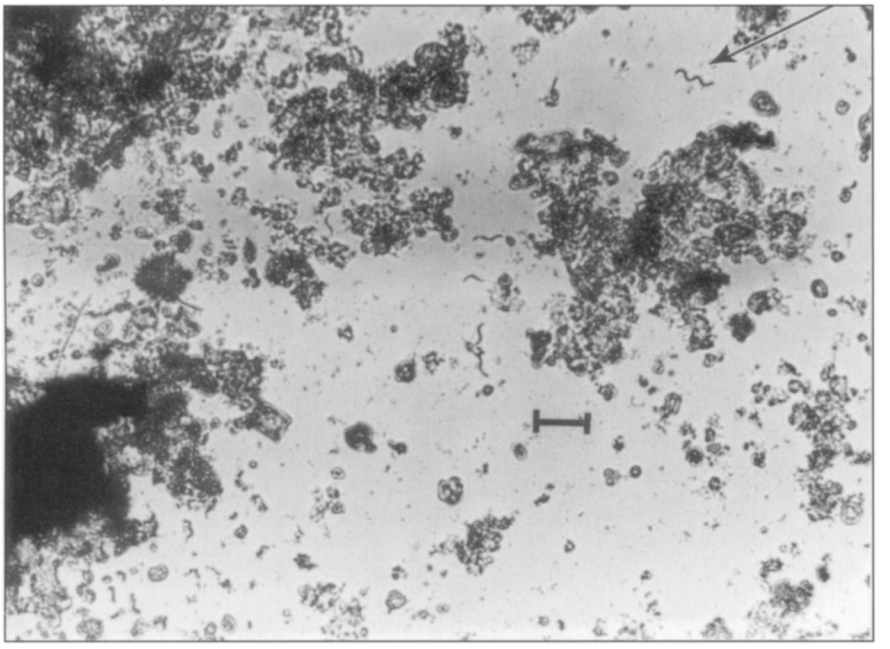

Figure 2. Biofilm development over three weeks.
(a) Week 1: Several spiral-shaped bacteria (10μm = bar) are seen.

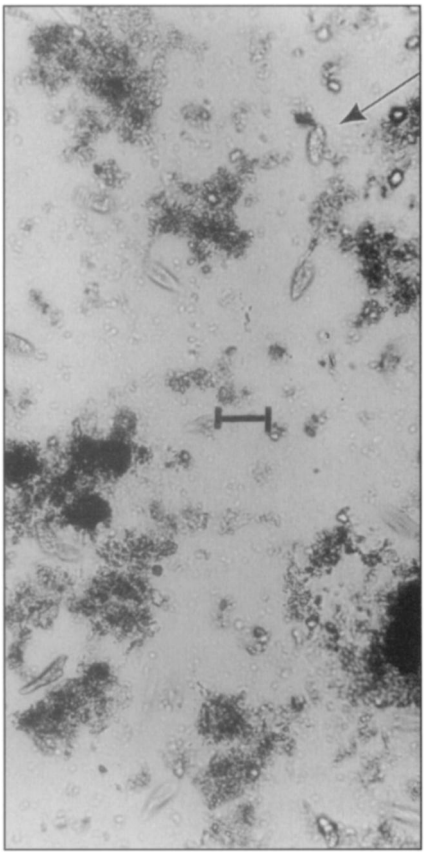

Figure 2. (b) Week 2: Protozoa (20μm = bar) colonize the biofilm.

4. Note the changes that take place over a period of time with respect to the type and abundance of macro and microorganisms. You may need to modify your fixing and staining methods depending on how long the slides have been immersed in the column. For example, during the first days of observation, Gram staining may be more successful as more bacteria colonize the slide. As time passes and the biofilm develops (and more microbes encrust on the slide), a wet mount, with or without the simple stain, would be more effective for viewing the larger eukaryotes. Bacteria are usually the first to colonize, followed by an assortment of algae, protozoans and invertebrates. Figure 2 illustrates biofilm development over a period of three weeks.

Discussion

The Winogradsky Column

The Winogradsky column is a miniature ecosystem in which microorganisms and nutrients interact over time. As oxygen diffuses downward from the surface, fermentation products from the breakdown of cellulose and hydrogen sulfide diffuse upward from the reduced lower zone (Prescott et al. 1996).

As microbial metabolites migrate within the column in response to various chemical gradients, groups of organisms accumulate in accordance with their required conditions in much the same way that they do in nature, thereby simulating the vertical microbial distribution that may be found in lakes, ponds, or in the intertidal zone. Figure 3 is a diagram of the Winogradsky Column (modified from Prescott et al. 1996).

In the lower region, anaerobes such as *Clostridium* degrade cellulose and produce fermentation products such as organic acids, alcohols and hydrogen.

These fermentation products together with sulfate (from the calcium

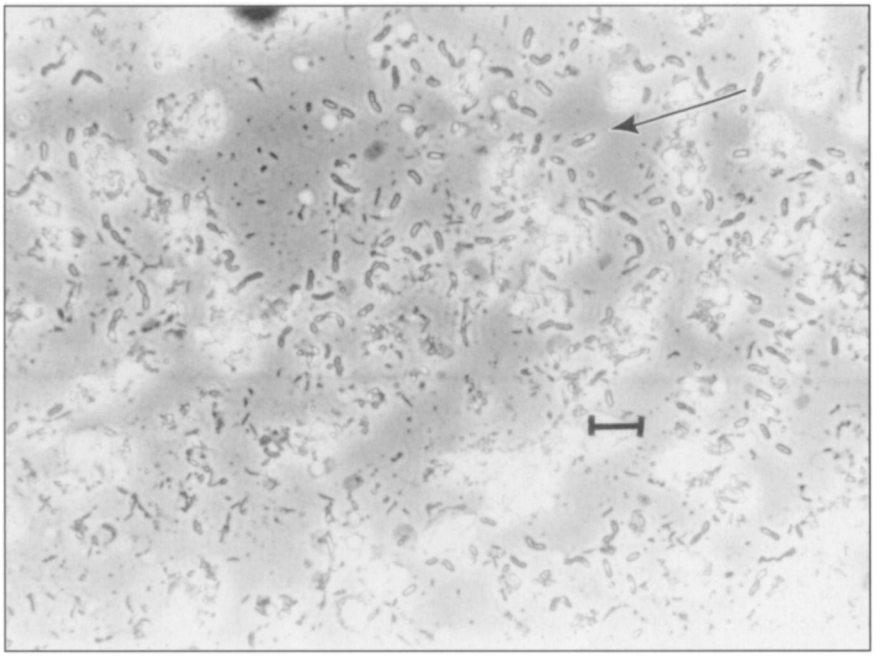

Figure 2. (c) Week 3: Numerous rod-shaped bacteria (10μm = bar) are noted.

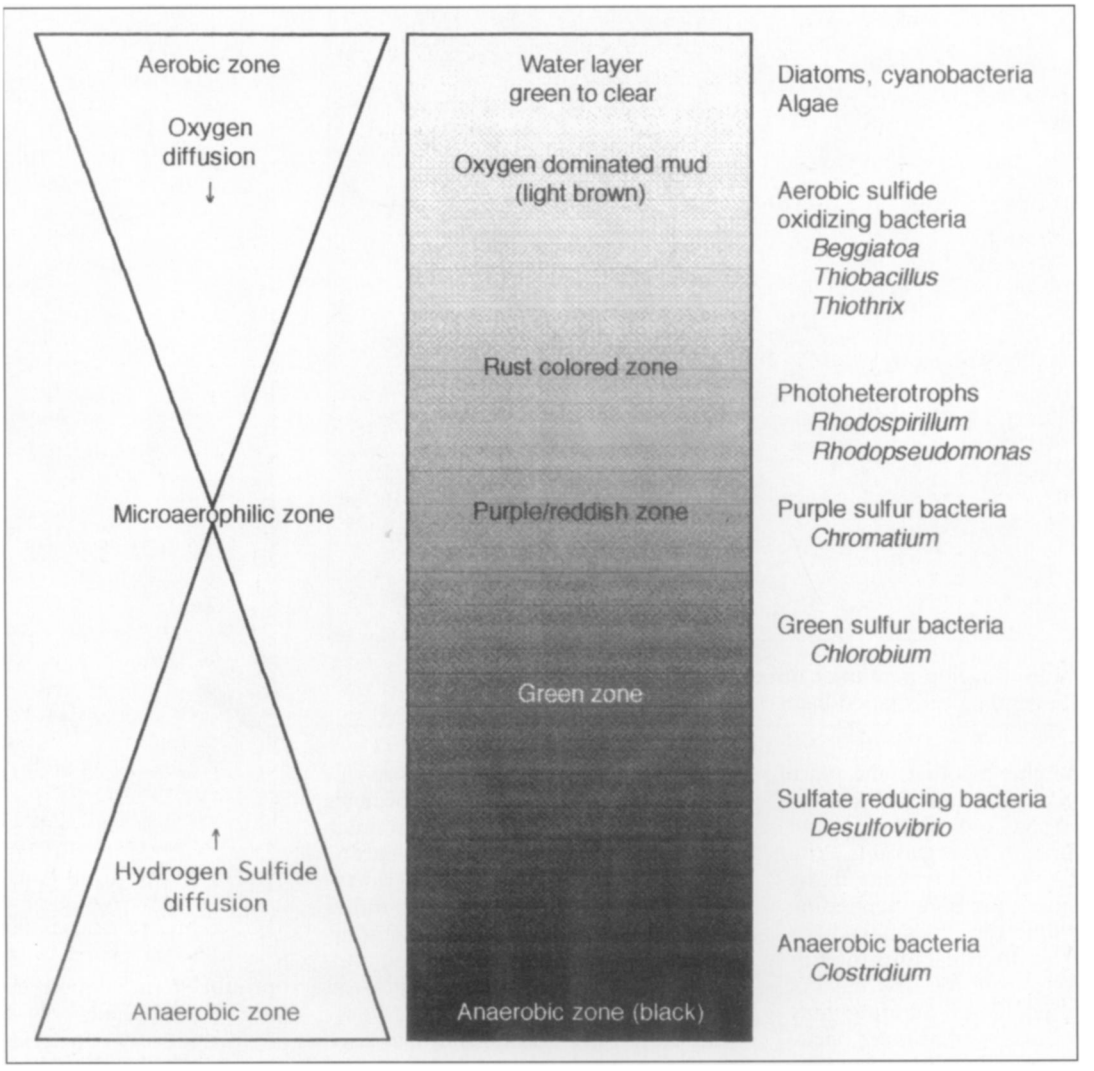

Figure 3. The Winogradsky Column (modified from Prescott et al. 1996).

sulfate enrichment) are used by sulfate-reducing organisms such as *Desulfovibrio* to produce hydrogen sulfide. The development of sulfate-reducing bacteria appear as blackened areas in the lower portion of the column and may even blacken zones throughout the sediment if sufficient aerobic bacteria are present to deplete the oxygen supply.

The sulfides are then used by anaerobic photosynthetic populations such as green sulfur, *Chlorobium*, and purple sulfur, *Chromatium* bacteria. Evidence of this is seen as purple and green patches in areas throughout the column as these phototrophs respond to gradients of light and sulfide. In nature, purple and green sulfur bacteria may be found in any fresh or marine waters as long as there is a sufficient supply of hydrogen sulfide and the water is clear enough so that light penetrates to the anoxic (anaerobic) zone (Madigan et al. 1997).

Ferric compounds may precipitate and appear as brown or greenish-gray deposits. The predominant iron-reducing bacteria within gleyed soils appear to be *Bacillus* and *Pseudomonas* (Atlas & Bartha 1993.)

Higher up in the column, in the microaerophilic zone, rust-colored patches will appear, generally from the photoheterotrophs, such as the purple nonsulfur bacteria (*Rhodospirillum* and *Rhodopseudomonas*). These organisms trap light energy and use organic molecules as both electron and carbon sources.

At the mud-water interface, where both hydrogen sulfide and oxygen are found, various aerobic, sulfide-oxidizing organisms such as *Beggiatoa, Thiothrix* and *Thiobacillus* may colonize. This water is frequently quite turbid.

Algae and cyanobacteria appear quickly in the upper portion of the water column, where sunlight is abundant. By producing oxygen, these organisms help to keep this zone aerobic. This watery top layer contains an interesting diversity of microbes—green algae, cyanobacteria, various aerobic bacteria, fungi and protozoa.

Nutrient Cycling

Nutrient cycling of elements between the atmosphere, water and soil results in the biogeochemical zonation of habitats that can be seen in the column. Although the column is not connected to the environment, cycling of elements does occur as macro and microorganisms degrade various organic substances found in the soil/mud and in the "enrichment materials" that have been added. While the column mimics the natural wetland in several ways, the dynamic interplay found in the natural environment is missing. For example, additional elements are not being continuously added to the system as they

are in nature, nor are microbes being "transported" into the system by runoff, wind or other forces.

In the column, carbon, hydrogen and oxygen are cycled primarily by photosynthesis and respiration. Under anoxic conditions, organic carbon may be recycled in the fermentation process. As alcohols and acids accumulate, they may be metabolized further with the simultaneous reduction of nitrate or sulfate.

Changes in the oxidation states of sulfur are mediated by microorganisms, establishing the sulfur cycle (Atlas & Bartha 1993). For example, algae and many heterotrophic microorganisms may utilize sulfate, incorporating it into protein. Other organisms may reduce this sulfate to produce hydrogen sulfide. Hydrogen sulfide may then be subject to microbial oxidation under aerobic conditions, or may be used by the anaerobic photosynthetic sulfur bacteria (Atlas & Bartha 1993). Hydrogen sulfide gas may diffuse through the column, as it does within natural sediments, resulting in the characteristic odor associated with anoxic soils. As part of this exercise, students are asked to describe the odors created within the column.

In the nitrogen cycle, nitrogen from the atmosphere moves through various organisms and back into the atmosphere. The critical steps of nitrogen fixation, nitrification, and denitrification are all mediated by bacteria and are responsible for this cyclic flow (Atlas & Bartha 1993).

The cycling of iron involves the oxidation-reduction reactions that reduce ferric iron to ferrous iron and oxidize ferrous iron to ferric iron. Ferric iron reduction is a common occurrence in soils, bogs and anoxic lake sediments (Madigan et al. 1997).

Although students are not asked to identify the microorganisms in the sediments according to specific genera, they are able to observe visual changes that correspond to the diversity in the column. They are able to microscopically identify groups of microbes (such as bacteria, algae, fungi and protozoa) from the water column using their textbook and other laboratory resource materials.

Biofilms

Biofilms develop on surfaces immersed in natural, aqueous environments. These surfaces may be aquatic plants and animals or submerged objects such as wood, plastic, metal, concrete or rocks. Biofilms form rapidly in fluid environments and increase over time, especially where there is nutrient accumulation (Madigan et al. 1997).

The development of biofilms is initiated by microorganisms forming a monolayer attachment on the surface. Over time, this film becomes more complex with layers of organisms of different types colonizing the surface. Depending on the energy sources available, photosynthetic microbes, facultative chemo-organotrophs, or sulfate-reducing microorganisms may be present (Prescott et al. 1996).

The formation of biofilms has broad implications not only in natural hydric systems, but also in industrial microbiology and in public health. Heated water supplies and potable water systems may form slimy aggregates. In air-conditioning and other water system units, biofilms may serve to protect pathogenic bacteria from the effects of chlorination and other antimicrobial agents. Biofilms may also colonize the surfaces of medical equipment such as urinary or intravascular catheters and dialysis units and may serve as reservoirs of infection. In our own bodies, biofilms form plaque that leads to tooth decay. Biofilms can also accumulate on contact lenses, where bacteria may cause eye irritations and infections (Prescott et al. 1996).

Classroom Applications

Each Winogradsky column is unique and serves as an ideal experiment for inquiry-based laboratory activities. Students can be imaginative and collect water from neighborhood habitats—ponds, lakes, streams, puddles, birdbaths, or tidal creeks; use different soil types; and supplement the soil with various cellulose sources that may include shredded newspaper, grass clippings, sawdust or wood clippings. The variety of organisms and pigmentation in the column varies depending upon the nature of the soil and the nutrients contained in the sediments. Hence, there is no predetermined result in this experiment.

In this study, we collected feedback from the teachers about their students' reactions to the Winogradsky column and the biofilm experiments. We also collected the students' laboratory journals containing experimental data and perceptions. In addition, the second author observed classes where the experiments were conducted. Summarizing from various sources of students' comments, the Winogradsky column and biofilms exercise is a positive experience in light of the following instructional merits:

1. The exercise enhances the understanding of scientific principles and processes. The Winogradsky column serves as a source of many different types of microbes involved in nutrient cycling. Many of these organisms play an essential role in the transformations of carbon, nitrogen, sulfur and iron. Information-processing skills are developed as students predict and explain their results.
2. Cooperative learning groups were used to organize and manage the lesson's activities. This classroom management method promoted peer relationships and created positive interdependence among students to achieve the lesson outcome. The predictions, summarized as the group's prediction, demonstrated the students' ability to synthesize knowledge and experiences about the characteristics of wetland soil (i.e. color, texture and odor) from earlier lessons. The knowledge gained from the Winogradsky and biofilms activity was not acquired from rote memorization but from conceptual learning that required the use of prior activities and the accommodation of the new information into the existing mental structure about wetlands.
3. The Winogradsky column with biofilms is an investigative activity that may be used over an extended period of time. Students learn and practice multiple science process skills such as observing, predicting, inferring, generalizing and interpreting data. The Winogradsky laboratory activity provides students with manipulative practice in setting up the column and with the intellectual challenge in finding out if their predictions are observed in the developed column. The relevance of this laboratory activity is maintained over a long period as students rely on these established procedures of inquiry to find the best explanation for their observations. In addition, this exercise integrates earth science and geochemical cycles in the life science concept of interdependence of organisms.
4. The changes in the Winogradsky column, such as the appearance of color and mottle formations, are models and visual anchors for identifying anaerobic and aerobic processes and for describing the role of microorganisms in nutrient cycling. Based on what they

observe, students develop an understanding of decomposition and chemical transformations that serves to augment what they read from the textbook or hear in a lecture.

5. The students' observations of microorganisms that colonize the biofilms reinforce and expand the concept of ecological succession. As one student wrote in her journal, "It is like a parade of microbes—they follow one another and they multiply until there are many of different kinds."

Nutrient cycling is one of the concepts in the unifying principle of *Interdependence of Organisms* included in the Content Standard for Life Science in grades 9 through 12. Teaching Standard A suggests that "teachers of science plan an inquiry-based science program for their students" (*National Science Education Standards 1996*). Nutrient cycling is an abstract concept to most junior high and high school students, many of whom may not yet have reached the level of formal reasoning. The observations that are made as the column develops provide students, who may be in transition from concrete to formal reasoning, with an observable model of nutrient cycling and microbiological succession. During the long-term observations, students visualize the gradual changes that take place during the chemical transformations and identify the role of various microbes involved in the process.

Our study illustrates that the Winogradsky column and biofilms is a simple, inexpensive and safe exercise that junior high and high school students can perform using sediments from their local environment. Active participation by the students adds to the interest and relevance of the experiment and integrates critical thinking with an investigative approach. Personal involvement increases excitement and discussion and provides an opportunity for students to experience how scientists function in the real world.

Acknowledgments

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Appendix A

Procedures for preparing the wet mount, simple stain, and Gram stain

(Morholt & Brandwein 1986)

Wet Mount Technique

1. Place the slide containing the biofilm on a flat surface. Since the biofilm accumulates on both sides of the slide, one side should be wiped clean.
2. Handle the coverslip carefully and place the edge of the coverslip on the slide so that it touches the edge of the water.
3. Rapidly lower the coverslip onto the slide to prevent forming and trapping air bubbles.
4. Place the slide on the microscope stage and observe under low power. Note the size, shape and arrangement of microorganisms. Record your observations by drawing and writing a brief description.
5. As an optional activity, examine the slide under high dry and oil immersion. Some microbes are motile, while others exhibit Brownian movement. Record your observations.

Appendix B

Simple Staining Technique

1. Allow the slides to air-dry on a flat tabletop.
2. Heat-fix the organisms. To heat-fix the smear, hold the slide with a slide holder and pass the slide back and forth three times immediately over the flame of a Bunsen burner. Heat-fixing kills bacteria and they adhere to the slide.
3. Flood the slide with methylene blue. Allow the stain to react with the organisms on the slide for 1 minute.
4. Using a slide forceps, tilt the slide so the stain runs off into a staining pan or sink.
5. Using water in a squeeze bottle, wash off the excess stain as the slide is tilted over the pan or sink.
6. Blot the slide gently with bibulous paper or a paper towel to remove the water. Do not rub the top part of the slide because that will remove the stain.
7. Place the slide on the microscope stage and observe under the low power.
8. Note the size, shape and arrangement of microorganisms. Record your observations by drawing and writing a brief description.
9. As an optional activity, repeat using high dry (40X) and oil immersion (100X) lenses.

Appendix C

Gram Staining Technique

1. Allow the slides to air-dry on a flat tabletop.
2. Heat-fix the smears.
3. Flood the slide with crystal violet, and allow it to react for 1 minute. All the bacteria will stain blue/purple.
4. Hold the slide with a slide handle or forceps, tilt it and drain the dye off into the staining pan or sink.
5. Using the squeeze water bottle, rinse the slide with a gentle stream of water.
6. Flood the slide with iodine. Allow the iodine to react for 1 minute.
7. With a slide forceps, tilt the slide and allow it to drain.
8. Immediately rinse the slide with water from your wash bottle.
9. Decolorize the slide for about 30 seconds by allowing the alcohol to run over and off the smear. The Gram positive bacteria retain the blue/purple color; the Gram negative bacteria lose their stain and become colorless.
10. Rinse immediately with water from the wash bottle to stop the decolorizing action.
11. Flood the stain with safranin counterstain for 1 minute. Drain the slide.
12. Rinse the slide thoroughly with water from the wash bottle. The Gram positive bacteria retain the blue/purple color; the Gram negative bacteria stain a pink/red color.
13. Blot the stained slide in a booklet of bibulous paper or between layers of paper towels. Do not rub, because that will remove the smear.
14. Place the slide under the microscope. Place a drop of oil immersion on the slide and observe under the oil immersion lens (100X).
15. Identify the bacteria as Gram positive or Gram negative. Record your observations by drawing and writing a brief description.

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