Curriculum Units by Fellows of the Yale-New Haven Teachers Institute
2010 Volume III: Geomicrobiology: How Microbes Shape Our Planet

How Microbes Help Ecosystems

Curriculum Unit 10.03.02
by Julia Biagiarelli

Introduction

On the 40 th anniversary of Earth Day 2010, this is what the headlines read:

> Oil Rig Sinks In Gulf of Mexico; 11 Still Missing
> NEW ORLEANS April 22, 2010, 04:13 pm ET
> Officials are saying that an explosion on an oil rig off the coast of Louisiana has the potential to be a major spill. The platform burned for more than a day after a massive explosion Tuesday. It sank into the Gulf of Mexico on Thursday (Fleming).

What does an oil spill have to do with microbes A lot, according to National Geographic News reporter, Christine Dell'Amore's report from Port Sulphur, Louisiana: "Gulf Oil Spill Is 'Butter' for Microbes." The story quotes Christopher Reddy, a marine chemist at the Woods Hole Oceanographic Institution (WHOI) in Massachusetts. "Any self-respecting bacteria are going to want to eat it." (Dell'Amore 2010).

There are hundreds of species of bacteria, so, what kind eats oil? Where do they come from? Have they been used before? Do they occur naturally? What are other ways that microbes can help ecosystems? This unit will be focused on exploring and answering these questions as well as teaching middle school students the basics about microbiology.

Objective

Through this curriculum unit, How Microbes Help Ecosystems, students will develop knowledge of the connections between the Science taught in the classroom and the world around them. They will also be able to use this knowledge throughout the academic and arts curriculum at Betsy Ross and as they transition into high school courses. This unit will be set up so that it can also be modified and adjusted to work for children at all grade levels and for children with specific IEP goals.

This unit will be taught to a sixth grade General Science class of about twenty to twenty five students at Betsy Ross Arts Magnet Middle School in New Haven, CT which is co-taught by a certified regular education Science teacher and a certified Special Education teacher. The students in the class have mixed abilities and experiences because it is a Magnet school. There will be between three to five students who have Special Education IEPs. About twenty five percent of the students in the school come from surrounding suburban schools. Another twenty five percent live in the surrounding neighborhood and the other fifty percent live in other parts of the city of New Haven. The sixth grade science course is a general science curriculum which covers both life sciences and earth sciences.

This unit is intended to deepen students' knowledge of biology, earth science, and ecosystems with a focus on the role of microorganisms in soil ecosystems. In this process I will create a curriculum unit that involves students in hands on projects, research and science journal writing. It is also my intention that students, through participating in the lessons and by producing written work, will be able to apply this knowledge to their participation in the annual school science fair.

Background Information

Basic Microbiology and the Tree of Life

When scientists first began to classify living things there were simply "Plants" or "Animals." However, with the invention of the microscope and further study of cells the Five Kingdom system of Animals, Plants, Fungi, Protista and Bacteria was developed. This all changed in the early 1990's after discovery, through DNA technology, of Archaea, organisms that were once thought to be part of the Bacteria "Kingdom." Hence a three branched system called The Tree of Life was developed.

In "Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya," Carl Woese explains the reason for changing how living things are classified:

> Molecular structures and sequences are generally more revealing of evolutionary relationships than are classical phenotypes (particularly so among microorganisms). Consequently, the basis for the definition of taxa has progressively shifted from the organismal to the cellular to the molecular level. Molecular comparisons show that life on this planet

divides into three primary groupings, commonly known as the eubacteria, the archaebacteria, and the eukaryotes. The three are very dissimilar, the differences that separate them being of a more profound nature than the differences that separate typical kingdoms, such as animals and plants. Unfortunately, neither of the conventionally accepted views of the natural relationships among living systems--i.e., the five-kingdom taxonomy or the eukaryote-prokaryote dichotomy--reflects this primary tripartite division of the living world. To remedy this situation we propose that a formal system of organisms be established in which above the level of kingdom there exists a new taxon called a "domain." Life on this planet would then be seen as comprising three domains, the Bacteria, the Archaea, and the Eucarya, each containing two or more kingdoms. (The Eucarya, for example, contain Animalia, Plantae, Fungi, and a number of others yet to be defined). Although taxonomic structure within the Bacteria and Eucarya is not treated herein, Archaea is formally subdivided into the two kingdoms Euryarchaeota (encompassing the methanogens and their phenotypically diverse relatives) and Crenarchaeota (comprising the relatively tight clustering of extremely thermophilic archaebacteria, whose general phenotype appears to resemble most the ancestral phenotype of the Archaea.

The "Domains" of Bacteria and Archaea, being that they are microorganisms, will be studied in this Unit.

Bacteria

Bacteria are single celled prokaryotic organisms. Their cells contain five main structures a nucleoid, which is different from a eukaryotic cell nucleus, because the nucleoid consists of DNA only and does not have a nuclear membrane; ribosomes, cell membrane, cell wall and surface structures which may or may not be part of the cell wall (Todar 2010).

The first known observation of bacteria occurred in 1676 when Antonie van Leeuwenhoek experimented with lenses and designed a microscope. He observed what he called, "animalcules" or "little animals." He wrote about his observations in several letters to the Royal Society. Other scientists were able to confirm his results, but it took about two hundred years until microscopes were developed enough for the science of microbiology to begin development (Madigan and Martinko, 9-10).

Development of the microscope has been essential to the study of bacteria being that they are usually only a few micrometers long. Their shapes vary. They can be rods, spirals, spheres, or even squares (Madigan and Martinko, 3), (Dennis 2010).

Nearly every habitat on Earth, including those with very extreme conditions, contains bacteria. They are found in soil, acidic hot springs, water, organic matter, living bodies of other organisms, oil slicks, deep in the Earth's crust and even in radioactive waste. Bacteria are also extremely prolific in these various habitats. One gram of soil typically contains about forty million bacterial cells. Fresh water usually contains about one million bacterial cells per milliliter. It is estimated that the Earth contains five nonillion, which is 5×10^{30} bacterial cells. Therefore it is not surprising that most species of bacteria have not been studied and only about half have been reproduced in laboratory work (Madigan and Martinko, 6).

Archaea

Since the early 1990's archaea have been recognized as one of the three basic domains in the Tree of Life. Archaea, are also single-celled prokaryotes, and include microorganisms that can live in oceanic hydrothermal vents or in concentrated brines, or in animal guts, or even inside of their host organism's cells. Although the most extreme environments are usually dominated by archaea, they are also the dominant organisms in vast expanses of the ordinary ocean water column and can be found in most any stagnant freshwater ditch or swamp. It is believed that in the very history of life they evolved from bacteria. They perform many diverse and surprising functions including complex chemical reactions. They also have varied physical appearances. Some take on basic geometric shapes like triangles and squares. Others look like amorphous blobs or like sophisticated networks of interconnected structures (Howland, 1).

Scientists group archaea into several categories including: Thermophiles, Methanogens, Halophiles, and others. Thermopilic archaea live in high temperature environments such as hot springs and near volcanic vents. Methanogenic archaea, as their name suggests, produce methane gas and live in anaerobic mud or animal guts where no oxygen is present. Halophilic archaea live in hypersaline environments such as the Dead Sea where most other organisms could not survive (Howland, 2-4).

Useful Microorganisms

Scientists are discovering, on a daily basis, how useful microorganisms are to ecosystems. As stated in the opening of this unit, bacteria are being used to help clean up oil spills. Bacteria, and other organisms, have also been long known for their important role in decomposing organic matter which returns nutrients to the earth for use by other organisms. Bacteria are also essential in the fixation of nitrogen, converting nitrogen from the atmosphere into ammonia, which is essential to create nucleotides for DNA and amino acids for proteins in living organisms. Anaerobic microorganisms perform putrefaction, the decomposition of animal proteins which is an important role in "trash removal" and "recycling" of organic material (Madigan and Martinko, 6). Recent studies have also discovered a connection between precipitation and the types of protein that certain strains of bacteria produce (Robbins). Also, it is becoming more and more apparent that microorganisms, often referred to as "Probiotics," play an essential role inside of larger organisms such as the human digestive tract and can be used to treat various ailments (Madigan and Martinko, 7).

Oil Oxidizing Microorganisims

Petroleum products can be oxidized aerobically by several varieties of bacteria, molds, and yeasts. These organisms use oil as electron donors, or energy sources, in a process called bioremediation which changes the oil into CO_2. The activity of these microorganisms is increased at certain temperatures and in the presence of adequate nitrogen and phosphorous which act as fertilizer for the bacteria. If conditions are ideal, bacteria can oxidize up to 80% of the non volatile components of the oil spill (Madigan, and Martinko 651,652).

Decomposers

There are two main groups of microorganisms called chemoorganotrophs and methanogens that decompose the organic carbon of dead organisms into carbon dioxide (CO_2) and methane (CH_4). Methanogens use the waste products of the organotrophs, CO_2 and acetate to make methane, they do not break down complex organic matter directly. The CH_4 is then oxidized by organisms called methanotrophs into CO_2. As shown below in the Carbon Cycle, all carbon biomass eventually returns to the form of CO_2 (Madigan, and Martinko 633).

Microbial Flora in Humans

In humans and other animals, the skin, oral cavity, respiratory tract, intestinal tract and urogenital tract, being exposed to the environment, are nearly always inhabited by microorganisms. These microorganisms perform a variety of processes essential to maintaining health. In human intestines, vitamin B_{12} and vitamin K are produced by the metabolic reactions of intestinal flora. Also, in the gut, microbial flora contribute to the absorption of essential steroids (Madigan, and Martinko, 701-710).

Symbiotic Relationships between Microbes and other Organisms

Coined by German botanist Heinrich Anton de Bary in 1879, symbiosis is defined by biologists as: "life together." In symbiotic relationships two organisms live in close association with each other. These relationships are categorized into three types depending on the role each member plays in the association: mutualism, commensalism and parasitism.

Mutualism is a symbiotic relationship where both organisms derive benefits from the other. This includes the microorganisms living on the skin and in the digestive tracts of humans. There are more bacterial cells living on and within the human body that there are actual cells in the human body. Mutualistic relationships are either obligate, where the relationship is essential to at least one of the organisms or facultative, beneficial relationships, but not essential for survival.

Commensalism generally means that both organisms receive neither benefits nor harm from the other. However, it is not as clearly defined a category because, through further study, benefits and or harm to one of the species involved is often discovered.

Parasitism occurs when an organism feeds or is sheltered by another organism without any benefit to the host. Often the parasitic species will develop into a pathogen, causing harm to its host organism (Hartnett 2010).

Ecosystems

A community of living things in a non-living environment that occurs in a specific location where a series of biogeochemical cycles and energy transformations take place is called an Ecosystem. These systems have boundaries that often overlap and are usually devised for the convenience of a particular study of the organisms living there. Ponds, lakes, oceans, forests, estuaries, and grasslands are examples of ecosystems.

Ecosystems are organized into non living, or abiotic, and living, or biotic components. The abiotic components include: sunlight, temperature, precipitation, water, soil and chemical elements and compounds. The biotic components include: primary producers, herbivores, carnivores, omnivores and detritivores. Microbes are usually members of the primary producers or the detritivores. Primary producers, being the organisms that use energy and elements to create biomass that other organisms can feed upon, while detritivores, sometimes called decomposers, are organisms that consume dead organisms returning essential nutrients to the system. Also within these systems are symbiotic relationships between microbes and other organisms (Madigan and Martinko, 6).

Long Island Sound

The ecosystem of Long Island Sound (the Sound) is an estuary consisting of several different habitats. In the Sound, fresh water from rivers and marshes mixes with salt water from the ocean. There are several major rivers flowing into the Sound coming from sources to the north in Massachusetts, Vermont and New Hampshire which provide eighty percent of the fresh water in the Sound. What makes the Sound unique is its two connections to the sea. Where the east side of the sound meets the Atlantic Ocean it is called, "The Race." The West side of the Sound connects to the sea via the East River.

As in most estuaries, a great diversity of plants, animals and microorganisms use the resources in the Sound for food, nesting, breeding, and as a nursery. In 1987 Long Island Sound was designated as a National Estuary (U. S. EPA).

Habitats of Long Island Sound

Within the ecosystem of Long Island Sound there are multiple habitats including: Salt Marshes, Tidal Flats, Rocky Intertidal Zones, Sandy Beaches, the Subtidal Zone, which is beyond the low tide level and constantly submerged, the Benthic Zone, which is the sea floor and the Pelagic Zone, which is the open water area. Plants, animals and other organisms have adapted themselves to survive and thrive in all of these habitats. (Wahle).

Microorganisms in Long Island Sound

Plankton is the most prolific organism in salt water environments. This includes photosynthetic plankton, such as bacteria like Prochlorococcus and algae, called phytoplankton. Phytoplankton play an important role in the survival of all life in Long Island Sound. Because they convert energy form the sun into biomass that is then consumed by other animals, they form the basis of the food chain. Another important function is their ability to convert elemental nutrients such as nitrogen into usable forms making it available to other organisms in the food chain. Also important is their role in the carbon cycle. Through the process of photosynthesis phytoplankton remove CO_2 from the water and release O_2 into the atmosphere. Then, when the phytoplankton die the CO_2 which was converted to carbon as biomass goes to the bottom of the ocean keeping a healthy balance of CO_2 and O_2 in the atmosphere (Hoyle), (enotes.com).

Bacteria can be found in all parts of the water column, on the surface of the sediments and as sediments themselves. Where they live in the water column is dependent on their individual requirements for oxygen or sunlight in the case of photosynthetic primary producers. Aerobic bacteria, because they require oxygen live closer to the surface whereas anaerobic bacteria, that do not require oxygen live in deeper portions of the water column, usually in the sediments below the sediment-water interface. Most water columns contain oxygen throughout except the Black Sea, dead lakes and Long Island Sound during summer hypoxia. Most bacteria in the water column live independently, although some live in symbiotic relationships with other water organisms. For example, bioluminescent bacteria live inside of some jellyfish and can been seen glowing at the surface of the water, of the Sound, near the coast at night (Hoyle).

Sulfate-reducing bacteria are organisms that respire sulfate by releasing hydrogen sulfide (H_2S) as a waste product. They are dominant microbes in marine systems, such as Long Island Sound, due to the high sulfate content of seawater. The H_2S is the source of the very distinctive "low tide smell."

Other bacteria in the Sound include anoxygenic phototrophs. These bacteria undergo photosynthesis in which O_2 is not produced. They are also among the first to have

harnessed energy from the sun over 3 billion years ago (Hunter et. al. p.32).

Cyanobacteria are also phototrophs. They are organisms that obtain their energy from light, and are important in Long Island Sound because of their ability to fix nitrogen (see Nutrient Cycles below). They were once called "blue-green algae," although they are not algae--which are eukaryotes more similar to green land plants and found in the Domain Eukarya . In the shallow waters of the Sound, during warm weather months, mats of cyanobacteria can be found. Cyanobacteria are oxygen producing photosynthesizers. These bacteria are also among Earth's earliest phototrophs and were responsible for converting the atmosphere to its present high-oxygen state. (Madigan and Martinko 397-399).

Nutrient Cycles

Walt Whitman said:

> "I bequeathe myself to the dirt, to grow from the grass I love;
> If you want me again, look for me under your boot-soles."

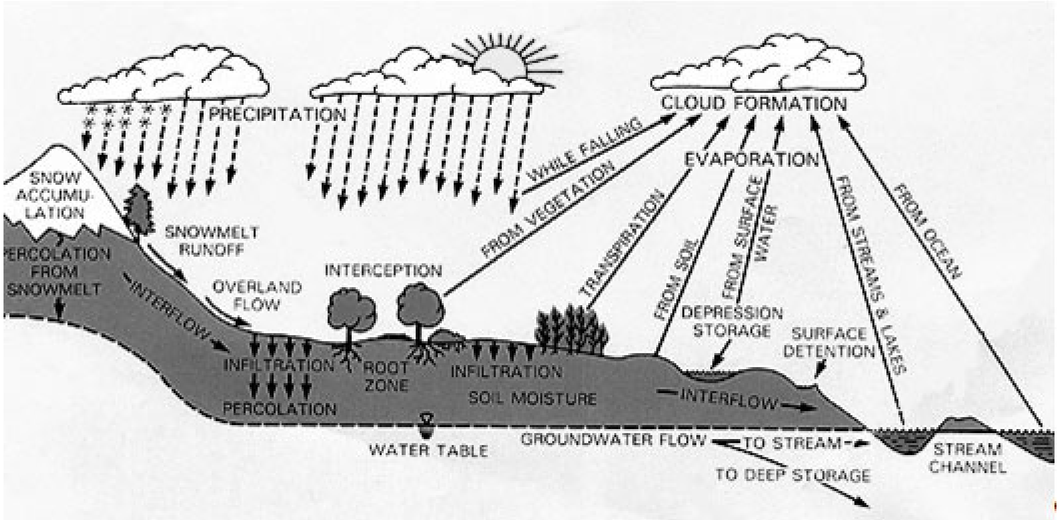
Ecosystems and the cycles of nutrients are not as simple as Whitman describes in the above quote, however, it gives the reader an image of living things undergoing changes. Scientists describe these changes as Nutrient Cycles.

The elements that are essential for living organisms: oxygen, carbon, nitrogen, phosphorus and sulfur are generally stable and remain preserved in the ecosystems of Earth undergoing changes called cycles. Water, although it is not an element, is essential for all known life, and also is mostly preserved through cycles. The cycles of water and the aforementioned elements can involve the simple process of the substance changing states and locations, or they can involve a series of chemical changes in which they are integrated into different molecular compounds. Microbes play important roles in the functioning of these cycles often as primary producers through photosynthesis and respiration or as detritivores through fermentation and respiration, and through other chemical processes (Sengbusch 2010).

Water Cycle

The amount of water on the earth is finite. Water can be cycled through different states and through ecosystems but it cannot be reproduced. The Water Cycle includes the processes of evaporation, transpiration, condensation, precipitation and collection. During evaporation and transpiration liquid water is changed into water vapor. Condensation occurs when water vapor returns to a liquid state. Precipitation happens when the condensed water droplets or ice crystals fall to earth. Collection refers to the areas on earth where water collects such as ponds, lakes oceans and streams. (Kidzone 2010).

WATER CYCLE DIAGRAM



http://rst.gsfc.nasa.gov/Sect16/Sect16_4.html

Oxygen Cycle

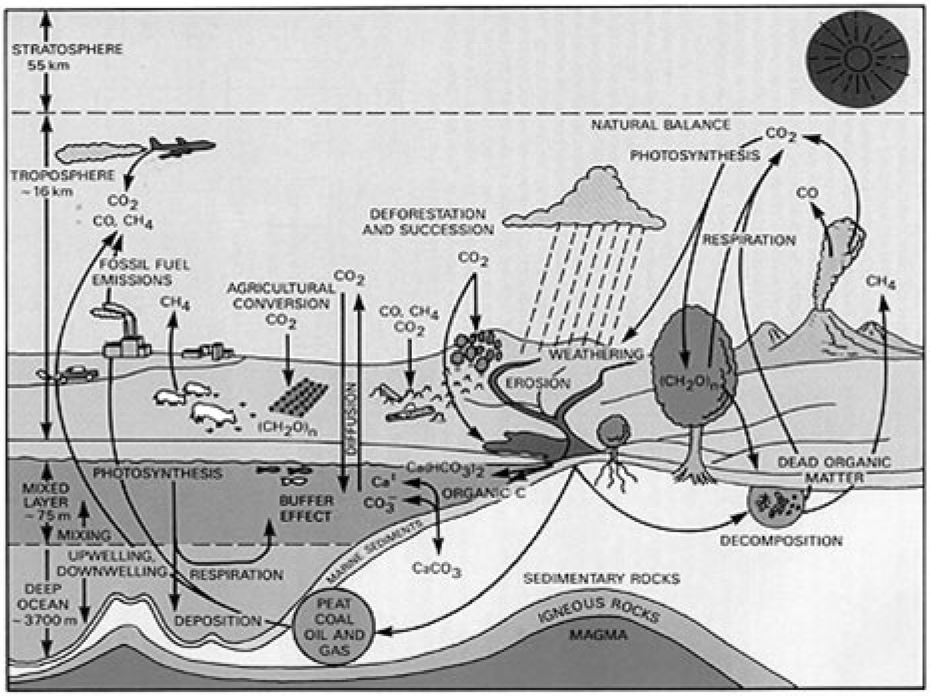
Plants and photosynthetic bacteria play an essential role in the oxygen cycle primarily through the process of photosynthesis. Only one two-thousandth of the entire earth's atmospheric oxygen is produced by plants each year the remainder comes from photosynthetic bacteria. In turn, animals and other living things use that oxygen for essential life processes and release other gases, mainly CO_2 back into the atmosphere which plants use again for photosynthesis. (Sengbusch 2010).

Earth's early atmosphere, however, was anoxic or without oxygen. Gases in Earth's early atmosphere included: carbon dioxide, nitrogen, ammonia, and methane. In the early atmosphere of Earth, the type of photosynthesis that we are familiar with, which produces oxygen, did not occur. There was another type that still occurs today all around us. It is a much simpler process and is known as anoxygenic photosynthesis because it does not produce O_2. This takes place in green and purple sulfur bacteria, which use H_2S, which is produced by sulfate-reducing bacteria mentioned above, in place of H_2O used by oxygen-producing photosyntesizers like green plants and algae. These two types of bacteria are important drivers of Earth's Sulfur Cycle. In both types of photosynthesis, the organism uses light to make ATP, the main energy carrier of cells (Madigan, and Martinko 29, 129, 302).

Carbon Cycle

The carbon makes up about 50% of the biomass of each living organism. It is also contained in earth's atmosphere, the ocean, rocks and soil. Carbon and oxygen atoms are combined as carbon dioxide (CO_2) molecules in the air. CO_2 is used by autotrophs, including plants and photosynthetic bacteria primarily in the Calvin Cycle for CO_2 fixation. CO_2 is not used in the light reactions of photosynthesis, it is used in the Calvin Cycle which converts inorganic carbon in the form of CO_2 into organic carbon or biomass that makes up the tissues of the plant. Then, it is available as food for organisms higher on the food chain. When plants die they may become buried and become fossil fuels after many millions of years. When these fuels are burned carbon dioxide returns to the air. (UCAR 2010).

CARBON CYCLE DIAGRAM

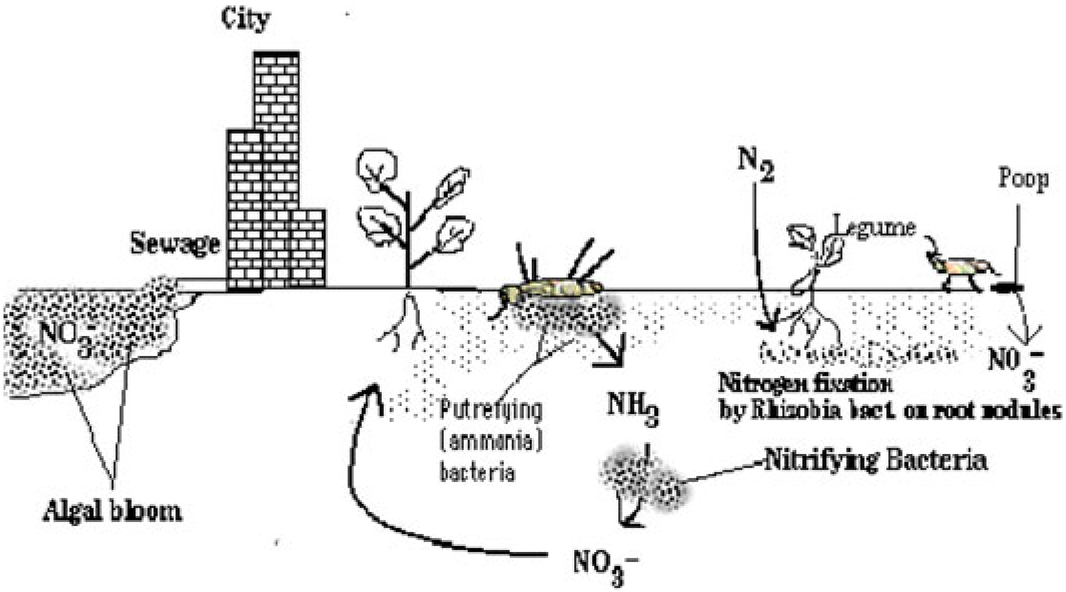


http://rst.gsfc.nasa.gov/Sect16/Sect16_4.html

Nitrogen Cycle

Earth's atmosphere is nearly 80% Nitrogen (N_2). Organisms need it to produce proteins and DNA. However, living things cannot make use of nitrogen gas directly from the atmosphere. Microbes in the soil, called diazotrophs and aquatic microbes like cyanobacteria are essential for changing or "fixing" atmospheric nitrogen into ammonium (NH_4^+), which is the form that plants use to make proteins. Some plants, mainly legumes, have nitrogen-fixing bacteria in their root nodules, also clovers, which we will explore in a hands-on exercise. Once nitrogen is in a usable form in plants, other organisms that eat plants also have access to nitrogen. After plants, animals and other organisms die, microbes and other decomposers feed on them changing their proteins back into nitrogen gas that is released into the atmosphere. The formula for nitrogen fixation is: $N_2 + 8H \rightarrow 2NH_3 + H_2$. (Madigan, and Martinko, 641), (Kids Know it Network 2010).

NITROGEN CYCLE DIAGRAM



http://soil.gsfc.nasa.gov/NFTG/nitrocyc.htm

Activities

Because the students at Betsy Ross Arts Magnet School come from a variety of backgrounds, the unit will begin with a brainstorming and question and answer session to assess students' prior knowledge of microorganisms. Open ended questions such as: What is a Microbiologist? What are germs? What are microbes? Can bacteria be good for you? What living things are helpful to people? How are microbes helpful to the Earth? Where can we find the smallest living things? Have you ever eaten bacteria? Have you ever eaten Fungi? Etc.

Students will be given a Science Journal where they can record everything including class notes, lab observations, questions, independent research and illustrations. It will be set up in a similar format as the interactive notebooks that they use for each academic subject. The interactive notebook utilizes a right brain/left brain approach to gathering information. In the open notebook the page to the left is for illustrations, 3-D vocabulary inserts, charts and graphs, while the page on the right side is for basic facts, and linear style note taking. The notebook is an ongoing tool in the classroom containing a Table of Contents, Vocabulary Lists, Formulas and References to sources of information (Gannon 2010), (Squidoo 2010).

ACTIVITY #1 What's That Smell?

Materials:

· 8 small glass beakers or containers (100mL-200mL capacity)

- Dark construction paper
- Tape
- Label stickers
- Markers
- 2 samples of Tap water (10mL-20mL)
- Live sample containing sulfate reducing bacteria which can be found in black mud (small sample, 25-50 mL is enough) from Long Island Sound (see Activity #2) or other marine source kept in a closed container for a few hours is long enough.
- Sample of rotten eggs
- Freshly made mixture of yeast and warm water (10mL-20mL)
- Sample of recently baked bread (warmed)
- Sample of soil taken where plants are growing (remove bulk of plants and roots)
- Live sample containing Streptomyces, (can be obtained from a small sample of soil) the bacteria that produces geosmin, the chemical compound that smells like dirt.

Procedure:

PART I-preparation

Prepare samples before beginning activity with students. First cover the sides and bottom of the beakers with the dark paper, so that the students cannot see inside. Place a label on each beaker and number them from 1 to 8.

Then place, each in its own beaker, the following samples numbered 1-4: soil, bread, rotten eggs, and one of the tap water samples. Cover the beakers with filter paper or cloth (so that the smell comes through but they cannot see the samples).

Repeat for samples 5-8: sulfur reducing bacteria, yeast, streptomyces coelicolor, and another tap water sample.

Prepare microscope slides or use premade slides of the yeast, the two bacteria samples and the tap water.

PART II-student observation using smell only

Prepare students for lab activity. Have microscopes and beakers with samples set up, and make sure each student has his or her interactive notebook ready.

Start with samples 1-4, have them smell each sample and record in their notebooks their own description of what samples 1-4 each smelled like. Repeat with samples 5-8.

Next, have them match 1-4 with 5-8 and when their final choices are made, remove the filters or cloth from the top and some of the paper if necessary, so that they can see what they were smelling (keep number labels affixed so that they can refer to their notes to compare their descriptions with what they see). Have them draw and name what they see after the samples have been revealed.

PART III-student observation using microscopes

Have students view slides of each sample and draw their observations in their notebooks.

PART IV-mini lesson on odors produced by chemical processes of microorganisms

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Open by asking students, "Do you know why dirt smells like dirt?" "Why does yeast smells like yeast?" "Why do rotten eggs smell like rotten eggs?" Have students write their ideas in their notebooks while the teacher writes them on the board (or whatever presentation surface is used in the classroom). Most likely they will guess correctly that each smell comes from the microorganisms they just observed. Then give a brief explanation, having students take notes, of the chemicals that cause each smell.

Dirt smells like dirt because of chemicals called "geosmins" which means "earth smell" (Greek: geo-earth, osmi-smell). These chemical compounds of carbon, oxygen and hydrogen, are produced when bacteria, called streptomyces coelicolor, ingests dead plant biomass. There are many different kinds of streptomyces coelicolor that are very beneficial to humans. Most antibiotic medicines are made from certain varieties of streptomyces coelicolor.

Temperatures between 105 ^{o}F and 115 ^{o}F are ideal for yeast growth and reproduction. Sugar, a chemical compound made from carbon, oxygen and hydrogen, is food for the yeast. As the yeast uses the sugar for energy it releases CO_2 and ethyl alcohol. The smell and taste of fresh baked bread comes from the combination of those two substances.

Hydrogen sulfide (H_2S) is produced by anaerobic bacteria, usually in moist environments where oxygen is absent such as the mud at the beach which is revealed at low tide. The sulfur is what makes it smell like rotten eggs. The bacteria feed on the biomass of dead organisms to obtain energy for their own growth and reproduction and release the H_2S into the air. Small amounts of H_2S can be very smelly, however, the smell of more concentrated amounts cannot be detected by human smell and is very toxic and flammable (Cane 2010), (H. Kimberly 2010), (Wordinfo 2010), (Glass 2010), (Madigan and Martinko 390-394).

ACTIVITY #2 Microorganisms in a Winogradsky Column

Sergei Winogradsky was a Russian scientist who created this miniature environment for studying microorganisms in the late 1800s. Once these columns are created they can be stored for several months and used for a variety of laboratory activities for all grade levels (Fox 2010).

Materials:

· Gloves
· Buckets
· Hand Trowels/Shovels
· Water Shoes (or old sneakers that will be thrown out after activity)
· Observation/Data sheets (to be attached to interactive notebooks)
· Aprons (or disposable ponchos)
· Empty plastic bottles (12 to 20 ounce)
· Spoon for stirring
· Carbon source (shredded newspaper)
· Sulfur source (hardboiled egg yolks)
· Water (obtained at source)
· Soil (obtained at source)
· Rubber Bands
· Plastic wrap

Procedure:

Soil samples for Winogradsky Columns can be obtained from any location however, those obtained from a water source are best. In this activity samples will come from the estuary environment of Long Island Sound just below the low tide line better to get from salt marsh pond mud (Subtidal Zone). Choose a location where students can easily access samples and where there are no restrictions for use. (Avoid Wildlife Sanctuaries and private property).

Upon arrival at the Subtidal Zone find a muddy spot that, preferably, does not have many rocks. Using buckets, gather mud (enough to fill each student's plastic bottle about 75% full) being careful to avoid rocks, sand, and sticks. Gather a small amount of water from the same location (students need only a few centimeters of water at the top of their plastic bottles above the mud).

In a separate location, such as picnic benches at the gathering site or back in the classroom lab, prepare individual Winogradsky columns in plastic bottles. First mix in about a handful of shredded news paper and a tablespoon of egg yolks for every two bottles that will be filled with mud. Add a little more water if the mixture is too thick. Have students fill each bottle about three quarters full of mud mixture, gently tapping down mud after each spoonful to keep air bubbles out of column. Then add about one quarter inch of water to the top (don't stir it in). Cover top of each bottle with plastic wrap and fasten with rubber bands. Place in indirect light at room temperature. After about three to six weeks the different layers of microorganisms will become visible. Columns are now ready to be used for observations. Take caution when removing plastic wrap from the column. Always do all unwrapping and gathering of specimens under a fume hood to prevent inhalation of sulfur gases. Top off column with water lost due to specimen removal or evaporation. Rewrap the column with plastic wrap immediately after removing specimens.

In a Winogradsky column different organisms will develop in different layers depending on their individual needs for oxygen, sulfur and sunlight. Layers in individual columns will vary depending on the water source, amount of light, temperature and nutrients in the mud.

Below is a sample of how the layers form. At the top where the sunlight can penetrate the photosynthetic organisms can thrive. This is also the area where oxygen is present due to the production of O_2 from photosynthesis. Therefore, as the amount of sunlight decreases, so does the amount of oxygen. As the sunlight and oxygen decrease the sulfur content increases. In these layers the sulfur producing organisms are present.

It is important to note that a Winogradsky column is not a true representation of the natural ecosystem. It serves as a mini laboratory to obtain specimens for study which can be a useful tool in the classroom.

The diagram below is an example of how the layers can form in a Winogradsky column, will not all form exactly alike. Most, however, will have distinctive zones usually identifiable by colors or odors. This will make is easier to identify the individual types of bacteria and other microorganisms.

Layers of a Winogradsky Column:

<table>
<tr><td colspan="5">Plastic Wrap</td></tr>
<tr><td rowspan="2">High Oxygen</td><td rowspan="2">Low Sulfur</td><td>Air</td><td></td><td rowspan="2">Sunlight</td></tr>
<tr><td rowspan="2">Aerobic Zone</td><td>Sheathed Bacteria</td></tr>
<tr><td rowspan="2">Decreasing oxygen</td><td rowspan="4">Increasing Sulfur</td><td>Cyanobacteria, diatoms, Protists</td><td rowspan="2">Decreasing Sunlight</td></tr>
<tr><td rowspan="3">Microaerophilic Zone</td><td>Photoheterotrophs

Purple non-Sulfur Bacteria</td></tr>
<tr><td rowspan="4">Low Oxygen</td><td>Purple Sulfur Bacteria</td><td rowspan="4">Low Sunlight</td></tr>
<tr><td>Green sulfur Bacteria</td></tr>
<tr><td rowspan="2">High Sulfur content</td><td rowspan="2">Anaerobic Sediment</td><td>Sulfur Reducing Bacteria</td></tr>
<tr><td>Clostridium</td></tr>
</table>

ACTIVITY #3 Clover Root Nodule Exploration

Materials:

- Freshly gathered clover plants with roots (choose those with obvious pink root nodules)
- Scalpel or razor blade
- Microscope
- Microscope slides
- Cover slips
- Observation notebooks
- Gloves
- General Stain

Procedure

Shake off excess soil from clover roots and rinse with deionized water and alcohol. Using a scalpel or a razor blade, break open a root nodule. (The tissues inside the nodule should appear pink indicating the presence of the plant protein, leghaemoglobin, that is necessary to fix nitrogen). Smash nodule or chop into very small pieces, place a small amount on a slide and mix with a drop of water to create a wet mount. Place cover slide over specimen and examine wet mount under a microscope. To make cells more visible, a general stain can also be used. For staining, first heat fix the specimen to the glass slide by allowing the sample mixed with the water drop to dry onto the slide by passing the slide back and forth over a flame (specimen side up and not in contact with the flame). Next, flood heat-fixed specimen with a general stain (e.g. Gram's crystal violet, methylene blue) and allow it to sit for one minute. Then gently rinse the slide with tapwater to remove excess stain (until water runs clear). Add a water drop and cover slide for observation under the microscope. Rod shaped bacteria should be visible under the microscope. Record observations in Interactive Notebook.

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APPENDIX A

Web Resources recommended for elementary grade teachers:

http://www.kidzone.ws/water/

http://eo.ucar.edu/kids/green/cycles6.htm

http://www.kidsgeo.com/geography-for-kids/0161-the-nitrogen-cycle.php

http://www.kidsgeo.com/geography-for-kids/0165-biomes.php

interactive-notebooks.wikispaces.com/

www.squidoo.com/interactive-notebook

http://www.teacherweb.com/SC/LadysIslandMiddleSchool/Gannon/AP6.stm

http://indianapublicmedia.org/amomentofscience/the-smell-of-dirt/

http://www.microbiologybytes.com/video/Streptomyces.html

APPENDIX B:

Science Standards

National:

U. Unifying Concepts and Processes - Unifying concepts and processes help students think about and integrate a range of basic ideas which builds an understanding of the natural world.

A. Science as Inquiry - Science as inquiry requires students to combine processes and scientific knowledge with scientific reasoning and critical thinking to develop their understanding of science.

C. Life Science - Life science focuses on science facts, concepts, principles, theories, and models that are important for all students to know, understand, and use.

http://www.education.ne.gov/science/Documents/National_Science_Standardspdf.pdf

State of Connecticut:

6.2 -- An ecosystem is composed of all the populations that are living in a certain space and the physical factors with which they interact.
6.4 -- Water moving across and through earth materials carries with it the products of human activities.
7.2 -- Many organisms, including humans, have specialized organ systems that interact with each other to maintain dynamic internal balance.

http://www.sde.ct.gov/sde/lib/sde/pdf/curriculum/science/PK8_sciencecurriculumstandards2009.pdf