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Welcome to Nanoscience

INTERDISCIPLINARY ENVIRONMENTAL EXPLORATIONS

GRADES 9–12

NSTApress
National Science Teachers Association

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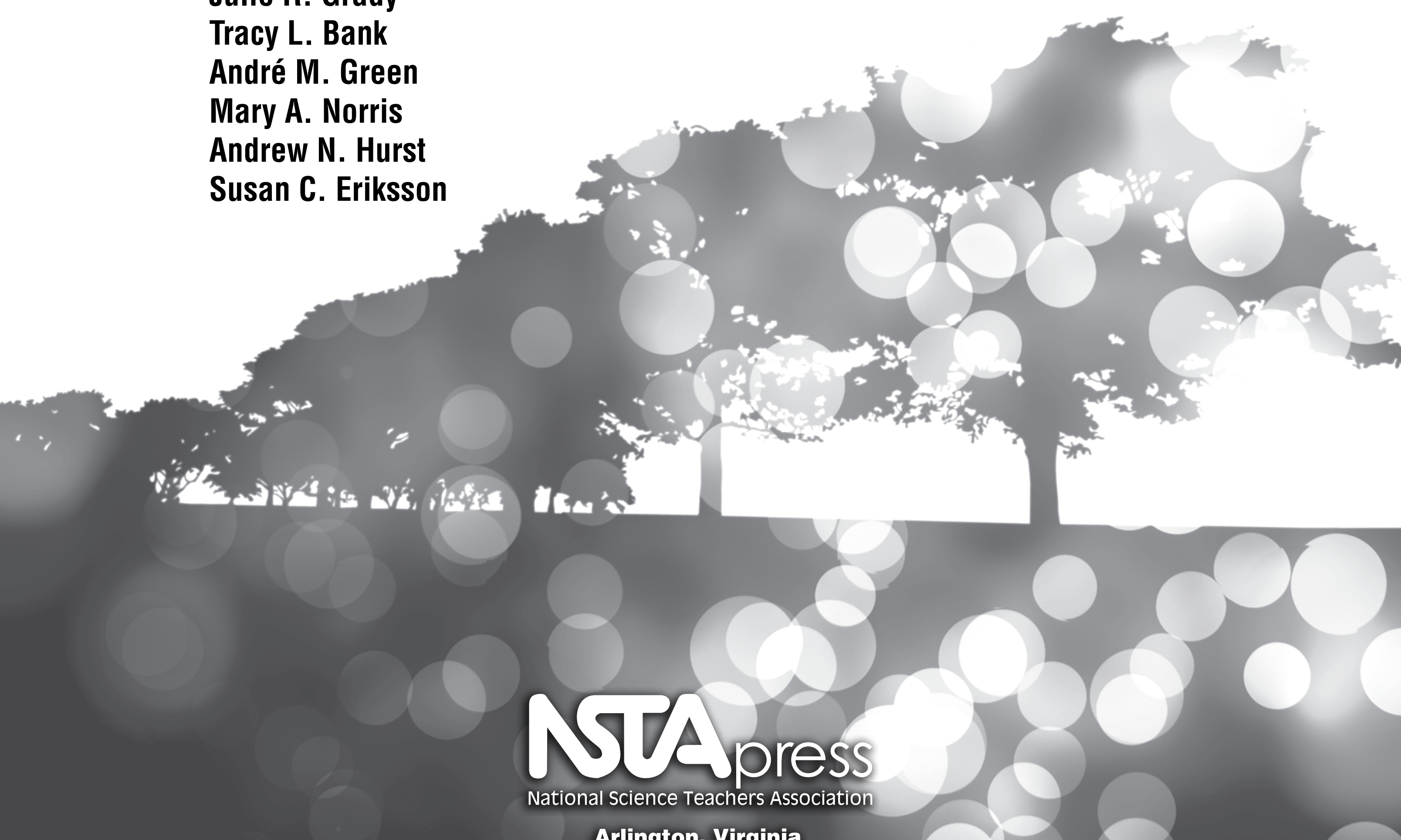

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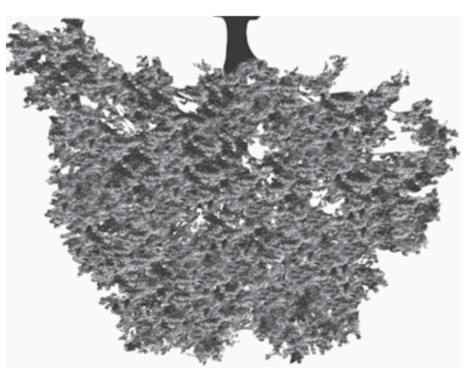
CONTENTS

The Nano2Earth Curriculum

Nano2Earth (pronounced "nano-to-Earth") is a secondary school science curriculum that brings nanoscale science and technology to life in the context of Earth and environmental sciences. Nanoscale science and technology, working together with environmental science issues, transcends traditional scientific knowledge and processes presented in high school chemistry, biology, geoscience, and environmental science classes today. Nevertheless, every aspect of the curriculum addresses one or more of the National Science Education Standards (NSES). Nano2Earth originated as an outreach project in the Department of Geosciences at Virginia Tech. *Welcome to Nanoscience* was a collaborative project four years in development. It was conceived, written, and classroom-tested by five high school science teachers from southwest Virginia, four professors from Virginia Tech, and several graduate students (see Working Group on Nano2Earth and the Nanobiogeochemistry Secondary Science and Math Curriculum Project, p. ix). This material is based on work originally supported by the National Science Foundation (NSF) Nanoscale Science and Engineering Program under contract EAR-0103053, and subsequently by NSF and the Environmental Protection Agency (EPA) under NSF Cooperative Agreement EF-0830093, Center for the Environmental Implications of NanoTechnology (CEINT). Any opinions, findings, conclusions, or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review and no official endorsement should be inferred.



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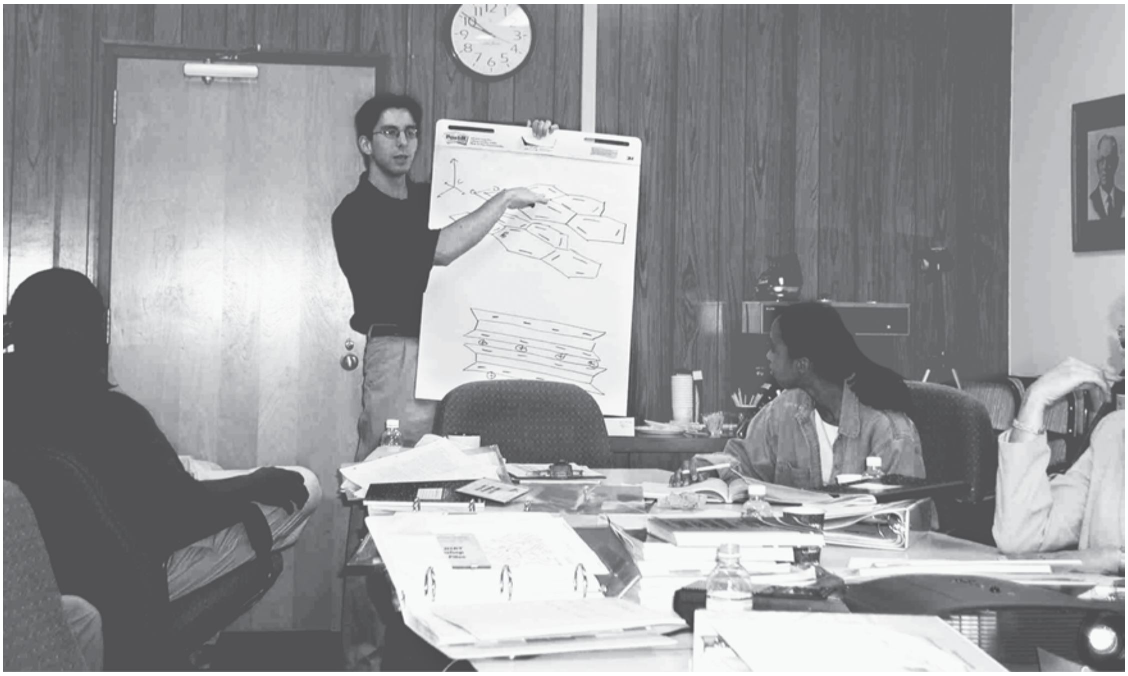
Tips For Using This Book

The secondary school curriculum presented in this book is designed to introduce the new, revolutionary fields of nanoscience and nanotechnology to high school students. This curriculum is the first in the country (as far as we know) to introduce these two subjects using an environmental science approach, which makes the curriculum appropriate for biology, chemistry, and Earth science courses.

This book is divided into two parts. Part I is made up of chapters 1–5. Chapters 1–3 provide background material for the teacher, answering questions such as the following: What is nanoscience and technology? What are the important historical and societal aspects of nanotechnology? How is nanoscience related to environmental science? Chapter 4 describes how education in nanoscience and nanotechnology addresses the National Science Education Standards. Chapter 5 describes the curriculum.

Part II is the Nano2Earth curriculum itself, consisting of five lessons. Teachers may use the entire curriculum or pick and choose among its several parts depending on their preferred emphasis, the course level, and available time. The curriculum is meant to be flexible, with numerous entry and exit points. Teachers



Andrew Madden explains to the Nano2Earth team how nanoscale forces of interaction are related to the properties of mineral surfaces.

can use aspects of the curriculum for as little as one day, explore the entire package for a few weeks, or choose an in-between length of time. For example, Lesson 5, "Nanoforces in Nature" includes two scenarios. The choice of scenario would depend on whether Lesson 3 or Lesson 4 was done beforehand.

While most required materials are readily available or inexpensive, two lessons include technology or supplies that the teacher should consider before starting:

1. Activities in both Lesson 3 and Lesson 4 require probeware or a dissolved oxygen (D.O.) probe (Lesson 3) or a light-sensing probe (Lesson 4).
2. Activities in Lesson 4 require fluorescent microbeads that must be purchased—no substitutes have been identified (see the lesson description on pp. 81–84 for details).

Appendixes include excerpts from the NSES, a correlation chart relating AP Environmental Science Themes with Nano2Earth lesson content, and a glossary of key terms.

We encourage comments and suggestions. Please send them to Ms. Ellen Mathena (*mathena@vt.edu*). These will help us produce the next edition of Nano2Earth. In addition, questions concerning the use of this curriculum should also be sent to Ellen for distribution to the appropriate team member.

Lesson 5 in this book uses a computer simulation program. This program can be downloaded at *www.nsta.org/download/nanosim.exe.*

Full color versions of the figures in this book can be downloaded at *www.nsta.org/dowload/WelcometoNanoscienceimages.pdf.*

Part I

Nanoscience History, Context, and Curriculum Overview

Chapter 1

What Are Nanoscience and Nanotechnology? A Nano Primer

The prefix *nano* simply means one billionth. So, 1 nanometer (nm) is 10^{-9} m. To help put this size in perspective, one-tenth of 1 nm is approximately the size of an atom. For example, 10 gold atoms, lying side by side, would be about 1 nm long. So why should a fundamental branch of science—one that is applicable to all sciences—be named using the *nano* prefix? What is so special about this length scale? The answer lies in the physical properties of any substance that is this small.

Let's consider the melting point of gold. Find gold's melting point in any reference book for metals; it is listed at exactly 1,064°C (1,947°F). This temperature can be easily verified by placing a gold nugget in a high-temperature furnace and increasing the temperature. When 1,064°C (1,947°F) is reached, the nugget changes shape and forms a ball of liquid gold. Let's try the experiment again, but this time, instead of a gold nugget that we can see and easily handle, let's melt a nugget that is only a few nanometers in diameter (obviously, we'll need special equipment and methods for this, but it can be done). Surprisingly, gold's melting temperature in the second case is only 427°C (800°F). Have we made a mistake?

More experimentation would show that no mistakes were made, and we would actually find that the melting temperature of gold particles depends on the size of the particles being heated. So why do all the reference books list 1,064°C (1,947°F) as gold's melting temperature? Because for all practical purposes, this melting temperature is accurate. If we continued our melting experiments with a range of gold particle sizes, we would find that all the gold particles that we can see melt at this temperature, from large nuggets to very small nuggets. However, when the nugget size is reduced to several tens of nanometers, we would start to measure a lower melting temperature. In the nanosize range, the smaller the particle, the lower the melting temperature.

This example allows us to easily and precisely define nanoscience and nanotechnology. *Nanoscience* is the field of science that measures and explains the changes of the properties of substances as a function of size. Like the melting of gold, the properties of any substance will remain constant as its size gets smaller and smaller—that is, until the size is reduced to the nanoscale (depending on the substance and the property being measured, roughly 10–100 nm). In the dimensional nanoscale, any physical property measured will continuously change with size, and often dramatically so. *Nanotechnology* is the application of property modifications that happen at the nanoscale to some beneficial endeavor—and what a warehouse of beneficial endeavors there are! As described in the next chapter, the promise of nanoscience is so great and the application of nanotechnology so vast that they are projected to change our world, similar to the current biological revolution occurring in genomics.

It is important to realize from the outset that all substances, including solids, liquids, and gases, show property changes through the nanosize regime. In addition, not all three dimensions of a substance need be in the nanoscale. For example, a *nanoparticle* (such as our small, low-melting temperature gold particles described previously) is small in all three dimensions. What if we simply confine one dimension to the nanoscale? With two dimensions unlimited, and one confined, we have created a *nanofilm,* which is a film with a thickness in the nanoscale. Will its physical properties be affected relative to the same substance with no size restrictions? Absolutely.

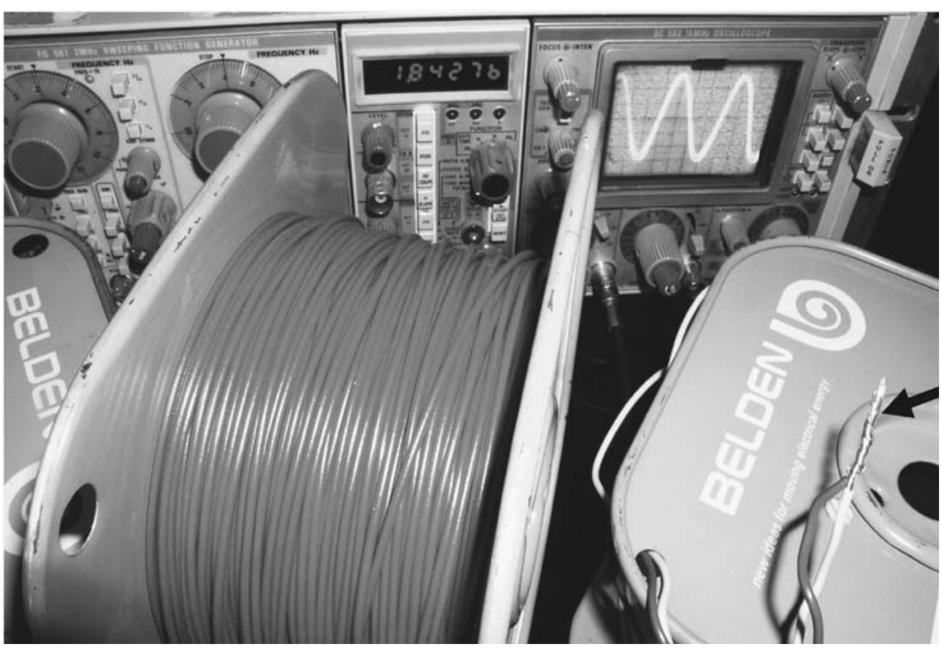


PHOTO COURTESTY OF MARK FORTNEY

Two aluminum wires twisted together (see arrow at right) provide excellent electrical conduction from one wire to the other. Yet the aluminum metal wires are covered with aluminum oxide, an insulator. How is good electrical conduction achieved through the nonconductive aluminum oxide? Nanoscience explains this phenomenon.

Consider the following example: Imagine that you have two wires made of aluminum, a metal that conducts electricity well. Imagine connecting each wire to the terminals of a battery, and then touching the surfaces of the wires together. Naturally, a circuit is formed and an electrical current will flow from one wire to the other. However, the surface of the wires is not conductive aluminum (Al) as one might assume, but instead a thin film of aluminum oxide (Al_2O_3), which covers the aluminum metal. This is because the surface of aluminum—whether the aluminum makes up a wire, a soda can, or an airplane wing—oxidizes in air to an aluminum oxide. The apparent problem in our electrical conduction experiment is that aluminum oxide is a well-known insulator; that is, it does not conduct electricity. So how can a current flow from one wire to the other? The answer lies in the physical dimension of the thin film of aluminum oxide. The coatings on the aluminum wires are very thin, typically 1 nm thick. In this size range the electrical properties of aluminum oxide have changed. When conducting electrons perpendicular to the thin film (that is, across the film from one wire to the next), the aluminum oxide acts more like a conductor, and, therefore, the electrons are free to pass from one wire to the next. In other words, because of the dimensions and geometry of the aluminum oxide, it behaves more like a conductor than an insulator, and good electrical contact between the two wires

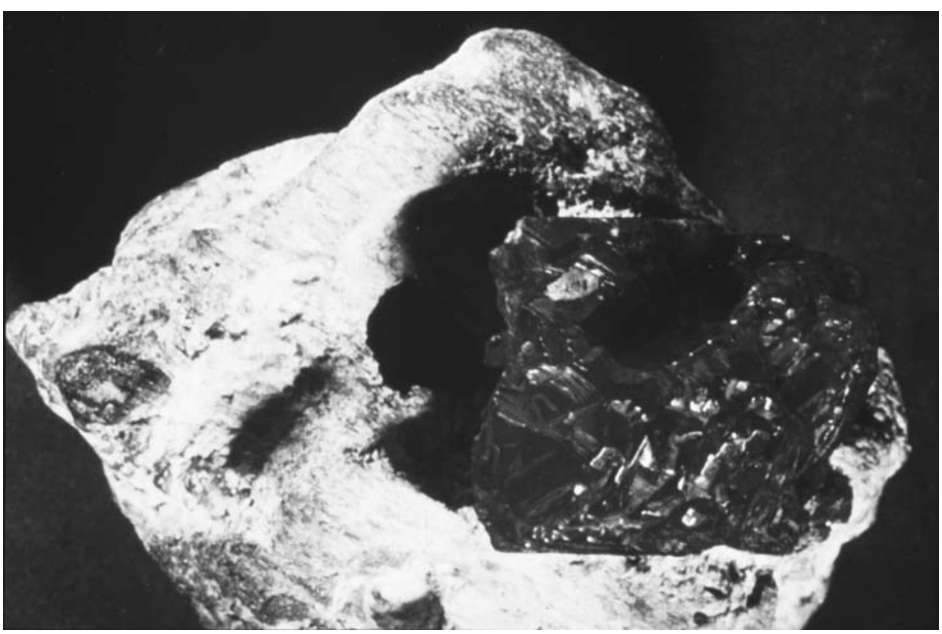


PHOTO COURTESY OF RICHARD BAMBACH

One of the most valuable of all gemstones, blood-red ruby is aluminum oxide with the same composition as the thin film that covers aluminum wire. Rubies do not conduct electricity, but the thin film of the wire does, thanks to the special properties of nanofilms.

is the result. Although this is something we take for granted—or were never aware of in the first place—it is a dramatic result. One simply needs to imagine what electronics would be like if bulk properties also applied to thin films!

In summary, in the examples presented above, we have defined the nanoscale and nanoscience. We have seen surprising results for two "ordinary" phenomena that we thought we knew everything about, but which were both shown to have surprising twists. These deceptively simple twists are what the nanorevolution is all about. This revolution is described in the next chapter.

Chapter 2

Historical and Societal Aspects of Nanoscale Science and Technology

The Origins of Nanoscience

Nanoscale science is typically defined (for reasons given in Chapter 1) to be relevant between 1 nm and perhaps 100 nm. With the advent of x-rays in the late 1800s, crystallographers were working at the nanometer scale or smaller as atomic arrangements in crystals were first determined in 1912. However, scientific historians say the earliest beginnings of nanoscale science and technology began in 1959, the year that Richard Feynman—a quantum physicist and one of the 20th-century's greatest scientists—gave a speech to the American Physical Society entitled "There's Plenty of Room at the Bottom." Feynman was fascinated by the notion of scaling, and in this speech he imagined that a single bit of information could be stored in a nanospace (specifically a 125 atom cluster), an exceptionally bold prediction at that time. At that scale of miniaturization, he estimated that all the text ever written in books in the history of the world could be stored within a cube 0.2 mm on a side (thus his lecture title).

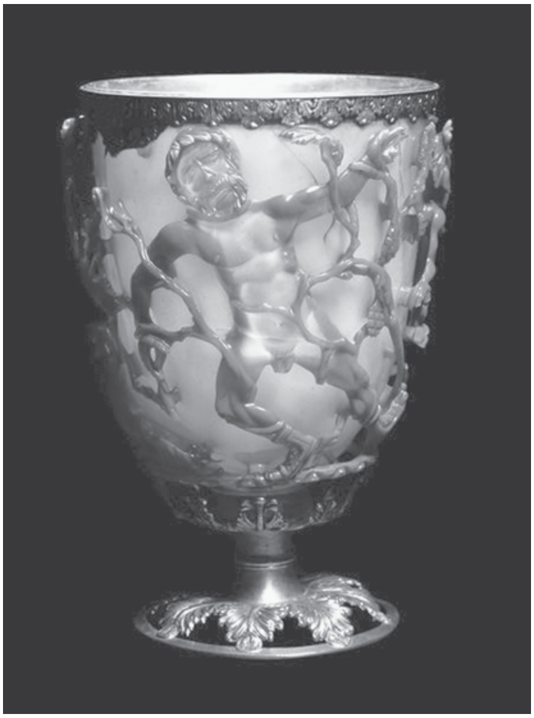


PHOTO COURTESY OF THE TRUSTEES OF THE BRITISH MUSEUM

The Lycurgus Cup (4th century, Roman). The cup is made of common silica glass, except that it contains nanocrystals of gold. The nanocrystals cause the glass to appear ruby-red when the light source is inside the cup (transmitted light; as shown) and entirely yellowish-green when the light source is outside the cup (reflected light).

Feynman's genius was his realization that *all things do not simply scale down in proportion*, which is now considered the cornerstone of nanoscience. He was predicting that when materials were scaled down to the nanometer size range, they would behave differently, which could be turned into an advantage. Near the end of his talk, he posed the ultimate challenge when he said: "I am not afraid to consider the final question. Can [we] arrange atoms the way we want, all the way down?" The general reaction to his comments was amusement, as statements like these were considered scientifically radical, not necessarily visionary, at the time. For example, in the 1950s one of the great theoretical physicists of the last century, Erwin Schrödinger, predicted that we would never experiment with just one atom or molecule. However, in the late 1980s the direct manipulation of individual atoms by humans became a reality. Unfortunately, Feynman did not live long enough to witness this monumental achievement.

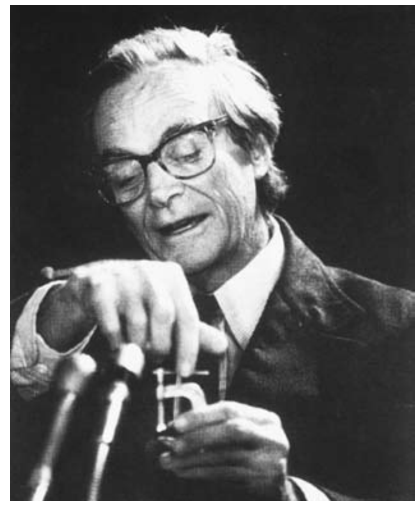


PHOTO COURTESY OF ENGINEERING & SCIENCE MAGAZINE

Physicist Richard Feynman, (1918–1988)

How Much Interest Has Nanoscale Science and Technology Attracted?

Developing a new field of science is very expensive. Without the significant investment of large amounts of capital, beneficial technology that comes from the new science won't materialize. Federal funding agencies, such as the National Science Foundation, the Department of Energy, and the Department of Defense, are keenly aware of this. So to accelerate the commercial development of nanotechnology, which has enormous commercial potential and benefits to society, the National Nanotechnology Initiative (NNI) was launched by the Clinton administration in 2000. Over the following decade, the U.S. government pumped nearly 12 billion dollars into nanoscience research and the development of nanotechnology, and the investment continues today. Other countries also initiated impressive research programs. The corporate world has gotten involved as well and in some cases corporations have been leading the charge. Giants such as IBM, Motorola, Dow Chemical, and other science-based corporations are cumulatively investing billions of dollars in nanoscale science and technology. Just as important, hundreds of new and relatively small nanotechnology-based companies have been established. All of this excitement is another indication of the young, but clearly recognizable, nanoscience and technology revolution.

How Has Nanotechnology Already Affected Our Everyday Lives?

Before the "nano" label became so popular over the last decade, scientists and engineers were hard at work developing useful products that use nanoscale particles and thin layers with properties specifically targeted for various purposes. Nanosize particles of zinc, cerium, and indium oxides are already used in electronics, fuel additives, optics, and personal care products such as sunblock and cosmetics. Stain- and wrinkle-resistant pants sold by Eddie Bauer, Lee Jeans, Gap, and other retailers use nanotech fiber and coating technologies.

In the computing world, nanotechnology has already had a great impact on data storage. Several years ago, computer disk drives were quickly approaching the theoretical limit in data density for the existing technology of magnetic storage—20 to 40 gigabytes (GB) per square inch—but nanotechnology provided a major change in hard disk design. On the surface of a disk, a layer of the metal ruthenium three atoms thick (which is much less than 1 nm) is sandwiched between two much thicker magnetic layers. This allows for smaller than previously obtainable magnetic domains in the layers above and below the ruthenium, while remaining stable over time. As a result, data could be stored at much higher densities, which allowed for the creation of 400 GB desktop drives (equivalent to the data on 80 DVDs or 600 CDs), 200 GB drives in notebook computers, and 6 GB drives in small handheld devices. Today, small handheld devices can hold hundreds of GBs, enough for tens of thousands of songs and hundreds of hours of video. Even these numbers will seem small in a few years; however, the point is that nanotechnology provided the quantum leap in disk design needed to continue to propel industry forward.

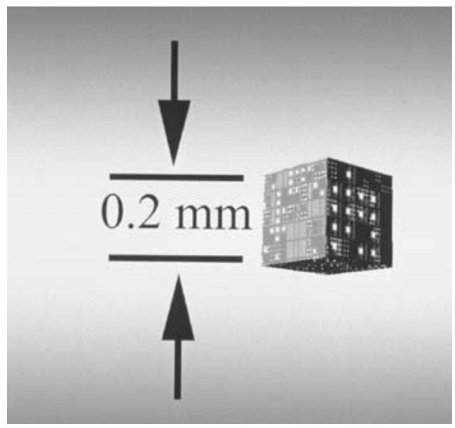


PHOTO COURTESTY OF DR. MICHAEL HOCHELLA

Richard Feynman estimated that all the text ever written in books in the history of the world could be stored within a cube 0.2 mm tall.

The most sweeping nanotechnology application occurred in the heterogeneous catalysis industry. Heterogeneous catalysts promote chemical reactions on surfaces and nanosize particles. This technology has played a key role in leading a multitrillion dollar chemical industry worldwide, and the technology is used in hundreds of applications, from refining oil to breaking down toxic car emissions through catalytic converters.

What Could Nanotechnology Mean to Society in the Long Run?

In its report *Nanotechnology: Shaping the World Atom by Atom*, the National Science and Technology Council stated that nanotechnology "stands out as a likely launch pad to a new technological era because it focuses on perhaps the final engineering scales people have yet to master" (NSTC 1999). Nanoscience has applications in all areas of science, and nanotechnology has applications for most fields of technology, including robotics, chemical and mechanical engineering, medicine, computing, and so on. Because of these widespread applications, it is widely anticipated that the future impact of nanotechnology will eventually far exceed the impact of the silicon-based integrated circuit (computer technology as we know it today). Like the present molecular biology revolution (e.g., genomics, proteomics) and

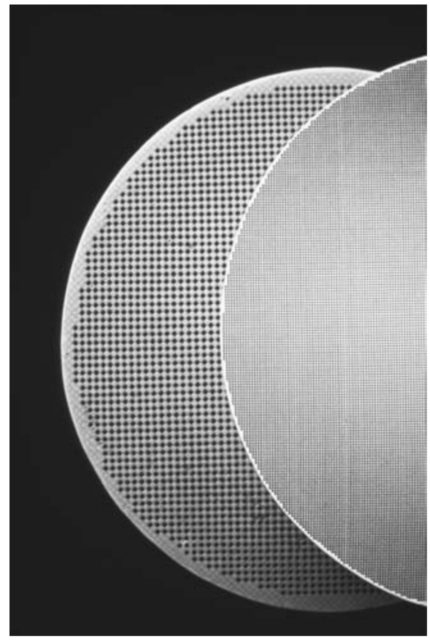


PHOTO COURTESTY OF CORNING, INC.

Automotive catalytic converters produced by Corning, Inc. Nanoparticles of platinum or rhodium reside on the surface of the honeycomb ceramic structure. As hot exhaust gases pass through the channels, toxic nitrous oxides and carbon monoxides are converted to nitrogen and carbon dioxide gases.

BACKGROUND PHOTO COURTESY OF U.S. ARMY
FOREGROUND PHOTO COURTESY OF BELL LABORATORIES

In the background is ENIAC, the first fully operational large-scale electronic digital computer with its 18,000 vacuum tubes, 70,000 resistors, and 10,000 capacitors (Philadelphia, 1948). The inset in the foreground shows William Shockley (sitting) and John Bardeen and Walter Brattain (standing behind) in the same year, shortly after their invention of the solid state transistors that would lead to modern computers.

other health-related sciences, the importance of nanoscale science and technology is so sweeping and so vast that boundaries cannot be reliably defined, and limits cannot be clearly foreseen. Scientific historians know all too well that technology predictions are notoriously inaccurate. Shortly after the invention of the solid state transistor at Bell Labs (the invention that made modern computers possible), experts published predictions in the March 1949 issue of *Popular Mechanics*: Futuristic calculators (computers) would add 5,000 numbers per second and weigh only 1,400 kg, while consuming 10 W of power. Today, a laptop computer weighing just 1 kg can add millions of numbers per second using about 1 W of power. So who can reliably say today what a quantum computer—built from the bottom up using nanotechnology—will be able to do in the future, or which fields will be most dramatically affected by nanoscience? Besides unimaginably powerful computing devices, one can easily anticipate great advances in medical diagnostic tools, chemical sensors, communication devices, environmental restoration methods, construction materials, and cancer treatments, just to name a few. It is this small scale—where we have the ability to put molecules and atoms to new uses—that fuels the hope and hype surrounding nanotechnology. What seems certain is that nanotechnology will make a dramatic and lasting impact on every scientific field and also in every major area of modern technology. The nanorevolution is here to stay.

PHOTO COURTESY OF INTEL CORPORATION

An eight-inch diameter "wafer" of modern computer chips, before they are separated to go into individual machines or computers. Each silicon chip may contain more than one billion solid state transistors.

Reference

National Science and Technology Council (NSTC). 1999. *Nanotechnology: Shaping the world atom by atom.* Available online at *http://itri.loyola.edu/nano/IWGN.Public.Brochure.*

Chapter 3

The Link Between Nanoscale Science, Technology, and a Vital Environmental Issue: Groundwater Pollution

Nanoscale science and technology play central roles in our understanding of how Earth works and in the environmental science field of study. All living things—from bacteria to humans—have components that exist, and key chemical reactions and physical processes that occur, in the nanoscale-size range. The same is true for the nonliving world (i.e., within water and in and on minerals and rocks). In addition, new types of sensors and detectors based on rapidly emerging nanotechnologies are having major impacts on how we model and understand important natural reactions that influence the environment. Student investigations described in the Nano2Earth curriculum touch on all these concepts.

PHOTO COURTESTY OF DR. ROGER BECKIE, UNIVERSITY OF BRITISH COLUMBIA

Water well in a Bangladesh village. A red spout indicates that the water is contaminated with naturally occurring arsenic. Many communities continue to use these wells because they do not see the effects of arsenic poisoning for some time.

The curriculum described in this book introduces nanoscale science and technology through investigations of groundwater pollution. As a freshwater source, groundwater is second in abundance only to water found in glaciers and polar ice. Groundwater is critically important as a freshwater source to much of the Earth's population; however, when it is polluted, it becomes a severe and dangerous liability with hundreds of millions of lives at stake from waterborne illnesses. This chapter provides teachers with critical background information on the nature of groundwater and the reasons why nanoscale science and technology are so relevant to groundwater pollution.

The "Critical Zone" of the Earth

The critical zone of our planet is the place where the land meets the fluid portions of the Earth: the hydrosphere and the atmosphere. The place where we live provides us with freshwater, agriculture, and many vital natural resources, such as soil, timber, and mineral deposits. Considering all the geo- and bio-aspects of this zone, it is probably the most heterogeneous and complex portion of the entire Earth. Understanding this area is an important key to intelligently sustaining the planet for human habitation. Earth processes within the critical zone can be conveniently divided into three principal and overarching categories based on the physical, geochemical, and biological processes that occur there. The following categories are strongly cross-linked, each one affecting the others to various degrees:

- biological activity: because of organism nutritional requirements, this process drives a significant amount of the chemical cycling within and between water, soil, rocks, and the atmosphere
- weathering (the breakdown of minerals): this process is constantly at work in the critical zone, generating soil, producing mineral by-products, and redistributing elements among water, rocks, and organic materials
- fluid transport: this process is the critical component in water resources supply and management, including everything from flooding to landform development

Below, we look at a few of these critical zone processes as a way of introducing the Nano2Earth curriculum.

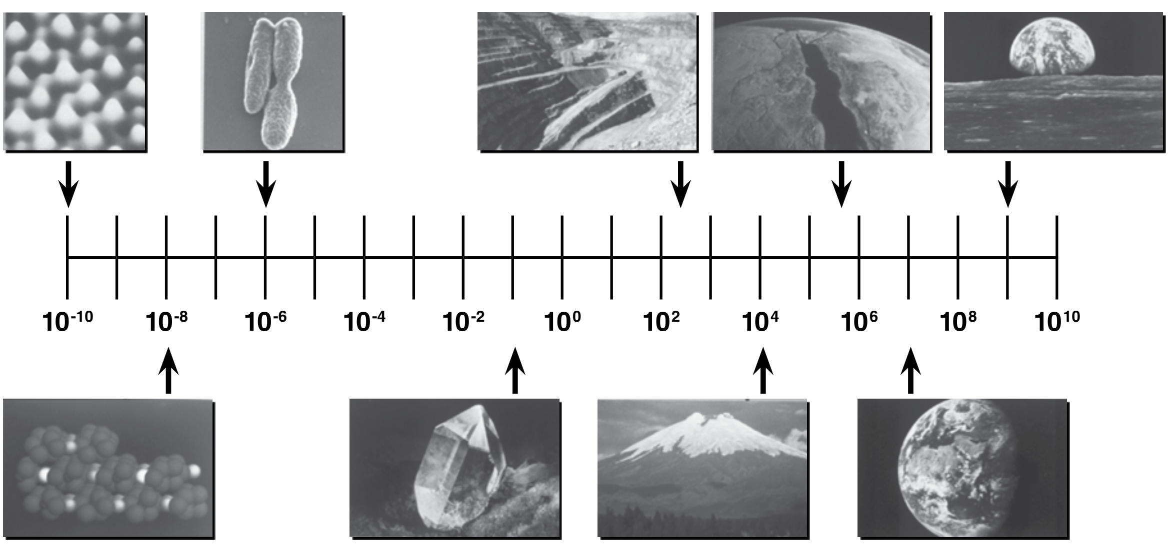
Water/Mineral/Bacteria Nanoscience

Nearly all aspects (both geo- and bio-processes) of weathering, soil, and water/rock interaction sciences are inexorably linked to nanoscience. Within the Earth's near-surface, minerals and living matter that are breaking down or decaying, as well as minerals that have just started to form, are often in the nanoscale size range. Further, when simple and complex organic molecules, bacteria, and all flora and fauna in the critical zone interact with the mineral components present, nanoscale processes occur everywhere. Understanding the nanoscale helps provide a complete picture of these exceptionally complex systems.

The biggest scientific problem is that one needs to understand how things work at this small, awkward scale. One needs to understand the nanoscale because processes that occur at the easy-to-see micron (one millionth or 10^{-6} m) and larger dimensions simply do not directly relate to the nanoscale (one billionth of a meter or 10^{-9} meters). Within the nanoscale, entirely different processes are possible (see Chapter 1); processes found nowhere else in the dimensional range of the Earth.

One of the most important interactions that occurs during weathering and in soils is mineral-bacteria association. The bacteria—individual micron-size cells enclosed within membranes made of an elegant arrangement of complex organic molecules—interact with surfaces of any one of thousands of minerals, each with its own chemical composition, atomic structure, and surface shape. This interaction takes place over nanometers of distance as their surfaces approach one another, come in contact, and separate. Recently, all of these forces of interaction—between fully viable (normally functioning) bacteria and various mineral surfaces in water—have been carefully and exactly measured using a variation of atomic force microscopy, or AFM (see Lesson 5, p. 105). Measurements of this type speak to the heart of nanoscience because the observer is looking at nanonewtons of force over nanometers of distance. As a bacterial cell and a mineral surface are brought closer together, the water layer between the two objects becomes thinner and thinner, and eventually is subject to the same

THE SCALE OF EARTH SCIENCE



The Earth-Moon system has a dimension of 10^9 m. But the inner space of this system is more vast then its outer space by one additional order of magnitude; at 10^{-10} m, we are in the realm of atoms as seen in this figure by scanning tunneling microscopy (far left image; Pb and S atoms on a galena—PbS—surface). Molecules (collections of atoms) such as enzymes that are an important part of the machinery of life have dimensions on the order of 10 nm, or 10^{-8} m. Typical bacteria have lengths on the order of 1 micrometer (10^{-6} m) as pictured.

consequences of any nanoscale substance, influencing and being influenced by the cell wall/membrane of the bacterium and the mineral surface. This aspect of nanoscience will have applications for bacterial movement in groundwater. For example, if the bacterium attaches to a mineral surface, it is effectively removed from groundwater flow. If the bacterium does not attach, it moves with the groundwater flow, which is of great concern if that bacterial species is pathogenic (disease-producing).

Metal Transport Nanoscience

Toxic metals and metalloids, such as lead, cadmium, chromium, and arsenic, are naturally present in the environment but can also be artificially introduced into the environment via inadvertent release from industrial processes such as mining or manufacturing. The movement of these toxic substances in the environment is of great concern. Metal mobility ultimately depends on (1) the chemical reactivity of any particular metal, and (2) the part of the environment through which that metal is moving (e.g., soil, groundwater, rivers or lakes, the atmosphere).

A fundamental question that can be easily overlooked is whether the metal in question is moving in the environment as an aqueous (water-based) species (as a single metal atom surrounded by water molecules and, therefore, "dissolved"), within or on a metal oxide nanoscale particle (called a nanoparticle), or perhaps attached to an organic molecule. If the metal in question passes through a submicron filter (e.g., a filter that traps particles larger than 0.2 microns, which is 200 nm), then it is often assumed to be dissolved. However, if in reality the metal is attached to the surface of a particle 10 nm in size, it will pass through the filter, but it is not dissolved. That is, metals can be and presumably often are transported within or on nanoparticles, not as aqueous species. These metals interact with the environment very differently depending on how they are transported. Another layer of complexity can occur if a toxic metal is attached to a nanoparticle surface, but then interactions with bacteria release the metal from the surface. Lesson 3 (see p. 55) in this curriculum explores this possibility in some detail.

Chapter 4

Nano2Earth Curriculum and the National Science Education Standards

With the Nano2Earth curriculum, students learn about nanotechnology and nanoscience by using inquiry to study groundwater pollution. The curriculum is designed to meet the National Science Education Standards (NSES) for content in grades 9–12 (NRC 1996). The following content standard categories are addressed in the curriculum: Unifying Science Concepts and Process, Science as Inquiry, Physical Science, Life Science, Earth and Space Science, Science and Technology, Science in Personal and Social Perspectives, and History and Nature of Science. (See Appendix 1 for excerpts of NSES addressed in the Nano2Earth curriculum.)

Side view of a scientist loading an atomic force microscope (AFM), a major tool in nanotechnology.

Unifying Content and Processes

Nano2Earth is designed to help students make connections among the traditional scientific disciplines of biology, chemistry, Earth science, and physics. For example, while learning about groundwater pollution, students will explore the biogeochemistry of microbe-mineral interactions at the nanoscale. Within this context students learn about unifying concepts and processes as identified in the NSES (NRC 1996, p. 115):

- Systems, order, and organization
- Evidence, models, and explanation
- Constancy, change, and measurement

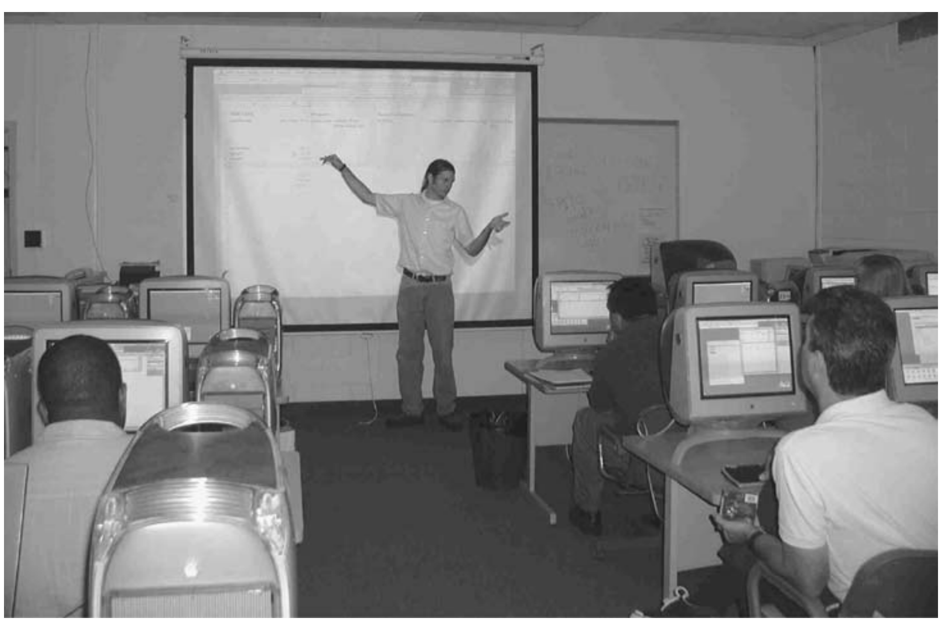
- Evolution and equilibrium
- Form and function

Within the Nano2Earth curriculum, these unifying concepts and processes are

1. understanding the Earth as an interconnected biological and physical system with different levels of complexity;
2. using evidence and mathematical modeling to interpret microbe-mineral interactions;
3. investigating change in biogeochemical systems under laboratory conditions;
4. using measurement and "scaling" from the macro- to the nanoscale; and
5. examining how form and function apply to the interaction of bacteria with mineral surfaces.

Conducting Scientific Inquiry

The Nano2Earth curriculum is designed to engage students in the process of conducting scientific inquiry. The pedagogy and teaching strategies are framed within the context of nanotechnology and nanoscience research on groundwater



The Nano2Earth team discusses issues in processing atomic force microscope data to determine nanoscale forces of interactions between objects.

pollution. As outlined in the NSES (NRC 1996, p. 175), the abilities necessary to do scientific research are the following:

- Identify questions and concepts that guide scientific investigations
- Design and conduct scientific investigations
- Use technology and mathematics to improve investigations and communications
- Formulate and revise scientific explanations and models using logic and evidence
- Recognize and analyze alternative explanations and models
- Communicate and defend a scientific argument
- Understandings about scientific inquiry

Each lesson begins with an activity that engages students' prior knowledge by asking questions or brainstorming ideas about topics related to groundwater pollution and nanotechnology. Students actively participate in laboratory scientific investigations by exploring microbe-mineral interactions and the transport of bacteria in groundwater, as simulated by the measurement of fluorescent beads transported through a sand column. Students learn how to use mathematical models to interpret and graph real scientific data that has been collected by atomic force microscopes, which are used to measure the nanoscale forces involved with microbe-mineral interactions. Students are also involved in web searches and investigating sources of groundwater pollution in their communities. Similar to scientists doing cutting-edge research, students are encouraged to consider alternative explanations and models for explaining their data and what they know about microbe-mineral interactions.

Physical Science

Using nanotechnology and nanoscience, the Nano2Earth curriculum addresses the NSES in Physical Science. The Physical Science NSES addressed in the curriculum include the following (NRC 1996, p. 176):

- Structure and Properties of Matter
- Chemical Reactions
- Motions and Forces

By examining images of mineral surfaces at the nanoscale, students learn about the structure of matter from new perspectives. Because research has shown that nanosize particles behave differently in the nanoscale than in the macroscale, students are exposed to new ways of thinking about the structure and interactions of matter. Students investigate chemistry at the nanoscale by creating conditions in the laboratory to demonstrate the microbial reduction of iron(III).

Students will then interpret data collected from atomic force microscopes that measure the forces (e.g., van der Waals, electrostatic, hydrophobic) between bacteria and mineral surfaces, which can be less than one nanonewton.

Life Science

The NSES in Life Science include (NRC 1996, p. 181) the following:

- Interdependence of Organisms
- Matter, Energy, and Organization in Living Systems
- Behavior of Organisms

By investigating the microbe-mineral interactions at the nanoscale, students learn how matter and energy cycles and flows through different physical and biological systems. The adaptive response of bacteria to living in anaerobic conditions results in the extraction of iron(III) from mineral surfaces in the process of microbial respiration. Students learn how groundwater pollution is influenced by the interaction of microbes and minerals.

Earth Science

In support of the NSES in Earth Science, the Nano2Earth curriculum emphasizes an Earth systems approach and specifically addresses the standard for learning about Geochemical Cycles (NRC 1996, p. 189). Iron respiration of microbes is very important in geochemical cycling. In fact, microbes influence the distribution of many common elements on a globalscale. Students learn how microbe-mineral interactions are related to significant metal contamination (e.g., lead, chromium) and release of arsenic in the groundwater. Understanding microbe-mineral interactions in groundwater requires students to learn how geological, biological, and chemical systems interact.

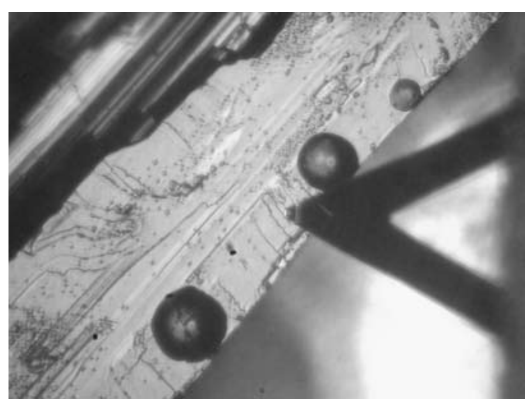
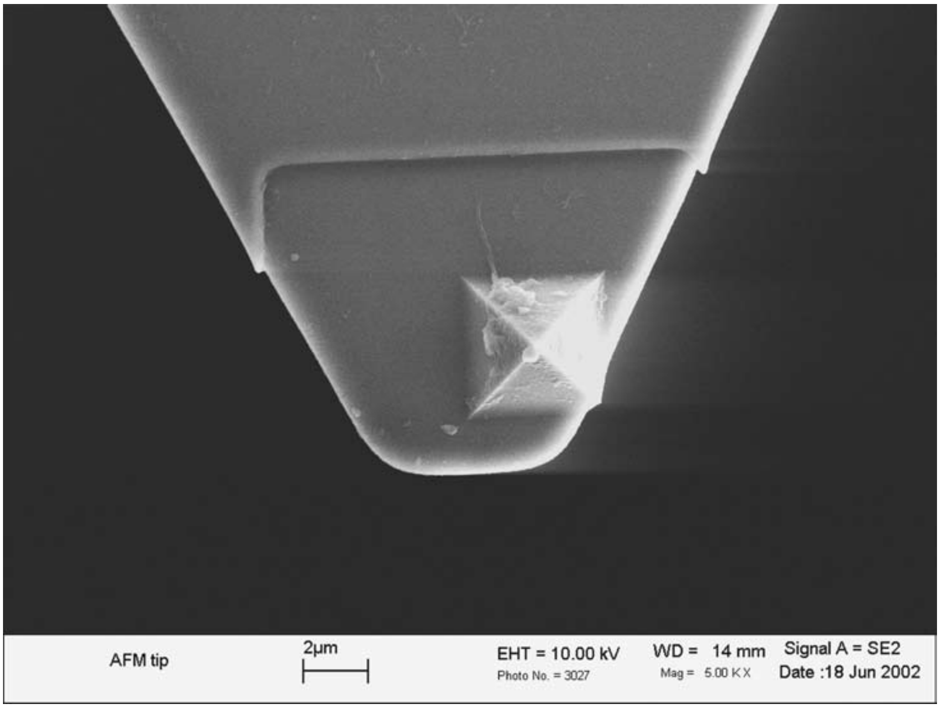


PHOTO COURTESY OF DR. ROBERT WEAVER

This image shows a sample of halite (sodium chloride salt) inside the sample chamber of an atomic force microscope (AFM). A laser spot can just been seen at the apex of the triangular AFM cantilever. Details on AFM are discussed in Lesson 5.



Scanning electron microscope image of an AFM cantilever, oriented upside down relative to its position in the instrument. Normally, the sharp pyramidal tip is located in the center. The tip's off-center position suggested the manufacturing process still needed improvement.

Science and Technology

Students address the NSES about Understanding About Science and Technology by learning how science and our understanding of nature have advanced through the development of new technologies in nanoscience. These new technologies include the atomic force microscope, which measures forces between microbes and mineral surfaces at the nanoscale. In the Nano2Earth curriculum, students learn how to interpret and make inferences from data collected by this instrument. Research in nanotechnology and nanoscience requires the contributions of scientists from many disciplines, including engineering. Students learn how nanotechnology has contributed to our knowledge of groundwater pollution.

Science in Personal and Social Perspectives

Science with Personal and Social Perspectives is another area of the NSES (NRC 1996, p. 193). Specific standards addressed in the Nano2Earth curriculum include:

- Natural Resources
- Environmental Quality
- Natural and Human-Induced Hazards
- Science and Technology in Local, National, and Global Challenges

Groundwater pollution has enormous implications on the global scale for personal and community health. Waterborne diseases and metal and chemical contamination of groundwater dramatically affects the ability of the Earth to sustain growing populations. Students will explore how the natural microbe-mineral systems adapt to changing environments, thus affecting water quality. Because groundwater pollution can be induced naturally or by humans, it is important for students to learn how research in nanotechnology advances our understanding of these processes. The advancement of research in nanotechnology can lead to an assessment of cost, benefits, risks, and possible solutions to groundwater pollution.

History and Nature of Science

The Nano2Earth curriculum provides an excellent opportunity for students to learn about the nature of science and cutting-edge scientific research from a historical perspective. Research in science education and the National Science Education Standards have emphasized the importance of students' understanding that science is a human endeavor in which

- explanations of nature are formulated and tested using observational and experimental
- evidence;
- theoretical and mathematical models are used to interpret scientific data;
- science is tentative and subject to change; and
- scientific explanations are constructed and defended based on interpretation of evidence, experimental procedure, and theoretical explanations (NRC 1996, p. 201).

With the Nano2Earth curriculum students will experience the nature of scientific research by learning about the development of the field of nanotechnology and by exploring microbe-mineral interactions in groundwater. Students will conduct laboratory experiments, examining evidence of bacteria respiration of iron under anaerobic conditions and exploring bacterial transport through porous substrate in groundwater. Using real scientific data from the laboratories of scientists in Virginia Tech's Department of Geosciences, students will analyze the nanoscale forces between bacteria and mineral surfaces through mathematical modeling and graphing. Since the results are often tentative and subject to interpretation and careful analysis, students will learn about the cutting-edge nature of scientific research in nanotechnology.

Reference

National Research Council (NRC). 1996. *National science education standards.* Washington, DC: National Academies Press.

Chapter 5

Nano2Earth Curriculum Overview

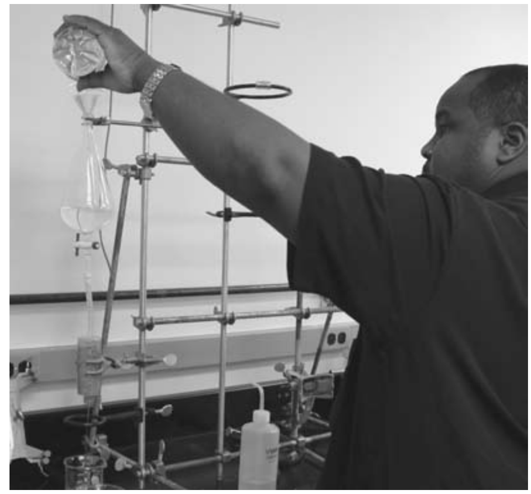
Curriculum Framework

The Nano2Earth curriculum is designed to promote student inquiry and learner-centered investigations of microbe-mineral interactions at the nanoscale. The curriculum is organized into five Lessons:

- *Lesson 1–Introduction to Nanotechnology* introduces students to nanotechnology through a scaling activity and a webquest of current events related to nanoscience research.
- *Lesson 2–Introduction to Water Pollution* engages students in water testing and investigating groundwater pollution through know-wonder-learn charts and webquests.
- *Lesson 3–Microbe-Mineral Interactions* involves students in a laboratory activity that promotes the reduction of iron(III) to iron(II) during the respiration of anaerobic bacteria by investigating interactions in a Winogradsky water column.
- *Lesson 4–Investigation of Bacterial Transport in Groundwater* involves students in a probeware laboratory activity investigation designed to simulate the interactions of bacteria with mineral surfaces in a water column.
- *Lesson 5–Nanoforces in Nature: Using Atomic Force Microscopy to Explore Microbe-Mineral Interactions* is designed for students to investigate nanoforces in nature by interpreting real scientific data from microbe-mineral interactions. Students also interpret simulated data of nanoforces involved in the Winogradsky water column lab (Lesson 3) and the bacterial transport in groundwater lab (Lesson 4).

The instruction and activities in each of the lessons are designed to follow the 5E Instructional Model (Figure 1, p. 25) Engage, Explore, Explain, Elaborate, and Evaluate (Bybee 1993). This model is designed to "actively involve students in the inquiry process by accessing prior knowledge, using strategies for scientific investigation of evidence, testing ideas, and reflecting on the results of investigations" (McLaughlin and Glasson 2003, p. 49). The 5E Instructional Model has been promoted as an instructional model for teaching inquiry-based science (NRC 2000).

Using the Curriculum



Demonstration of a column experiment during the design of Lesson 4 (Bacterial Transport). The sand column simulates sediment, while fluorescent beads are used to simulate the passage of bacteria. Dr. André Green is shown performing the experiment.

The curriculum is designed to introduce students to nanoscale science and technology by investigating microbe-mineral interactions in groundwater pollution. Teachers may choose to use the entire curriculum or select topics that fit into their existing course. For example, science teachers who want to introduce students only to nanotechnology may choose to complete Lesson 1 (Introduction to Nanotechnology). Earth, life, or environmental science teachers may choose Lesson 2 (Introduction to Water Pollution) if their curriculum goals include the study of water pollution. Lesson 3 (Microbe-Mineral Interactions) is ideal for chemistry classes studying microbial reduction of iron(III) but may also be selected by biology, Earth, or environmental science teachers for helping their students learn more about microbe-mineral interactions in anaerobic conditions and to highlight the differences between aerobic and anaerobic respiration. Lesson 4 (Investigation of Bacterial Transport in Groundwater) may be appropriate for chemistry and environmental science classes as it involves students in laboratory investigations that address particle transport and its effects on water quality. Teachers in all subjects may choose Lesson 5 (Nanoforces in Nature: Using Atomic Force Microscopy to Explore Microbe-Mineral Interactions) to apply nanotechnology to concepts from the previous lessons by interpreting real scientific data. Teachers may finish the lesson by having students complete one or both of the scenarios ("Arsenic Poisoning in Bangladesh" and "Bacterial Transport in Groundwater") with actual data that relate directly to the Winogradsky column or bacterial transport experiments. Because nanoscale science and technology are interdisciplinary in nature, the Nano2Earth curriculum also includes many scientific disciplines that are taught in secondary schools. Therefore, science teachers may decide to complete the entire curriculum to expose their students to the interdisciplinary nature of scientific research. The Nano2Earth curriculum is designed to correlate with the National Science Education Standards (see Table 1). Each of the five lessons is designed to take three to five days to complete.

FIGURE 1 5E Instructional Model

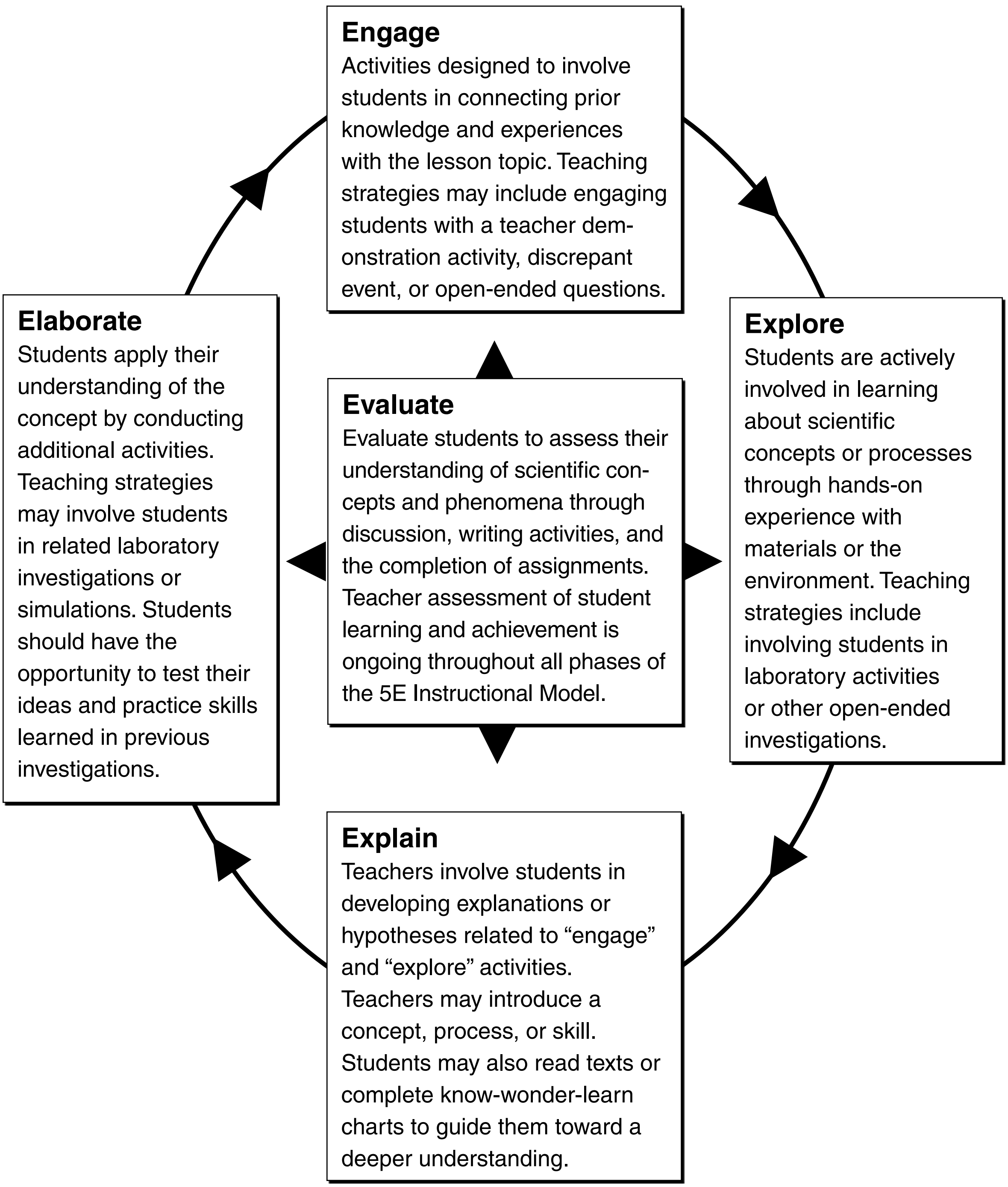


TABLE 1 Nano2Earth-NSES Lesson Correlation

Nano2Earth Lessons	NSES Addressed
1. Introduction to Nanotechnology **Engage:** Brainstorming **Explore:** Scaling Activity **Explain:** Why Is the Nanoscale Important? **Elaborate:** Current Event Webquest **Evaluate:** Current Event Presentations	Unifying Concepts • Measurement Science as Inquiry • Using Technology and Mathematics to Improve Communications Science and Technology • Understanding About Science and Technology • Science in Personal and Social Perspectives • Science and Technology in Local, National, and Global Challenges History and Nature of Science • Historical Perspectives
2. Introduction to Water Pollution **Engage:** Teacher Demonstration–Glass of Water **Explore:** Water Testing Activity **Explain:** Water Pollution K-W-L Chart **Elaborate:** Water Pollution Webquest **Evaluate:** K-W-L Chart and Writing Activity	Unifying Concepts • Evidence • Explanations Science as Inquiry • Identify Questions and Concepts that Guide Explanations Earth and Space Science • Geochemical Cycles Science in Personal and Social Perspectives • Natural Resources • Environmental Quality Natural and Human-Induced Hazards • Science and Technology in Local, National, and Global Challenges
3. Microbe-Mineral Interactions **Engage:** Prelab Questions **Explore:** Winogradsky Column Lab **Explain:** Discussion and Sharing of Results **Elaborate:** Groundwater Scenario and Inquiry **Evaluate:** Analysis and Conclusions	Unifying Concepts • Systems • Evidence • Models • Explanations • Changes • Measurement Science as Inquiry • Identify Questions and Concepts That Guide Explanations • Design and Conduct Scientific Investigations • Using Technology and Mathematics to Improve Investigations and Communications • Formulate and Revise Scientific Explanations and Models Using Logic and Evidence Physical Science • Structures and Properties of Matter • Chemical Reactions Life Science • Interdependence of Organisms • Matter, Energy, and Organization of Living Systems • Earth and Space Science • Geochemical Cycles • Science in Personal and Social Perspectives • Environmental Quality Natural and Human-Induced Hazards • Science and Technology in Local, National, and Global Challenges

TABLE 1 Nano2Earth-NSES Lesson Correlation

Nano2Earth Lessons	NSES Addressed
4. Investigation of Bacterial Transport in Groundwater **Engage:** Groundwater Pollution Scenario and Waterborne Diseases **Explore:** Bacterial Transport Column Lab **Explain:** Class Discussion and Questions **Elaborate:** Influence of Groundwater Chemistry (pH) on Bacterial Transport **Evaluate:** Groundwater Pollution Scenario Revisited	Unifying Concepts • Systems • Evidence • Models • Explanations • Changes • Measurement Science as Inquiry • Identify Questions and Concepts That Guide Explanations • Design and Conduct Scientific Investigations • Using Technology and Mathematics to Improve Investigations and Communications • Formulate and Revise Scientific • Explanations and Models Using Logic and • Evidence Physical Science • Structures and Properties of Matter • Motions and Forces Earth and Space Science • Geochemical Cycles Science in Personal and Social Perspectives • Environmental Quality Natural and Human-Induced Hazards • Science and Technology in Local, National, and Global Challenges

(Cont.)

TABLE 1 Nano2Earth-NSES Lesson Correlation

Nano2Earth Lessons	NSES Addressed
5. Nanoforces in Nature **Engage:** Introduction to the Atomic Force Microscope **Explore:** What Happens When We Bring Bacteria and Minerals Together **Explain:** Force Curve Computer Simulations **Elaborate:** Building a Model AFM **Evaluate:** Real-World Scenarios	Unifying Concepts • System • Evidence • Models • Explanations • Changes • Measurement • Form and Function Science as Inquiry • Identify Questions and Concepts That Guide Explanations • Using Technology and Mathematics to Improve Investigations and Communications • Formulate and Revise Scientific Explanations and Models using Logic and Evidence • Recognize and Analyze Alternative Explanations and Models • Understanding About Scientific Inquiry Physical Science • Structures and Properties of Matter • Motions and Forces • Earth and Space Science • Geochemical Cycles • Science and Technology • Understanding About Science and Technology • Science in Personal and Social Perspectives Earth and Space Science • Natural Resources • Environmental Quality • Natural and Human-Induced Hazards • Science and Technology in Local, National, and Global Challenges History and Nature of Science • Science as a Human Endeavor • Nature of Scientific Knowledge • Historical Perspectives

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McLaughlin, J., and G.E. Glasson. 2003. Connecting biotechnology and society. *The Science Teacher* (70) 4: 48–52.

National Research Council (NRC). 2000. *Inquiry and the national science education standards.* Washington, DC: National Academies Press.

Part II

The Nano2Earth Curriculum

Lesson 1

Introduction to Nanotechnology

Purpose

Students identify and compare the scale of different objects, and define *nanoscale* and *nanoscience*. The teacher introduces the history and applications of nanoscience.

Background Information and Lesson Overview

Imagining differences in scale can be very easy (a softball is bigger than a baseball) or very difficult (how can we imagine the size of a galaxy?). It is perhaps most challenging to build conceptual frameworks for objects that are too small to see. In fact, the world of inner space is more vast and daunting to the imagination than the entire world visible to us on Earth and the nearby solar system. Nanoscience is the field of science that measures and explains the changes of the properties of substances as a function of size; these changes occur in the range of approximately 1–100 nm. Nanotechnology simply takes advantage of this phenomenon by applying property modifications of this nature to some

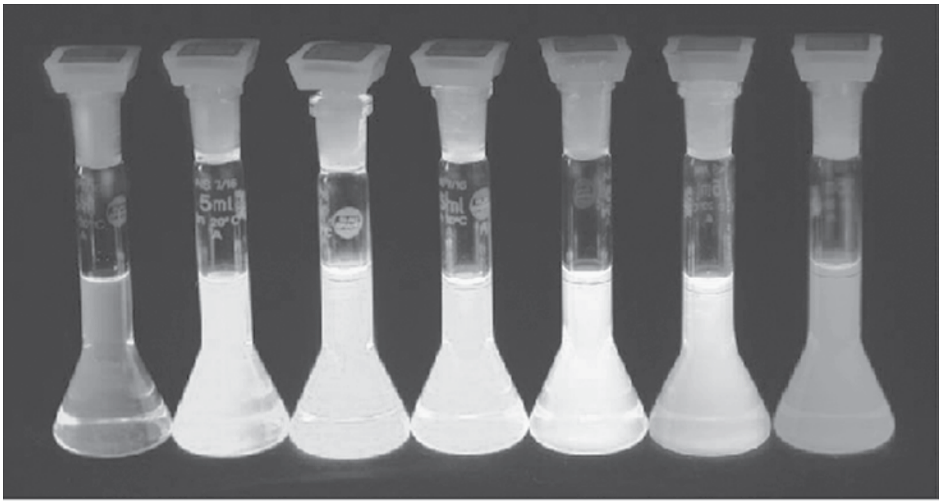

FIGURE 1: These flasks contain suspensions of nanosize CdSe particles known as "quantum dots." The color arises after UV illumination. The only difference between the flasks are the size of the particles, ranging from 2 nm (left) to approximately 5 nm (right).

beneficial endeavor. Chapters 1–3 provide a descriptive background for teachers of nanoscience, nanotechnology, and the roles of both in Earth and environmental science.

In this lesson, the engagement brainstorming activity will help bring out any preconceived notions students may have regarding the scale of objects. Then, the scaling activity provides an opportunity to compare and plot the scale of objects ranging from atoms to galaxies. The explain activity introduces Richard Feynman's 1959 visionary speech, demonstrating that what was then science fiction is now being realized. The current events webquest provides opportunities for students to investigate if we have come as far as Richard Feynmann had imagined. Finally, for assessment, current events information is shared through an in-class presentation.

For Further Information

- National Nanotechnology Initiative: *www.nano.gov*
- Nanotechnology Center for Learning and Teaching: *http://community.nsee.us*
- Nanotechnology: Big Things From a Tiny World: *www.nano.gov/Nanotechnology_BigThingsfromaTinyWorld-print.pdf*

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Stevens, S. Y., L. M. Sutherland, and J. Krajcik. 2009. *The big ideas of nanoscale science and engineering: A guidebook for secondary teachers.* Arlington, VA: NSTA Press.

National Science Education Standards (NSES)

Nano2Earth Lesson	NSES Addressed
Introduction to Nanotechnology **Engage:** Brainstorming **Explore:** Scaling Activity **Explain:** Why Is the Nanoscale important? **Elaborate:** Current Events Webquest **Evaluate:** Current Events Presentations	Unifying Concept • Measurement Science as Inquiry • Using Technology and Mathematics to Improve Communications Science and Technology • Understanding about Science and Technology Science in Personal and Social Perspectives • Science and Technology in Local, National, and Global Challenges History and Nature of Science • Historical Perspectives

Engage: Brainstorming

The class will brainstorm to identify and compare the scale of different objects. On a piece of paper or chalkboard, make a list of the largest and smallest objects students mention. Rank the objects according to estimated size. Ask students to add objects to the list that can be seen only with scientific instruments, such as telescopes and microscopes. Discuss how they would compare the size of these objects.

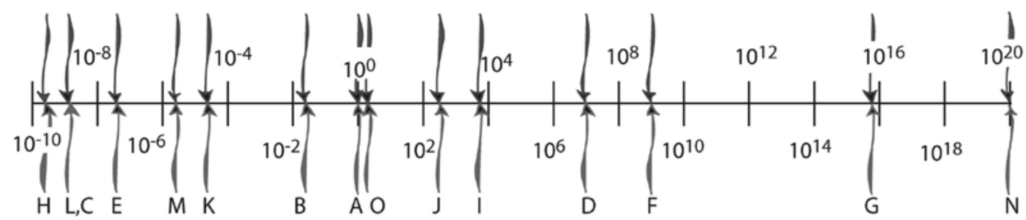
Explore: Scaling Activity

1. Key points to review: units and scientific notation
    - 1 meter (m) = the International System (SI) standard unit of length. All length scales are referenced to this length.
    - Scientific notation is related to "orders of magnitude," or numbers multiplied by powers of 10.
    - Multiplying 1 m by 10 equals a decameter, or 1×10^1 m. Multiplying 1 m by 1,000 equals 1 km, or 1×10^3 m ($1 \text{ m} \times 10 \times 10 \times 10 = 1{,}000$ m).
    - Multiply 1 m by 0.001 = 0.001 mm or 1×10^{-3} m

2. Introduce the term *nanometer (nm)* or 1×10^{-9} or a billionth of a meter. The nanoscale is considered 1–100 nm.

3. Instruct students to complete "The Scale of the Earth Sciences" student activity sheet (p. 38) by placing the letter corresponding to each object on the scale above the appropriate arrow. The upper set of arrows (Scale A) is for the students' estimations, while the lower set of arrows (Scale B) is for the students to write down the answers given by the teacher.

4. When students are finished, provide the correct answers from the list below and have them complete the scale of the actual size of the objects by writing the correct letter underneath the lower set of arrows). Note that both L and C are associated with the same arrow.



H.	Diameter of an atom	1×10^{-10} to 5×10^{-10} m
L.	A nanometer	1×10^{-9} m
C.	Diameter of a DNA molecule	1×10^{-9} m
E.	Diameter of a typical virus	1.5×10^{-8} to 5×10^{-7} m
M.	Diameter of typical bacteria	0.2×10^{-6} to 2×10^{-6} m
K.	The width of a human hair	6×10^{-5} m
B.	The length of your thumbnail	2×10^{-2} m
A.	A meter	1 m
O.	Your height	1.7 m
J.	Height of the Empire State Building	4.48×10^{2} m
I.	Height of Mount Everest	8.848×10^{3} m
D.	Diameter of the Earth	1.27×10^{7} m
F.	Diameter of the Sun	1.39×10^{9} m
G.	A light year	9.5×10^{15} m
N.	Distance across the Milky Way Galaxy	10^{20} m

5. Instruct the students to answer the summary questions (p. 39) and then review the correct answers.
   - Which part of the scale is considered the nanoscale?

     1–100 nm
   - What is the smallest part of the scale that your eye can see?

     About 10^{-5} m
   - What is the smallest part of the scale that a classroom microscope can see?

     About 10^{-6} m
   - On the scale, is your height closer to Mount Everest or to a nanometer?

     Mount Everest

- What distance separates 10^0 and 10^2? What distance separates 10^2 and 10^4? (Your answers should not be the same.)

  99 m / 9900 m

- Nanoparticles with diameters of 10 nm are common in the soil, air, and water around you. How many of these nanoparticles could you line up in a row along the width of a human hair? How many would fit lined up along the length of your thumbnail?

  Along a hair: 6,000; along a thumbnail: 2,000,000

- Assume that the size of one atom is 10^{-10} m. How many atoms fit in one nanometer? How about a cube with all dimensions 1 nm (1 nm^3 volume)? How many atoms would you expect in a cubic nanoparticle with all sides 10 nm?

  If one atom was 10^{-10} nm, then 10 atoms would fit in one nm, 1,000 atoms would fit in 1 nm^3, and 1,000,000 atoms would fit in 10 nm^3.

An additional opportunity for inquiry involves having the students develop their own similar questions.

Explain: Why Is the Nanoscale Important?

Have the students read "There's Plenty of Room at the Bottom" (p. 40). It contains excerpts from the lecture of the same name given by the physicist Richard Feynman at CalTech in 1959. It was first published in the February 1960 issue of Caltech's *Engineering and Science,* and at the time of this writing, the entire speech could be found online at *www.its.caltech.edu/%7Efeynman.*

1. Tell the students that at the end of the lecture, Dr. Feynman announced a $1,000 prize for the first people to make an electric motor only 1/64th inch cube and another $1,000 prize for the first person to write a passage from a book at a 1/25,000 smaller scale than the original text. Do the students think these feats have been accomplished, and if so, when?

FIGURE 2: The first page of *A Tale of Two Cities* by Charles Dickens minimized to 1/25,000th scale.

The two Feynman Prizes mentioned in the lecture were awarded in 1960 (making a 1/64th inch operating electric motor) and in 1985 (minimizing a page of a book at 1/25,000th scale so it could be clearly interpreted by an electron microscope). The prize-winning transmission electron micrograph, taken by Drs. Pease and Newman from Stanford University in 1985, is shown in Figure 2, p. 35. (*The text is the opening of* A Tale of Two Cities *by Charles Dickens.*)

2. Ask the students if they think it is currently possible to image and manipulate atoms.

   The answer is yes! Using a tool called the scanning tunneling microscope (STM), which the students will learn more about in Lesson 5, it is possible to both image and manipulate individual atoms of certain types (see Figure 3).

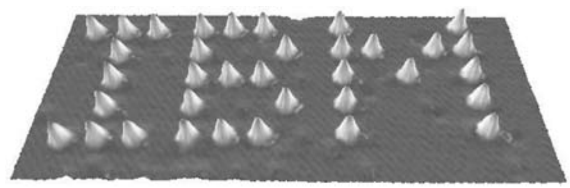


FIGURE 3: This image from Donald Eigler at IBM (1988) shows individual Xe atoms arranged with a scanning tunneling microscope, predecessor of the atomic force microscope, to form perhaps the world's smallest logo.

3. If it is possible to view and move individual atoms, why can't we build anything we want? If sources of the necessary atoms were available, could they just be organized in the arrangement of any material? These very questions are at the heart of current debate in nanotechnology. One of the original proponents of these ideas, Eric Drexler, considers these questions to be the future of nanotechnology. Known by Dr. Drexler as "molecular manufacturing" or "molecular nanotechnology," the possibility of creating nearly anything from constituent atoms may revolutionize human society. However, many scientists, including Dr. Richard Smalley, suggest that such synthesis is not possible. Dr. Smalley was awarded a Nobel Prize in Chemistry for his work in the discovery of carbon nanostructures. He (and others) suggests that bringing atoms in proximity to one another is not enough to cause the necessary bonding arrangements to occur in the resulting molecules. Present students with the idea of building materials "from scratch." What thoughts do they have for and against the possibility of building anything we want to?

Elaborate: Current Events Webquest

Assign students to find a current event on nanoscience. This can be done as a homework assignment or in the computer lab over the internet. Instruct students to seek recent articles in the newspaper, magazines, or on the web dealing with new technologies, applications, or products that are developed using nanotechnology. Links to nanoscale science and technology websites include:

- National Nanotechnology Initiative: *www.nano.gov*
- *http://dir.yahoo.com/Science/Nanotechnology*

Evaluate: Current Events Presentations

Students can summarize and present current events so you can assess their understanding of nanoscale sizes and potential applications of nanotechnology. Students should be able to describe the new technology, application, or products; identify the size or scale of the objects or products; and discuss the potential use of the new technologies, applications, or products to society.

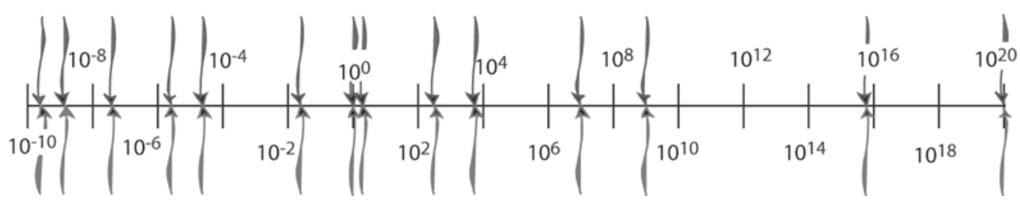
Nano2Earth Student Activity Sheet

The Scale of the Earth Sciences

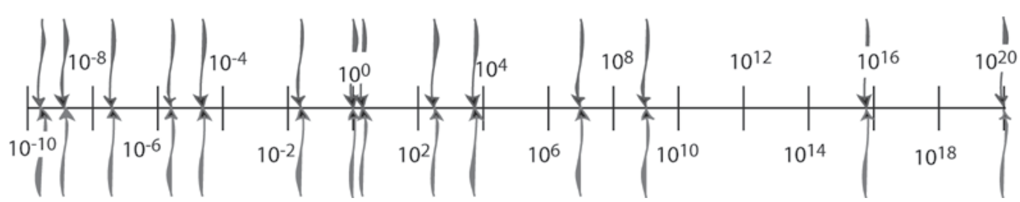
Below is a list of objects from all areas of the scale. Below that are two scales showing a wide range of measurements, from extremely small to extremely large. Estimate the size of each object, and place the corresponding letter on Scale A. Afterward, your teacher will provide the answers for you to write on Scale B. The scales are in meters.

A. A meter
B. The length of your thumbnail
C. Diameter of a DNA molecule
D. Diameter of the Earth
E. Diameter of a typical virus
F. Diameter of the Sun
G. A light year
H. Diameter of an atom
I. Height of Mount Everest
J. Height of the Empire State Building
K. The width of a human hair
L. A nanometer
M. Diameter of a typical bacteria
N. Distance across the Milky Way Galaxy
O. Your height

Scale A: Estimated size (meters)



Scale B: Actual size (meters)



Summary Questions:

1. Which part of the scale is considered the nanoscale?

2. What is the smallest part of the scale that your eye can see?

3. What is the smallest part of the scale that a classroom microscope can see?

4. On the scale, is your height closer to Mount Everest or to a nanometer?

5. What distance separates 10^0 and 10^2? What distance separates 10^2 and 10^4? (Your answers should not be the same.)

6. Nanoparticles with diameters of 10 nm are common in the soil, air, and water around you. How many of these nanoparticles could you line up in a row along the width of a human hair? How many would fit lined up along the length of your thumbnail?

7. Assume that the size of one atom is 10^{-10} m. How many atoms fit in one nanometer? How about in a cube with all dimensions 1 nm (1 nm^3 volume)? How many atoms would you expect in a cubic nanoparticle with all sides 10 nm?

Nano2Earth Student Reading Section

"There´s Plenty of Room at the Bottom"

(Excerpts from "There's Plenty of Room at the Bottom," a December 1959 lecture by Richard P. Feynman at the annual meeting of the American Physical Society at the California Institute of Technology)

I would like to describe a field, in which little has been done, but in which an enormous amount can be done in principle … a point that is most important is that it would have an enormous number of technical applications.

What I want to talk about is the problem of manipulating and controlling things on a small scale. As soon as I mention this, people tell me about miniaturization, and how far it has progressed today. They tell me about electric motors that are the size of the nail on your small finger. And there is a device on the market, they tell me, by which you can write the Lord's Prayer on the head of a pin. But that's nothing; that's the most primitive, halting step in the direction I intend to discuss. It is a staggeringly small world that is below. In the year 2000, when they look back at this age, they will wonder why it was not until the year 1960 that anybody began seriously to move in this direction.

Why cannot we write the entire 24 volumes of the Encyclopedia Britannica on the head of a pin?

Let's see what would be involved. The head of a pin is a sixteenth of an inch across. If you magnify it by 25,000 diameters, the area of the head of the pin is then equal to the area of all the pages of the Encyclopedia Britannica. Therefore, all it is necessary to do is to reduce in size all the writing in the Encyclopedia by 25,000 times. Is that possible? The resolving power of the eye is about 1/120 of an inch—that is roughly the diameter of one of the little dots on the fine half-tone reproductions in the Encyclopedia. This, when you demagnify it by 25,000 times, is still 80 angstroms in diameter—32 atoms across, in an ordinary metal. In other words, one of those dots still would contain in its area 1,000 atoms. So, each dot can easily be adjusted in size as required by the photoengraving, and there is no question that there is enough room on the head of a pin to put all of the Encyclopedia Britannica.…

That's the Encyclopedia Britannica on the head of a pin, but let's consider all the books in the world. The Library of Congress has approximately 9 million volumes; the British Museum Library has 5 million volumes; there are also 5 million volumes in the National Library in France. Undoubtedly there are duplications, so let us say that there are some 24 million volumes of interest in the world.

Nano2Earth Student Reading Selection

"There´s Plenty of Room at the Bottom"

What would happen if I print all this down at the scale we have been discussing? How much space would it take? It would take, of course, the area of about a million pinheads because, instead of there being just the 24 volumes of the Encyclopedia, there are 24 million volumes. The million pinheads can be put in a square of a thousand pins on a side, or an area of about 3 square yards.… All of the information which all of mankind has every recorded in books can be carried around in a pamphlet in your hand—and not written in code, but a simple reproduction of the original pictures, engravings, and everything else on a small scale without loss of resolution.

What would our librarian at Caltech say, as she runs all over from one building to another, if I tell her that, ten years from now, all of the information that she is struggling to keep track of—120,000 volumes, stacked from the floor to the ceiling, drawers full of cards, storage rooms full of the older books—can be kept on just one library card! When the University of Brazil, for example, finds that their library is burned, we can send them a copy of every book in our library by striking off a copy from the master plate in a few hours and mailing it in an envelope no bigger or heavier than any other ordinary air mail letter.

Source: Feynman, R. P. 1960. There's plenty of room at the bottom: An invitation to enter a new field of physics. *Engineering and Science* (Feb). *www.zyvex.com/nanotech/feynman.html.*

Lesson 2

Introduction to Water Pollution

Purpose

This lesson assesses students' prior knowledge about groundwater pollution and has students investigate types and sources of water pollution. Note that this introductory activity sets the stage for subsequent learning about sources of water pollution.

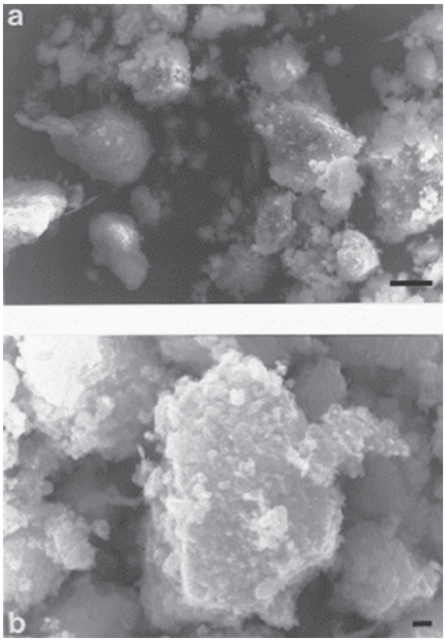


FIGURE 1: Two scanning electron microscope (SEM) images show sediments from a metal-contaminated river. The bottom image (b) is a close-up of the top image (a). The black bar in the lower right corner is 10 micrometers (10,000 nm) on the top image and 1 micrometer (1,000 nm) on the bottom image. Even at this scale, a diverse complex texture can be seen in the individual grains.

Background Information and Lesson Overview

Find out where your tap water comes from—groundwater, a local river or stream, a spring, a reservoir. All these water sources probably contain some or all of the following contaminants: the microbial contaminants *E. coli*, *Cryptosporidium*, *Giardia*, and *Salmonella* and the chemical contaminants arsenic, pesticides, radon, lead, and nitrates. These contaminants may originate from interaction of natural waters with rock. In addition to natural sources, contaminants may also be a result of human actions in industry and agriculture. The Environmental Protection Agency (EPA) sets maximum contamination levels (MCLs) for water sources. A list of drinking water contaminants (along with the specific problems they cause for humans) can be found at the EPA website, *www.epa.gov/safewater/mcl.html#mcls.* A list of websites with information about water pollution is also included on the "Water Pollution Webguest" student activity sheet (p. 52).

In the Introduction to Water Pollution activities, students work both individually and in small groups to analyze and present information about water pollution to the class. Students are introduced to the topic of water quality while considering water samples collected from different sources. The students explore the samples with a testing activity and use a Know-Wonder-Learn chart for the Explain and Evaluate components of the learning cycle. K-W-L charts also work well as engagement activities.

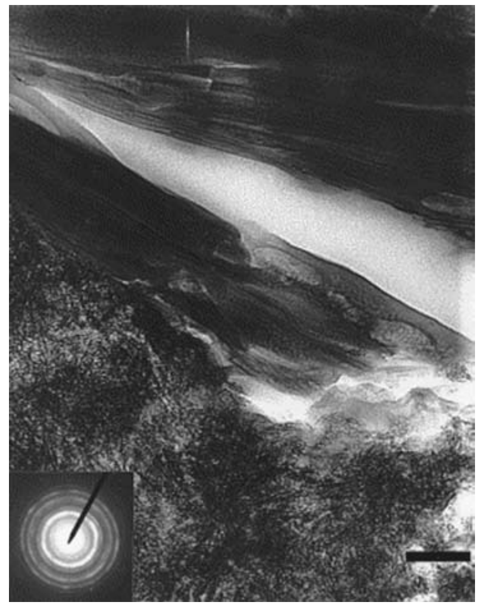


FIGURE 2: Other more powerful techniques such as Transmission Electron Microscopy (TEM) reveal the true story of the sediment at the nanoscale. Here the black scale bar in the lower right corner is 100 nm. The box inset to the lower left is a diffraction pattern, used to identify minerals. The results of this study helped identify which nanoscale minerals were serving as hosts to carry toxic metals downstream. For example, clay minerals dominate the center and top, while <10 nm grains of the iron oxide mineral hematite dominate the bottom.

For Further Information

The EPA and USGS websites are excellent resources to get background information regarding water resources and quality in the United States:

- *www.epa.gov/safewater/dwinfo.htm*
- *http://ga.water.usgs.gov/edu/waterquality.html*

National Science Education Standards (NSES)

Nano2Earth Lessons	NSES Standards Addressed
Introduction to Water Pollution **Engage:** Teacher Demonstration—Glass of Water **Explore:** Water Testing Activity **Explain:** Water Pollution K-W-L Chart **Elaborate:** Water Pollution Webquest **Evaluate:** K-W-L Chart and Writing Activity	Unifying Concepts • Evidence • Explanations Science as Inquiry • Identify Questions and Concepts That Guide Explanations Earth and Space Science • Geochemical Cycles Science in Personal and Social Perspectives • Natural Resources • Environmental Quality Natural and Human-Induced Hazards • Science and Technology in Local, National, and Global Challenges

Materials

- 4 beakers
- Tap water
- Bottled water
- Rainwater
- River/lake/creek/pond water
- Water quality test kits (available from Watersafe, *www.watersafetestkits.com,* or Ward's Natural Science, *http://wardsci.com,* which offers a variety of tests depending on the teacher's budget.
- Either a class set of internet-ready computers or information sheets printed out from computer.
- "Water Testing" student activity sheet (p. 49)
- "Water Pollution Know-Wonder-Learn (KWL)" student activity sheet (p. 51)
- "Water Pollution Webquest" student activity sheet (p. 52)
- "K-W-L Written Response" student activity sheet (p. 54)

Engage: Teacher Demonstration—Glass of Water

(Estimated time: 10 minutes)

Display in the front of the room a beaker of tap water; a beaker of bottled water; a beaker of rainwater; and a beaker of water from a river, lake, pond, or creek. Label the beakers 1, 2, 3, and 4 and do not tell students which beaker contains which water sample. Tell the students that each of the beakers holds water from a different source.

Ask students the following questions about each of the beakers:

1. Does this water look clean to you?
2. Would you drink this water? Why or why not?
3. What do you think was the source of this water?
4. What might be in this water? What evidence do you have for this?
5. How could we find out what is in this water?

Encourage the students to smell the water. Do not let the students drink the water.

Explore: Water Testing Activity

(Estimated time: 30 minutes)

1. Pass out the Water Testing student activity sheets (pp. 49–50). Instruct students to make a hypothesis about where each water sample originated.
2. Break students into eight equal groups. Assign each group one of the water quality tests—bacteria, lead, pesticides, nitrates, nitrites, chlorine, pH, and hardness. Give each group a small container of each of the four water samples and the appropriate test kit.

!

Science Safety Notes

- Students should not ingest water samples.
- Students should wear goggles when using water quality test kits.
- Note that bottled water may also contain contaminants.
- Read the kit instructions for disposal. Unless described otherwise, kit solutions are safe to rinse down the drain.

3. Instruct each group to circle on the activity sheet the water quality test they are performing.
4. Instruct each group to test the four water samples and record the group's data on the activity sheet.
5. Instruct students to properly dispose of the tested water samples.
6. When testing is complete, tell one person from each group to record the group's data on the board.
7. Direct students to copy onto their activity sheets the class data from the board.
8. Direct students to answer the questions on the activity sheet.

Explain: Water Pollution K-W-L Chart

(Estimated time: 15 minutes)

Know-Wonder-Learn (K-W-L) charts help students outline what they already know about a topic, explore what they want to learn about it, and perhaps most important, track their learning throughout a unit. Although K-W-L charts can be used on their own, they can be especially effective when used in conjunction with periodic reflections throughout a unit. Try one or both techniques as you lead your students through these lessons.

K-W-L charts can be constructed individually or you may want to make one chart for the whole class and begin filling it in as part of a class discussion. Students construct their charts at the beginning of the lesson, after the Engage activity. They should be able to fill in some boxes in the first column and perhaps some in the second column as well. After this initial construction, the class can revisit the chart every few days to add more information. If students find that their statements of prior knowledge were incorrect, they can revise these statements in the third column. K-W-L charts are about the learning process; they are not about being right! Below is a sample K-W-L chart for Water Pollution. A

What do I know about water pollution?	What do I wonder about water pollution?	What have I learned about water pollution?
Fertilizer runoff hurts the water.	Is there a type of fertilizer that does not hurt the water?	Fertilizer-laden runoff causes algal bloom and eutrophication in farm ponds.
Arsenic in water may be poisonous.	What is the source of the arsenic? Is there arsenic in my water? Am I being poisoned?	
	What does water pollution have to do with nanoscience?	

blank "Water Pollution Know-Wonder-Learn (K-W-L)" student activity sheet is also included (p. 51).

If students work on these K-W-L charts individually, you can collect them periodically to help you gauge student progress throughout the unit. Look for an increase in the depth of student thinking and student growth from beginning to end of the unit.

Elaborate: Water Pollution Webquest

(Estimated time: 30 minutes)

In this activity, students complete a webquest to find information about water pollution in their community. This activity works best if you have access to a class set of computers. Otherwise, you may print out information from the websites listed on the "Water Pollution Webquest" student activity sheet (p. 52) so students can find the answers to the questions. These websites were current at the time of publication but should be checked and updated if necessary. Do some research to find the source of your local water. The EPA website listed in the activity sheet includes information on local water sources and quality for many areas in the United States.

Many common toxic metals such as chromium, arsenic, and lead occur in public water supplies as a result of "erosion of natural deposits." This typically means that in the geologic past, various processes concentrated the metals in rocks. Subsequently, a fraction of the metals through natural processes leached out of the rocks into the groundwater.

Evaluate: K-W-L Chart and Writing Activity

(Estimated time: 30 minutes)

In the last section, students will synthesize what they have learned about water contamination by writing a response to the question given on the "Water Pollution Know-Wonder-Learn (K-W-L)" student activity sheet (p. 54).

Nano2Earth Student Activity Sheet

Water Testing Activity

Hypothesis

Each of the four samples comes from a different source: tap water, bottled water, stream/lake/pond/creek water, or rainwater. Where do you think each of the four water samples originates? Explain your reasoning. Record your answers below.

Science Safety: Do not drink water samples. Wear goggles when using water quality test kits. Note that bottled water may also contain contaminants.

Sample 1	Source:	________________	**Sample 3**	Source:	________________
	Reason:	________________		Reason:	________________
Sample 2	Source:	________________	**Sample 4**	Source:	________________
	Reason:	________________		Reason:	________________

Test	Sample 1	Sample 2	Sample 3	Sample 4
Bacteria				
Lead				
Pesticides				
Nitrates				
Nitrites				
Chlorine				
pH				
Hardness				

Nano2Earth Student Activity Sheet

Water Testing Activity

Questions:

1. Considering the test results, where do you think each of the water samples originates? Explain your answers.

2. Show this sheet to your teacher. The teacher will tell you the actual source of each sample. Compare the actual sources to your revised hypothesis in question 1. Based on the test results, which water seems "cleanest"?

3. Note any contaminants that occurred in the samples you tested. Considering where each sample originates, what do you think is the contaminant source of each one?

Nano2Earth Student Activity Sheet

Water Pollution Know-Wonder-Learn (K-W-L) Student Activity

L2

Introduction to Water Pollution

What do I know about water pollution?	What do I wonder about water pollution?	What have I learned about water pollution?

Nano2Earth Activity Sheet

Water Pollution Webquest Student Activity (Your Community)

Directions:

Use the websites below to find information about water quality in your community.

The Environmental Protection Agency (EPA)
www.epa.gov/safewater/mcl.html#mcls
This site includes regulations involving drinking water, lists of contaminants and their maximum levels, and potential health effects from contaminants.

EPA: Local Drinking Water
www.epa.gov/safewater/dwinfo.htm
The EPA site provides state-by-state information on drinking water sources and drinking water quality.

U.S. Geological Survey: Water Use, Water Sources, Water Pollution
http://ga.water.usgs.gov/edu/mwater.html
The USGS site provides information on water use, water sources, and water contamination, including what people are doing to prevent it. It also includes information on how contaminants leak into groundwater and on how storm sewers work.

How Stuff Works
http://people.howstuffworks.com/sewer.htm
This site describes what happens to your wastewater—where it goes and how it becomes clean again.

1. Drinking water comes from many sources—rivers, lakes/reservoirs, springs, groundwater, and cisterns. Some localities even use desalinated seawater.
   - What is the source of your drinking water?
   - If you get city or county water, how much does it cost?
   - How is it treated before it is piped to your house?
   - What do the treatments get rid of or add to the water?

2. Once water has been used, it may take one of many paths back into the local water supply.
   - What is the source of the water in a storm drain? How is this water treated before it drains back into the water supply?
   - What is the source of the water in a sewer pipe? How is this water treated before it drains back into the water supply?
   - What happens to the water that goes down the drain at your home or school?
   - What happens to the water that falls onto your roof when it rains?

Water Pollution Webquest Student Activity (Your Community)

3. Farmers and homeowners use many types of pesticides, herbicides, and fertilizers on their lawns and fields.
   - How do these contaminants end up in the water supply?
   - How long does it take for such contaminants to get into the groundwater?

4. If your drinking water comes from a municipal source, then it must meet federal and state guidelines for safe drinking water. Federal or state authorities routinely test the water for safety. However, if it comes from a well or a spring, it is up to you to test it.
   - What federal agency regulates drinking water in the United States?
   - What do the abbreviations “ppm” and “mg/L” represent?
   - What is the difference between “MCL” and “MCLG”?
   - Notice that the EPA divides contaminants into six categories: Microorganisms, Disinfectants, Disinfection Byproducts, Inorganic Chemicals, Organic Chemicals, and Radionuclides. Notice that the sources of these contaminants vary widely. Some are naturally occurring, some are generated by people, and some are both. The types of contaminants in your water depend on where you live. For each of these categories, find one contaminant that might occur in *your* water. Write the name of the contaminant, its source, the category it belongs to, and the potential health effects from ingestion of water that contains this contaminant. Organize your answers in a table like this one:

EPA Category	Contaminant	Source	Potential Health Effects
Microorganisms			
Disinfectants			
Disinfection byproducts			
Inorganic chemicals			
Organic chemicals			
Radionuclides			

Nano2Earth Activity Sheet

K-W-L Written Reponse Student Activity

Synthesize what you have learned and compose a written response to the following:

Over one billion people worldwide—almost one-sixth of the world population—lack access to safe drinking water and over two billion people lack basic sanitation facilities. In some countries in Africa and Asia, many children do not go to school because they must spend their time collecting water from streams, lakes, and wells for their families. According to WaterAid (*www.wateraid.org*), a child dies every 15 seconds due to water-related diseases. It is hard for most Americans to imagine living without clean drinking water and a place to go to the bathroom where the waste does not go directly into the drinking water supply. Yet even in America, over seven million people become sick from contaminated drinking water each year.

As the population of an area grows, the water supply becomes an increasing concern. Pretend you are a scientist who has been asked to give a presentation to the local board of supervisors. Given what you have learned about water quality and the water supply of your area, write a short response to be delivered to the board. Include a summary of the results of your water quality testing, any concerns the contaminants present for the community, and your recommendations for improving or maintaining the quality of your water supply. Your speech should be a minimum of two minutes long and may certainly be longer. Be sure to support your ideas with the data you have collected both experimentally and from the internet.

Lesson 3

Microbe-Mineral Interactions:
Using the Winogradsky Column to Demonstrate Bacterial Reduction of Iron(III)

Purpose

Students study the reduction of iron oxide minerals in a simulated anaerobic aquatic environment. Students discuss how the respiration of anaerobic bacteria may be involved with iron reduction. Students also consider problems related to excessive nutrients entering surface waters and what role students can play to help sustain the health of the Earth's waters.

PHOTO COURTESY OF NATIONAL PARK SERVICE

FIGURE 1: Different colors in the Grand Prismatic Spring of Yellowstone National Park are due to bacteria that survive by using chemicals from the hot spring water.

Background Information

The overreaching goal of this experiment is to integrate the sciences—Earth science, biology, and chemistry—to examine a process that is most likely unfamiliar to the students, the bacterial reduction of Fe^{3+} to Fe^{2+}. Students probably will have limited prior knowledge about water, water quality, bacteria, and respiration when beginning the lab, so the lab provides an opportunity for strengthening the student's knowledge and understanding of these concepts and topics. During this experiment students also use probeware, thus increasing their skill with using this type of technology.

If students are currently enrolled in a biology course or have had a biology course, they may be familiar with aerobic respiration, the complicated process by which organisms take up oxygen and produce carbon dioxide and water. For our purposes, we will consider *microbe* to be synonymous with *bacteria*. Bacteria get energy from this reaction, which enables them to grow and multiply. Carbon stored in high-energy compounds (such as sugars) provides electrons during this process and are considered electron donors. For aerobic bacteria, oxygen is the terminal acceptor of the electrons, but if oxygen is not present or is available in limited quantities, some other receiver must be available for the electrons. There are a number of possible receivers of the electrons, such as nitrate, sulfate, and iron(III) and manganese(IV). This experiment focuses on one possible acceptor of those electrons, the iron(III) ion.

Iron is found in almost any natural setting. It exists predominantly in two forms at and near the Earth's surface. In environments in contact with air, iron(III) dominates. When the environment is isolated from the atmosphere, such as in stagnant water or underground in rocks, iron(II) becomes more common. Iron(III) does not dissolve in water and is mainly found in iron(III) minerals. These minerals, such as goethite (FeO(OH)) and hematite (Fe_2O_3) are responsible for the yellow and red colors of soils all across the planet and even on Mars. On the other hand, iron(II) is much more soluble. The conversion of iron(III) to iron(II) is known as iron reduction; the valence state of iron is reduced as it accepts an electron. In the natural world, anaerobic bacterial respiration leads to iron reduction: The bacteria use the iron(III) in minerals as electron acceptors, partly dissolving the iron(III) minerals and releasing iron(II).

Iron reduction occurs in a variety of natural and anthropogenic settings, such as deep aquifers, lake and ocean sediments, swamps, oil spills, landfills—almost anywhere organic matter (food for the bacteria) drives aerobic respiration, removing all of the oxygen from water. If a landfill leaks into its surrounding environment, the abundance of organic matter within the leachate drives bacterial respiration. Oxygen is rapidly consumed through aerobic respiration, followed by anaerobic respiration processes such as iron reduction. In other words, these bacteria use the iron(III) as the electron receivers during the respiration process. Oil spills and eutrophic waters are additional anaerobic situations that may provide conditions favorable for iron(III) respiration by bacteria.

The schematic shown in Figure 2 (p. 57) illustrates expected results. In bottles containing fertilizer, the same process should occur but likely more rapidly and dramatically. Some Winogradsky columns are likely to develop pungent smells; much of the odor is likely due to an additional bacterial process known as *fermentation*. During fermentation, a variety of organic molecules are transformed, many of which have strong odors. In many columns you will probably notice a disappearance of the red/orange coloring of the sediment and water, related to the conversion of iron(III) to iron(II). Also, some Winogradsky columns may develop a black layer on top of the sediment. This results from another anaerobic process that can occur in waters containing sulfur: sulfate reduction. When

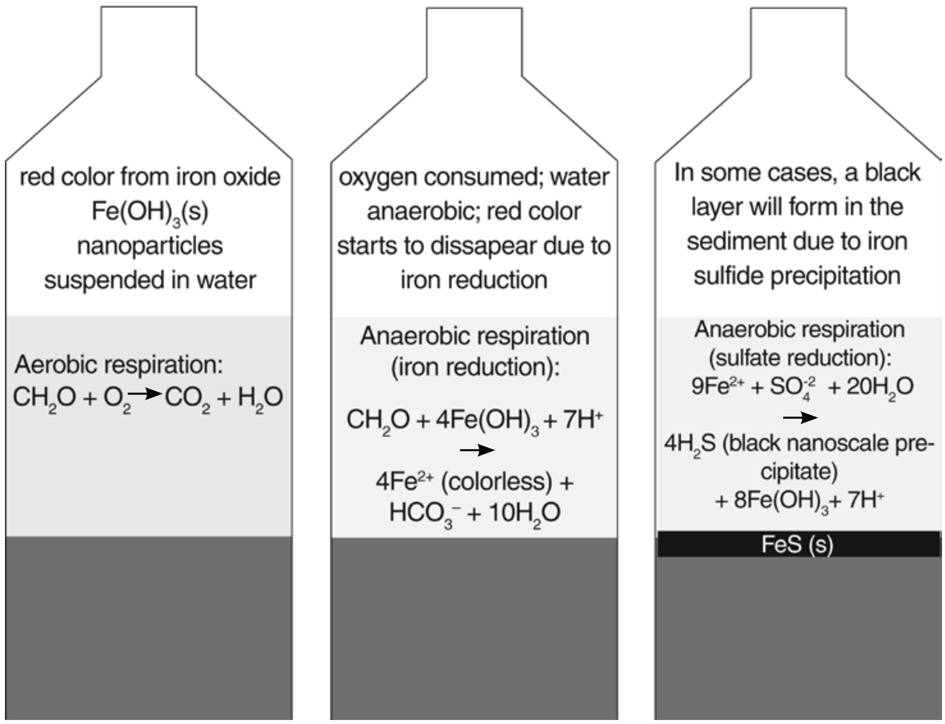


FIGURE 2: Example of expected results from Winogradsky column experiments.

sulfate is reduced to sulfide (S^{+6} in SO_4^{-2} to S^{-2}), it may combine with Fe^{2+} from iron reduction to produce nanoparticulate solid FeS minerals that are typically black.

How can you be sure that these processes are caused by bacteria? Unfortunately, to "see" iron reduction and sulfate reduction due to bacteria occurring inside the Winogradsky columns, sophisticated instrumentation or expensive procedures would be required. No direct observations or testing of bacteria are included in the lesson, partly for safety considerations. Direct observations of these types of processes require nanotechnology and are at the cutting edge of how scientists are investigating our environment. Lesson 5 provides an opportunity to perform these observations with a computer-simulated atomic force microscope.

Why is iron reduction so important to our understanding of the environment? One reason is that anaerobic respiration processes such as iron and sulfate reduction dramatically impact the quality of natural waters. For example, people living in many places around the world contend with high levels of arsenic in their water supplies. Although arsenic occurs naturally, drinking water containing even low concentrations of arsenic can lead to serious detrimental health effects. Perhaps no other place in the world is experiencing the problem as seriously as Bangladesh, where high levels of arsenic have been measured in well water. A scenario at the end of this lesson explores this example of how biology, chemistry, and geology combine to contribute to a tragic environmental problem (see p. 77). One of the leading scientific explanations for the elevated levels of arsenic in this region is the action of iron-reducing bacteria.

Over the last several decades, development organizations assisted in the installation of a tremendous number of groundwater wells, allowing the population of Bangladesh to avoid using potentially disease-contaminated water from rivers and lakes for their daily use. Readily available well water led to increased irrigation of crops, which in turn increased the amount of organic matter cycling into the rocks below. Scientists hypothesized that the organic matter inputs to the subsurface led to anaerobic conditions, promoting iron reduction. As the iron(III) oxide minerals dissolved, arsenic that was bound to their surfaces was released into the groundwater and was drawn up into public supply wells.

For Further Information

- Smith A. H., E. O. Lingas, and M. Rahman. 2000. Contamination of drinking-water by arsenic in Bangladesh: A public health emergency. *Bulletin of the World Health Organization* 78 (9). *http://asrg.berkeley.edu/00SmithContamDWBngldsh.pdf*
- Sustainable Development Networking Programme. 2003. Bacteria solution to groundwater arsenic. *www.sdnpbd.org/sdi/issues/environment/arsenic_farhana.php*
- World Health Organization. 2000. Arsenic in drinking water. *www.who.int/mediacentre/factsheets/fs210/en/index.html*

National Science Education Standards (NSES)

Nano2Earth Lessons	NSES Standards Addressed
Microbe-Mineral Interactions **Engage:** Prelab Questions **Explore:** Winogradsky Column Lab **Explain:** Discussion and Sharing of Results **Elaborate:** Groundwater Scenario and Inquiry **Evaluate:** Analysis and Conclusions	Unifying Concepts • Systems • Evidence • Models • Explanations • Changes • Measurement Science as Inquiry • Identify Questions and Concepts That Guide Explanations • Design and Construct Scientific Investigations • Using Technology and Mathematics to Improve Communications • Formulate and Revise Scientific Explanations and Models Using Logic and Evidence Physical Science • Structures and Properties of Matter • Chemical Reactions Life Science • Interdependence of Organisms • Matter, Energy, and Organization of Living Systems Earth and Space Science • Geochemical Cycles • Science in Personal and Social Perspectives • Environmental Quality Natural and Human-Induced Hazards • Science and Technology in Local, National, and Global Challenges

Engage: Prelab Questions

(Estimated time: 25 minutes)

The prelab questions on page 70 are designed to get the students to think about what they may already know about bacteria, respiration, and water quality. Arrange students into small groups to discuss the questions and follow up with a student-led whole-class discussion.

1. Where do you think the dissolved oxygen that is present in rivers, streams, ponds, and lakes originates?

   The dissolved oxygen present in water on the Earth's surface comes from oxygen in the air that is dissolved in the surface water and from the production of oxygen by water plants during their photosynthesis process.

2. Which type of bacteria, aerobic or anaerobic, do you think would be best adapted for survival in the water column of a stream? Which type of bacteria is best adapted to survive deep in the muddy region of the stream? Make sure you can explain your choice.

   Since dissolved oxygen is present in the water, aerobic bacteria would be successful surviving in this oxic region of the water. Since little dissolved oxygen is present within the muddy anoxic layer, anaerobic bacteria would be best adapted for this environment.

3. Make a prediction: What do you think will happen to the level of dissolved oxygen in a river if the river has an abundance of algal growth that is in the process of dying and decaying? Make sure you can explain your answer. Do you know what this process is called?

   Students will have their own predictions for this question depending on their prior knowledge. During the decay of overabundant algal growth, oxygen is used, which decreases the dissolved oxygen available for the other aquatic life dependent upon dissolved oxygen for survival. This decay process is called eutrophication. Eutrophication is a natural process, but if the level of eutrophication is excessive, the water becomes unhealthy for other organisms living in the water.

4. Make a prediction: Which type of bacteria, aerobic or anaerobic, would be best suited in the water environment that has an abundance of algal growth that is in the process of dying and decaying? Make sure you can explain your answer.

   Anaerobic bacteria would be best suited for a water environment that is excessively eutrophic because of the lack of oxygen.

Explore: Winogradsky Column Lab

(Estimated time: Varies from day to day)

During this activity, students will build a surface water ecosystem. The ecosystem will be used to

- observe general visual changes that take place in the Winogradsky column over time,
- simulate eutrophication,
- observe the effect of excess nutrients on the dissolved oxygen concentration,
- measure and follow changes in dissolved oxygen concentrations over a period of time,
- relate changes in dissolved oxygen to water quality and bacterial activity,
- observe changes in the bottle that students can use to infer changes in the concentrations of Fe^{3+} to Fe^{2+} when the column becomes anaerobic, and
- relate mineral-bacteria activity to water quality.

In this lab students create Winogradsky columns, which are simply 20–24 oz. plastic bottles containing natural water and sediment. You can collect the water/mud samples before class or students can collect them during class. Once the bottles are loaded, photosynthetic organisms should grow. They will begin to die and decompose as nutrients are exhausted. Sugar is added to speed up this process, so that students will be able to measure and observe drastic changes in only a few days. The oxygen will be consumed, creating an anaerobic environment. Students will use probeware to measure the amount of dissolved oxygen over a period of several days to monitor this transition. They will also add iron(III) chloride to create iron minerals in the water, which can be seen as a yellow-orange-red color. Students will observe how color changes in the water relate to the disappearance of oxygen. Finally, some students may add fertilizer to simulate the process of eutrophication.

Materials and Lesson Preparation

The procedure is outlined in the "Using the Winogradsky Column to Demonstrate the Microbial Reduction of Iron(III)" student activity sheet (p. 71); however, the following considerations should help make the lesson successful.

- Students will need to bring their own 20–24 oz. plastic bottle. It should be rinsed out thoroughly and must have the cap.
- The process is more efficient if each group has their own wide mouth funnel.
- Any small container can be used in place of the 50 ml beaker.
- If the students are not able to collect the mud and water, you will need to collect the mud and water ahead of time. These materials should be collected the morning that they will be used. Collect enough mud so students can fill their bottles about 2–3 in. high, and collect enough water to fill all of the bottles after students add the mud. If the class is building the columns inside the classroom, place newspapers in the workspace to protect furnishings and floor.
- Only one dissolved oxygen (DO) probe is necessary; however, if more than one is available, it will speed up the measuring of the dissolved oxygen concentration in the bottle contents. Using dissolved oxygen kits that depend on a color comparison will not work because the color of the mix interferes with testing. If DO probes are not available, students may complete the lab by observing color changes as an indication of mineral-bacterial activity.
- Students can use any household fertilizer for the nutrients they add to some of the columns. Miracle-Gro is one example of a household fertilizer.

Teacher Notes for Each Day of Experiment

Day 1

(The amount of time needed for Day 1 will depend on how much preparation you do ahead of time, as well as how many DO probes are available for the students.)

- Needless to say, this is a messy project! Place newspapers at each workstation. If possible, the bottles should be filled outside. Running water and a sink are necessary for cleaning up the outside of the bottles and the students. If you collected the mud and water before school, seal the bottles of water tightly.

- Each student will need to have his or her own directions about the use of the DO probe. With one probe available, each team can take its bottle to a station set up with the computer and probe to record the measurement. Oxygen will reenter the water in the bottle once it is opened; therefore, it is important to measure the DO concentration as soon as possible after opening the bottle. Students may want to open their bottles but then recap them until it is their turn to use the DO probe. Real-time reading of the DO level is fine. Let the probe stay in the muddy water until the DO concentration reading stabilizes. Have the students rinse off the probe with water after they are finished.
- Ask about half of the groups to add the fertilizer to their bottles.

Example of what students may write when observing bottles on Day 1

	Bottle without fertilizer	Bottle with fertilizer
DO concentration:	**7.5 mg/L**	**8.0 mg/L**

Other observations:

- Mud settles to bottom of bottle.
- Water is cloudy above the muddy layer.
- The water layer in the bottle with fertilizer has a greenish color.

Day 2

(Day 2 should immediately follow Day 1. This part of the activity will take 15–30 minutes depending on how many probes are available for student use.)

- Visual changes in the bottles: Further settling has occurred with the mud. The water level will be clearer. The color of the water layer probably has not changed much. Students may notice that the bottle feels harder due to the gas pressure building up in the bottle.
- Some gases from the decomposition process may have built up at this point, so it is vital that the removal of the cap be a careful, slow, and supervised process. Doing this outside may be preferable. Putting the bottle inside a sink may also help.
- Make sure the students wear chemical-splash goggles when using the iron(III) chloride.

Example of what students may write when observing bottles on Day 2

	Bottle without fertilizer	Bottle with fertilizer
DO concentration:	**6.9 mg/L**	**7.4 mg/L**

Other observations:

- The iron(III) chloride turns the small sample of water a deep yellow color.
- When added to the bottle, it will give the water a more yellow color. The bottle with fertilizer may have a more greenish-yellow color due to the fertilizer.
- Small bubbles may rise from the muddy layer when the bottles are opened.
- The distinct odor resulting from the decomposition process may also be present.

Day 3

(Day 3 should immediately follow Day 2. The amount of time needed for this part will depend on the number of DO probes available for student use.)

- Visual changes in the bottles: The bottle without the fertilizer will now have a red-orange color. The bottle with the fertilizer will not show any color change. The red-orange color is due to the iron(III) ions forming very small particles of iron hydroxides. Both bottles may feel harder because of the buildup of gases in the bottle.

Example of what students may write when observing bottles on Day 3

	Bottle without fertilizer	Bottle with fertilizer
DO concentration:	**1.9 mg/L**	**1.4 mg/L**

Other observations:

- The bottle without the fertilizer is now a red-orange color.
- The bottle with the fertilizer did not change color.
- Both bottles have a gas buildup inside the bottle.
- Bubbles rise from the mud when the bottle is opened. Distinct odors are evident from each bottle.

Day 4

(Ideally, Day 4 would be the day that the red-orange color has faded. This may not immediately follow Day 3. The amount of time needed for this day will depend on the number of probes available for student use.)

- Visual changes in the bottles: The red color in the bottle without the fertilizer has faded. The red-orange color fading is due to the iron hydroxides converting into iron(II) ions, which are soluble and colorless in water. The iron(II) is produced because the iron(III) has received electrons as a result of bacterial respiration processes. The bottle environment has become anaerobic, so oxygen cannot function as the final receptor of the electrons during respiration. The bottles feel harder because of gas pressure building up in the bottle.
- Cleaning up the bottles and contents: The bottle contents can be safely disposed of outside or in the trash going to the landfill. Cap the bottles and place in the trash or the teacher can choose to rinse them out to place in recycling.

Example of what students may write when observing bottles on Day 4

	Bottle without fertilizer	Bottle with fertilizer
DO concentration:	**0.8 mg/L**	**1.2 mg/L**

Other observations:

- The bottle without the fertilizer has lost its red color.
- There is little to no change in the color of the bottle with the fertilizer.
- The bottles are hard due to the buildup of gases.
- More bubbles rise from the mud when the bottle is opened.
- Odors are becoming stronger.

Explain: Discussion and Sharing of Results

(Estimated time: 20 minutes)

Have all students present their results to the class, including the DO concentrations and color changes over each day. Make a compilation of the results that can be seen by all of the class. These results will be necessary so students can answer the questions on the Analysis and Conclusions student activity sheet (p. 78).

FIGURE 3: Bottles with water samples.

Elaborate: Groundwater Scenario and Inquiry

(Estimated time: 30 minutes)

The Groundwater scenario (p. 77) extends the concepts from the Winogradsky column to a realistic environmental problem. If toxic substances, such as arsenic, are present in the environment, respiration of iron(III) minerals in soils and sediments becomes a problem. Many contaminants, such as arsenic, bind strongly to the surface of the iron oxides (they are adsorbed). However, if the iron(III) is reduced by bacteria, it becomes iron(II) and, thus, soluble in water. When the iron compounds dissolve, they release the arsenic from their surfaces (they desorb), so they will move into the groundwater.

Also, this is a good opportunity for student-driven inquiry "what-if" activities. The students could be challenged to present a hypothesis regarding the effect another variable (e.g., temperature, type of sediment, addition of salts) might have on the reactions inside the Winogradsky column.

Evaluate: Analysis and Conclusions

(Student questions on pp. 78–79)

(Estimated time: 20 minutes)

1. Why was the sugar added to the bottle?

   The bacteria convert the sugar (organic matter, carbohydrates) and oxygen to carbon dioxide and water during the respiration process. The addition of the sugar also helps to ensure that the oxygen is used up quickly, so that the lab *will only take a short time. This may represent an accelerated version of what might happen in a natural system.*

2. What happened to the concentration of dissolved oxygen during the time period that you observed the DO level?

   Students will have own answers for this question. The DO concentration in the bottle should have dropped.

3. Check with other lab groups to find out how the level of DO changed in their bottles during the experiment. Write down each group's result. Be sure to include whether or not they added fertilizer to their bottle. Do their changes agree with yours?

   All of the groups should have observed a drop in the DO.

4. What caused this change in the dissolved oxygen concentration during the experiment?

   The drop in DO level is caused by the oxygen being used by the aerobic bacteria during their respiration process and by other organisms during decomposition of organic matter in the bottle (such as dead bacteria and plant matter). Since the bottle is closed, there is no chance for the renewal of the oxygen in the water that would occur naturally when a creek is in contact with the air. Also, there are no water plants in the bottle actively replenishing the water with oxygen during the photosynthesis process.

5. Is there a difference in the dissolved oxygen change for the bottles that had fertilizer added and those without fertilizer? What do you think caused this difference?

   The DO level in the environment containing the fertilizer may drop sooner and lower because of the excess nutrients in the water encouraging a more rapid rate of bacterial respiration. The lack of these nutrients might have been limiting bacterial growth.

6. Fertilizer is considered a *nutrient* because it contains elements such as nitrogen and phosphorous that limit algal growth in bodies of water. Can you think of other nutrients that humans unintentionally add to rivers and lakes that stimulate the growth of algal blooms?

Nutrients that humans may add to water systems include excess fertilizer runoff from farms, yards, golf courses, agricultural runoff (animal manure), contents from leaky septic systems, and discharges from sewage systems.

7. When the algae dies and decomposes, it causes eutrophication in the water. What happens to the level of dissolved oxygen in the body of water when eutrophication occurs? What problems do you think would be associated with excessive eutrophication?

   During eutrophication, the level of dissolved oxygen decreases, thus, a reduced amount of oxygen is available for the aquatic life. This reduced amount of oxygen creates an unhealthy, or possibly toxic, environment for the aquatic life.

8. What part do you think you and your families can play in keeping excessive nutrients from finding their way into surface waters such as lakes, rivers, streams, and ponds?

   Students can encourage the correct and limited use of fertilizers in their yards or fields, encourage their parents to maintain an effective septic system, and keep their cattle and other livestock away from creeks and creek banks.

9. Adding the iron(III) caused the solution to initially become a deeper or darker yellow color. What happened to this color during the experiment? What could have caused this change in color?

   The dark yellow color changed to a red-orange, then disappeared. The yellow color was caused by the presence of Fe^{3+} in the water. The reddish-orange color was a result of the presence of small particles of iron hydroxide in the water. The disappearance of the color indicates that the Fe^{3+} is gone. It actually has converted into Fe^{2+}, which is soluble and colorless in water. This conversion of Fe^{3+} to Fe^{2+} during the reduction process (electrons being gained by the Fe^{3+}) causes the color to change from yellow/red to colorless.

10. During aerobic respiration, the oxygen is the terminal electron acceptor. When the bottle contents lacked oxygen, what do you think the terminal electron acceptor was during the respiration process?

    During the experiment the color of the water above the mud went from brownish to yellow to red/orange then back to brownish after the iron(III) chloride was added. The yellow color was due to the presence of the iron(III) ion is the water which was converted into iron(III) oxide (red-orange). The fading of *the red-orange color was due to the reduction of the iron(III) to iron(II). The iron(III) was the terminal electron acceptor.*

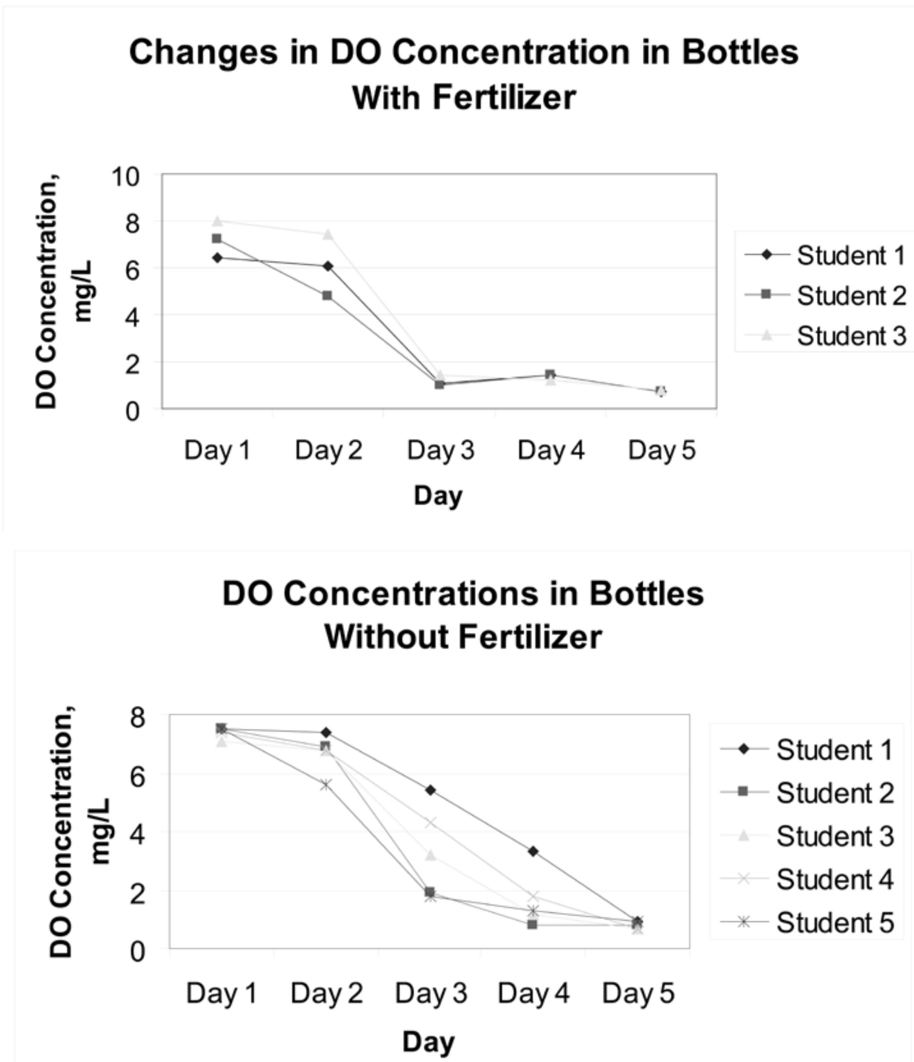


FIGURE 4: Sample set of class results.

Nano2Earth Student Activity Sheet

Using the Winogradsky Column to Demonstrate the Bacterial Reduction of Iron(III) Activity

Prelab Questions

Form groups of four students and discuss the following questions. Prepare to discuss your answers with the rest of the class.

1. Where do you think the dissolved oxygen that is present in rivers, streams, ponds, and lakes originates?

2. Which type of bacteria, aerobic or anaerobic, do you think would be best adapted for survival in the water column of a stream? Which type of bacteria is best adapted to survive deep in the muddy region of the stream? Make sure you can explain your choice.

3. Make a prediction: What do you think will happen to the level of dissolved oxygen in a river if the river has an abundance of algal growth that is in the process of dying and decaying? Make sure you can explain your answer. Do you know what this process is called?

4. Make a prediction: Which type of bacteria, aerobic or anaerobic, would best be suited in the water environment that has an abundance of algal growth that is in the process of dying and decaying? Make sure you can explain your answer.

Using the Winogradsky Column to Demonstrate the Bacterial Reduction of Iron(III) Activity

Experiment

Material and equipment needed for each group:

- clean, 20–24 oz. plastic bottle (with cap)
- wide mouth funnel
- mud and water from a pond, creek, lake, river, or stream
- ½ tsp. table sugar
- ⅛ tsp. (pea–size portion) iron(III) chloride, $FeCl_3$
- 50 ml beaker
- ¼ tsp. fertilizer (needed for half of the groups)
- scoops or spoons for muddy water

Material and equipment needed for the entire class:

- Vernier LabPro, dissolved oxygen (DO) probe
- buckets for mud and water
- window or grow lamp
- ¼ tsp. measuring spoon

Safety considerations:

- Wash your hands after building the column and after you have worked with the column.
- Chemical-splash goggles are needed on Day 2 and Day 3 because you will be working with iron(III) chloride, and fertilizer.

Procedure

Day 1

1. If your teacher has already prepared your mud/water mixture for you, you are ready to put it in the plastic bottle. Place the wide mouth funnel in the open end of the plastic bottle and use a spoon to add the mud/water mixture to the bottle. Add the mixture until it reaches about 3 in. Proceed to Step 3.

2. If your teacher has not prepared the mud/water mix, then you need to prepare it yourself. Go to a nearby pond, creek, or lake. At the edge of the water, use a heavy spoon to dig mud from the pond/creek/lake bottom and put it in a bucket or bowl. Add enough water so that you can

spoon the mud/water mix into a wide mouth funnel. Place the funnel into the opening of the bottle and spoon the mud/water mix into the bottle to a height of about 3 in. Add pond/creek/lake water to the bottle until the bottle is nearly full. Tightly screw the cap onto the bottle and bring it to class. Proceed to Step 3.

FIGURE 5: Collecting mud

3. Add pond water to the bottle until the bottle is nearly full.

4. Using the dissolved oxygen probe, follow your teacher's directions for measuring the dissolved oxygen concentration of the bottle environment. Record the results in the Day 1 data table on page 75.

5. Add ½ tsp. of table sugar (sucrose) to the bottle. Gently tilt the bottle to mix the sugar with the water in the bottle.

6. The teacher will let you know if your group is going to also add fertilizer to the bottle. If your bottle will contain fertilizer, add about ¼ tsp. to the bottle. Gently tilt the bottle to mix the fertilizer with the contents of the bottle.

7. Cap the bottle and place it near a window, but not in direct sunlight. If a window is not available, place the bottle about 2 ft. from a 40–60 W lightbulb or near a grow lamp.

8. Take a few minutes to observe the bottle. Record your description of the contents in the Day 1 data table on page 75.

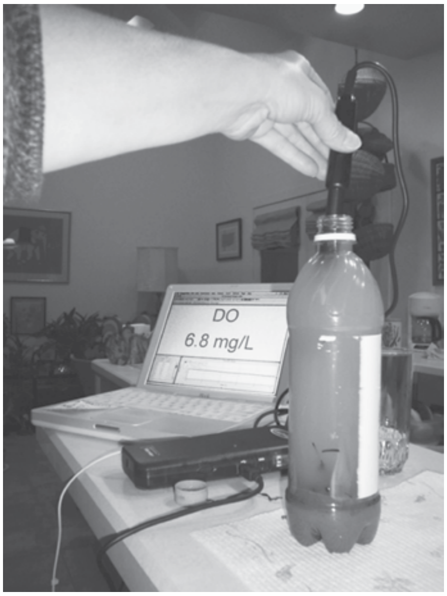


FIGURE 6: Measuring the dissolved oxygen concentration.

Day 2

1. Examine your bottle. What do you observe? What is different from Day 1? Record your observations in the Day 2 data table on page 75.

2. Place your bottle inside a sink. Very carefully and slowly, loosen the cap of the bottle. Make sure to keep under control any bottle contents that attempt to squirt out of the bottle. Remove the cap from the bottle.

3. Follow your teacher's directions to find the dissolved oxygen concentration of the bottle contents. Record the result in the data table.

4. Mix a pea-size portion, or ⅛ tsp., of iron(III) chloride with a small amount of water in a 50 ml beaker or small cup. Note the color of this Fe^{3+} solution and record it in the Day 2 data table. Add this solution to the bottle. You may need to gently pour out a little bit of the water in the bottle to make room for the iron(III) solution. Let the bottle sit for a few minutes

undisturbed. Note the color of the water in the bottle and record it in the Day 2 data table.

Day 3

1. Examine your bottle for changes since Day 2. Record your observations in the Day 3 data table.

2. Place the bottle in a sink and very carefully and slowly twist the cap off.

3. Measure the concentration of dissolved oxygen in the bottle contents. Record the value in the Day 3 data table.

Day 4

1. Examine your bottle for changes since Day 3. Record your observations in the Day 4 data table.

2. Place the bottle in a sink and very carefully and slowly twist the cap off.

3. Measure the concentration of dissolved oxygen in the bottle contents. Record the value in the Day 4 data table.

4. Follow your teacher's directions for cleaning up the bottles.

Using the Winogradsky Column to Demonstrate the Bacterial Reduction of Iron(III) Activity

Data Tables

Our bottle did / did not (*circle one*) have fertilizer added to it.

Day 1

Description of Bottle Contents	Dissolved Oxygen Concentration

Day 2

Description of Bottle Contents	Dissolved Oxygen Concentration	Color of Iron(III) Solution	Color of Bottle After Iron(III) Added

Nano2Earth Student Activity Sheet

Using the Winogradsky Column to Demonstrate the Microbial Reduction of Iron(III) Activity

Day 3

Description of Bottle Contents	Dissolved Oxygen Concentration

Day 4

Description of Bottle Contents	Dissolved Oxygen Concentration

Nano2Earth Student Activity Sheet
Groundwater Scenario

Your town's aquifer (naturally stored water underground) consists of sediments that were deposited in an ancient river system like the Mississippi delta. The sediments consist of quartz grains coated with iron oxides. Analysis of the sediments shows that they contain trace concentrations of arsenic, but when you analyze the groundwater, it does not contain arsenic. How would you explain this difference regarding the presence of arsenic?

The municipal landfill for your town has been collecting garbage for 50 years. In addition to paper, plastic, and metal, the landfill contains a lot of food and compost waste. In other words, the landfill is rich in organic matter. Rainwater percolates through the landfill and the leachate (mixture of rainwater and other dissolved materials from the landfill) easily gets into the groundwater.

Your family's well is located near the landfill. Recently you noticed that the water tastes like iron. You have the well water analyzed and find that it contains arsenic. Where do you think the arsenic is originating?

The diagram may help you imagine the scenario:

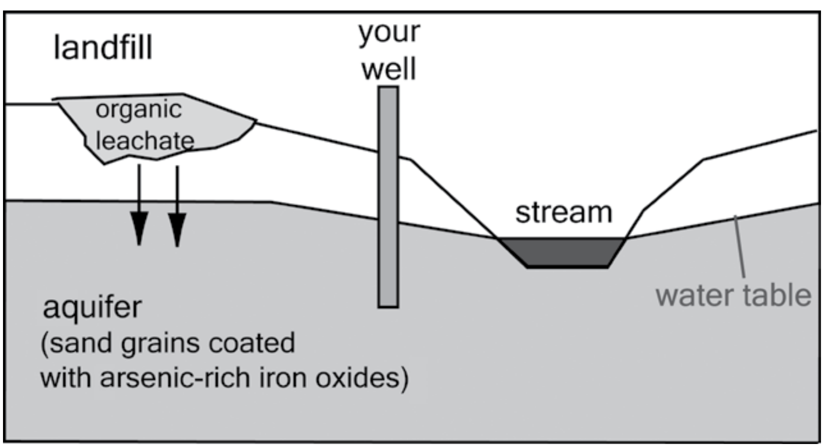


FIGURE 7: Groundwater scenario.

Equations that may help:

aerobic respiration:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O$$

iron(III) chloride dissolved in water:

$$FeCl_3 \rightarrow Fe^{3+} + 3Cl^-$$

yellow

iron(III) reacts with water:

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)^3$$

red-orange

possible anaerobic respiration process:

$$CH^2O + 4Fe(OH)^3 + 7H^+ \rightarrow 4Fe^{2+} + HCO_3^- + 10H_2O$$

red-orange colorless

Nano2Earth Student Activity Sheet

Analysis and Conclusions

1. Why was the sugar added to the bottle?

2. What happened to the concentration of dissolved oxygen during the time period that you observed the DO level?

3. Check with other lab groups to find out how the level of DO changed in their bottles during the experiment. Write down each group's result. Make sure to include whether or not they added fertilizer to their bottle. Do their changes agree with yours?

4. What caused this change in the dissolved oxygen concentration during the experiment?

5. Is there a difference in the dissolved oxygen change for the bottles that had fertilizer added and those without fertilizer? What do you think caused this difference?

Analysis and Conclusions

6. Fertilizer is considered a *nutrient* because it contains elements such as nitrogen and phosphorous that limit algal growth in bodies of water. Can you think of other nutrients that humans unintentionally add to rivers and lakes that stimulate the growth of algal blooms?

7. When the algae dies and decomposes, it causes eutrophication in the water. What happens to the level of dissolved oxygen in the body of water when eutrophication occurs? What problems do you think would be associated with excessive eutrophication?

8. What part do you think you and your families can play in keeping excessive nutrients from finding their way into surface waters such as lakes, rivers, streams, and ponds?

9. Adding the iron(III) caused the solution to initially become a deeper or darker yellow color. What happened to this color during the experiment? What could have caused this change in color?

10. During aerobic respiration, the oxygen is the terminal electron acceptor. When the bottle contents lacked oxygen, what do you think the terminal electron acceptor was during the respiration process?

Lesson 4

Investigation of Bacterial Transport in Groundwater

Purpose

Students explore the transport of bacteria in groundwater by performing a column experiment in a simulated groundwater environment. Students also investigate a groundwater scenario to learn about the influence of groundwater chemistry on bacterial transport.

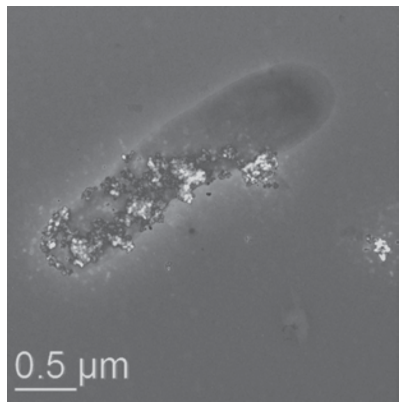


FIGURE 1: Transmission electron micrograph of the bacterium *Shewanella oneidensis* respiring 30 nm nanoparticles of the iron oxide mineral hematite. In the absence of oxygen, *Shewanella* is capable of using the ferric iron in hematite as a terminal electron acceptor. The outline of a single bacterium appears as a halo, while hematite nanoparticles appear as solid.

From Bose S., M.F. Hochella Jr., Y.A. Gorby, D.W. Kennedy, D.E. McGready, A. Madden, and B.H. Lower. 2009. Bioreduction of hematite nanoparticles by *Shewanella oneidensis* mr-1. *Geochimica et Cosmochimica Acta* 73: 962–976.

Background Information and Lesson Overview

Bacterial transport is an important area of research in the study of groundwater pollution. Because bacteria play an important role in causing disease, understanding particle transport and its environmental effects is important for addressing water quality, bioremediation, and global health issues. Bacteria are transported through porous materials as a function of physical and chemical properties of the microbes, minerals, and water. In this laboratory experiment, small microbeads, representing bacteria, are passed through a column of coarse sandy material. Effluent is collected and analyzed using adsorption or reflectivity by means of the light sensor probe. The results are graphed and interpreted to find the number of microbeads that become trapped in the column and the number that are passed through the system.

Following the lab, students will investigate a groundwater pollution scenario in which the concepts of surface charge and single displacement reactions are addressed in relation to the groundwater chemistry of particle transport. In Lesson 5, students will see how the nanoscale forces (electrostatic and van der Waals forces) between charged surfaces can be measured with an important tool of nanotechnology, the atomic force microscope (AFM). In preparation for Lesson 5, you may review the concepts of nanoscale science and technology (see Chapters 1–3).

Detecting the number of microbeads that pass through the column works when the light emitted from the source enters the solution and is (1) transmitted, (2) adsorbed, or (3) scattered.

$$\text{Total light} = \text{Transmitted} + \text{Adsorbed} + \text{Scattered}$$

The light-sensing probe measures transmitted light. The amount adsorbed and scattered light is expected to increase as the concentration of beads in the solution increases. Therefore, the transmitted light will accordingly go down.

$$\text{Transmitted} = \text{Total Light} - (\text{Adsorbed} + \text{Scattered})$$

For Further Information

- Harvey, R. W., L. H. George, R. L. Smith, and D. R. LeBlanc. 1989. Transport of microspheres and indigenous bacteria through a sandy aquifer: Results of natural and forced-gradient tracer experiments. *Environmental Science and Technology* 23: 51–56.
- Harvey, R. W. 1993. Fate and transport of bacteria injected into aquifers. *Current Opinion in Biotechnology* 4: 312–317.
- Johnson, W. P., M. J. Martin, M. J. Gross, and B. E. Logan. 1996. Facilitation of bacterial transport through porous media by changes in solution and surface properties. *Colloid and Surfaces A: Physiocochemical and Engineering Aspects* 107: 263–271.
- Maier, R. M., I. L. Pepper, and C. P. Gerba. 2008. *Environmental microbiology.* 2nd ed. San Diego, CA: Academic Press. Chapter 19: Bacterial Transport.

National Science Education Standards (NSES)

Nano2Earth Lessons	NSES Standards Addressed
Investigation of Bacterial Transport in Groundwater **Engage:** Groundwater Pollution Scenario and Waterborne Diseases **Explore:** Bacterial Transport Column Lab **Explain:** Class Discussion and Questions **Elaborate:** Influence of Groundwater Chemistry (pH) on Bacterial Transport **Evaluate:** Groundwater Pollution Scenario Revisted	Unifying Concepts • Systems • Evidence • Models • Explanations • Changes • Measurement Science as Inquiry • Identify Questions and Concepts That Guide Explanations • Design and Construct Scientific Investigations • Using Technology and Mathematics to Improve Communications • Formulate and Revise Scientific Explanations and Models Using Logic and Evidence Physical Science • Structures and Properties of Matter • Motions and Forces Earth and Space Science • Geochemical Cycles • Science in Personal and Social Perspectives • Environmental Quality Natural and Human-Induced Hazards • Science and Technology in Local, National, and Global Challenges

Materials

- Visible light sensor probe (CBL or computer-based laboratory, PASCO, LabPro)
- Fluorescent carboxylated microbeads (2 μm)—for the experiment, 0.1 ml will be needed for each group. These microbeads can be ordered from Polysciences, Inc. They are called Fluoresbrite Yellow Green (YG) carboxylate microspheres, catalog number 09847 for a 5 ml bottle (~$120). Alternatively, a 1 ml trial size may be ordered (~$43). If the

microbeads are outside your available budget, you should contact the company for possible donations or cheaper alternatives. If none can be found, you will likely have to skip this lesson and explore other aspects of the Nano2Earth curriculum.

- Bag of coarse sand ("playground") purchased at hardware or home supply stores. Before giving the sand to students, rinse it thoroughly to remove fine materials. Fine, suspended material can flush through the column during the experiment and interfere with the light sensor measurements.
- pH paper and 10 ml 0.1M HCl is needed for each group during the Elaboration activity.
- 16 oz. or 20 oz. plastic soda bottle (bottom may be cut off in advance), one per group
- Small flashlight or pen light, one per group

Science Safety

This activity requires group work. You will facilitate student progress and experimentation. Because the Elaborate activity uses weak hydrochloric acid, students are required to wear chemical-splash goggles. Carefully supervise cutting off the bottle bottoms and poking holes in caps. Practice cutting prior to demonstrating the process for students.

Engage: Groundwater Pollution Scenario

(Estimated time: 15 minutes)

Waterborne Diseases Student Activity

(Estimated time: 20 minutes)

Procedure

Engage students in learning about particle transport by discussing the "Groundwater Pollution Scenario" (p. 90) and sources of waterborne diseases. This activity should be done as a prelab. The purpose of the Engage activity questions (p. 90; answers below) is to get the students to think about what they may already know about bacteria and water quality. Students can be arranged into small groups to discuss the questions, followed by a whole-class discussion.

Students should also read and discuss the "Waterborne Diseases" activity sheet on page 91 (20 minutes).

Answers to Engage Activity Questions

1. What are the potential sources of the *E. coli* in your well?

   Potential sources of the E coli are your septic system, your neighbor's septic system, and farm animals.

2. Groundwater flows 1 m a day. Your neighbors live approximately 100 m down the hill from you. Look at the figure to see the direction of groundwater flow. How long would it take for bacteria from your well to reach your neighbors' houses?

   $v = d/t$, $v = 1$ m/d, $d = 100$ m; $t = v/d = 1$ m/d

   It would take 100 days to get to your neighbor's house.

3. What factors do you think might control how the bacteria are being transported in groundwater?

   Factors that control bacterial transport include: type of rock, porosity and permeability, structures or folds/faults in rock, preferential flow, type of bacteria, number of bacteria, chemical characteristics of groundwater, direction of water flow, and velocity of water flow.

Explore: Bacterial Transport Column Lab (Parts 1 and 2)

(Estimated time: 1.5 hours)

1. Set up everything for the activity (Part 1, pp. 92–97; Part 2, pp. 98–103) ahead of time except the actual column, which students will do. (45 minutes)

2. Students use the light probe to collect data. (30 minutes)

3. Using the data collected with the probe, students construct graphs to determine the concentration of particles (microbeads). (15 minutes)

Explain: Class Discussion and Questions

(Estimated time: 30 minutes)

Students should answer and discuss the questions found on the "Investigation of Bacterial Transport in Groundwater—Part 1: Particle Transport in a Sand Column" activity sheet (p. 92).

Answers to Explain Activity Questions

(Student questions on p. 97)

1. Based on your data, would adding more microbeads to the solution increase or decrease the light intensity value?

   Decrease

2. Why does light intensity decrease when the microbead concentration increases?

   Less light shines through because the fluid is filled with microbeads

3. Calculate the number of microbeads that remain in the column of sand.

   The number depends on results of experiments.

4. Why did some of the microbeads remain in the column?

   They get strained (filtered) or adhere/stick to the sand.

5. If a bacterium is 1–2 micrometers (1 µm = 10^{-6} m) in diameter, how does that mathematically compare to a nanoparticle? (1nm = 1 × 10^{-9} m)

   A bacterium would be 1,000 nm.

6. Smoke particles, viruses, and some bacteria are nanoparticles. If we used nanobeads instead of microbeads in our experiment, would you expect more or less particles to remain in the column? Why?

   Smaller (nano) particles would likely pass through the system to a greater extent than larger (micro) particles due to filtration. Nanoparticles and microparticles have different surface-to-volume ratios that may change the attraction/repulsion properties of the particles to the surface, but the effect of this on transport is not clear. This is an area of current scientific research.

Elaborate: Influence of Groundwater Chemistry (pH) on Bacterial Transport

(Estimated time: 2.5 hours)

Students follow the same procedure as in the Exploration activity, except they also need pH paper and 10 ml 0.1 M HCl for each group. The actual lab should take 35–40 minutes and data graphing should take about 15 minutes. Students should answer and discuss the questions found in the "Investigation of Bacterial Transport in Groundwater—Part 2: Influence of Groundwater Chemistry on Bacterial Transport" activity sheet. Questions are on pages 103–104.

1. Does adding 10 ml of HCl to the microbead solution raise or lower the pH?

   It lowers the pH.

2. What happens to the surface charges on the sand and microbeads when pH is lowered? What happens to the surface charges when the pH is raised?

   As pH decreases (becomes more acidic), the number of H^+ ions in solution increases. The free H^+ ions prefer to be associated with the negative charges on bacteria and mineral surfaces. Effectively, when solution pH is lowered, the surfaces of minerals and bacteria become more positively charged. When the pH is increased, the opposite happens—the surfaces become more negatively charged.

3. If you add a teaspoon of salt to the microbead solution, would you expect the surfaces to become more or less "sticky"?

   They would become more sticky as higher ionic strength increases attractive forces between the bacteria and sediment.

4. Calculate the number of microbeads that remained in the column of sand.

   The number depends on the results of experiments.

5. Do you think live bacteria would behave differently than the microbeads? Why? *Hint: Are ALL bacteria the same size and shape?

   Live bacteria would behave differently than microbeads—they are different shapes and sizes, and some bacteria produce proteins and other compounds that help them stick to surfaces.

6. Do you think sand accurately represents the rocks around your house and school? Explain.

   Answers may vary—it depends on the geology of the area.

7. List two differences between the sand column and the real rocks around your region.

   Answers may vary based on the geology of the area.

8. When storms occur and flooding is evident, there are often concerns about drinking water purity. Why?

   Several reasons. Floods can "strip" off bacteria from sediment into water. They can also bring runoff (e.g., from agricultural fields) to surface water. Also, because floodwater is derived from rain, the water is more dilute, and bacteria stick less to sediment under lower ionic strength.

Evaluate: Groundwater Pollution Scenario Revisited

(Estimated time: 1.5 hours)

To gauge the students' understanding of how changing pH and ionic strength may affect bacterial transport, pose the following questions in relation to the original scenario (Engage Groundwater Pollution Scenario activity). Encourage the students to draw diagrams supporting their answers (e.g., the charge at the mineral and microbe surfaces in solution).

1. Consider that the ground beneath the pasture and housing development are made of (a) quartz sand (such as sand found on a beach) or (b) iron oxide–coated soil (such as any red or yellow soil you have seen) How might bacterial transport be different in the two different soils?

   To answer this question, it is necessary to know that at the pH values of most soils and groundwater, quartz has a negative charge on the surface and iron oxide minerals have a positive surface charge.

2. Ask the students to design an experiment to test the effects of ionic strength on bacterial transport using the microbeads. They may come up with ideas such as adding different amounts of NaCl or another salt to the microbead solution. Have the students predict what the results of these experiments will be and draw graphs representing the expected trends. Then have the students do the experiments, repeating the previous procedures with their modifications. How do the results compare with their predictions?

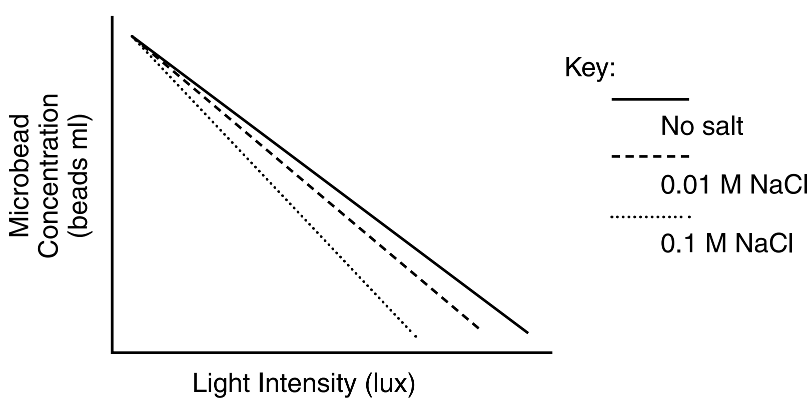


FIGURE 2: Example of student predictions.

Nano2Earth Activity Sheet
Groundwater Pollution Scenario

You have just moved into a subdivision in which all the houses have a well and a septic system (see Figure 3). Your house is located on the outskirts of the subdivision, right next to a cow pasture. You move in during the winter, when most of the cows are not out grazing, and things are fine. However, during the following spring, you get sick. The doctor tells you to analyze your well water for bacteria. You do, and it turns out that your well has *E. coli*, a pathogenic bacteria from fecal matter. You ask your three neighbors down the hill to have their well tested. Results show that their wells are fine.

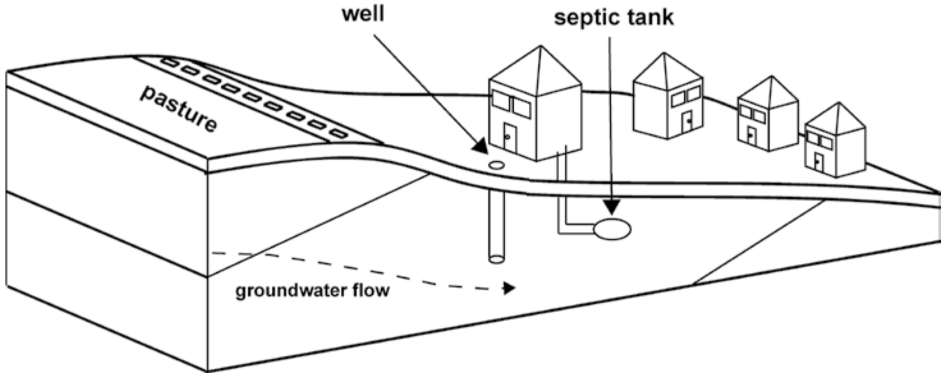


FIGURE 3: Groundwater pollution scenario.

Questions:

1. What do you think are the potential sources of the *E. coli* in your well?

2. Groundwater flows 1 m a day. Your neighbors live about 100 m down the hill from you. Look at the figure to see the direction of groundwater flow. How long would it take for bacteria from your well to reach your neighbors' houses?

3. What factors do you think might control how the bacteria are being transported in groundwater?

Nano2Earth Student Activity Sheet
Waterborne Diseases

Contamination of water supplies by pathogenic microorganisms is a leading cause of death in the world. It is estimated that 10 million people die every year from waterborne diseases, such as typhoid, cholera, and dysentery. According to the World Health Organization, 19% of deaths from infection worldwide are water-related, and water-related diseases contribute to the death of nearly 4 million children each year. Kofi Annan, then U.N. Secretary-General, said on June 5, 2003, "One out of every 6 people lives without regular access to safe drinking water. Worse, water-related diseases kill a child every 8 seconds, and are responsible for 80% of all illnesses and deaths in the developing world."

Waterborne Pathogens (from ***www.mwra.state.ma.us***)

Diseases	Responsible pathogen	Route of exposure	Mode of transmission
Cholera	*Vibrio cholerae* bacteria	gastrointestinal	often waterborne
Botulism	*Clostridium botulinum* bacteria	gastrointestinal	food/waterborne; can grow in food
Typhoid	*Salmonella typhi* bacteria	gastrointestinal	water/foodborne
Hepatitis A	Hepatitis A virus	gastrointestinal	water/foodborne
Dysentery	*Shigella dysenteriae* bacteria or *Entamoeba histolytica* amoeba	gastrointestinal	food/water
Cryptosporidiosis	*Cryptosporidium parvum* protozoa	gastrointestinal	waterborne; resists chlorine
Polio	polioviruses	gastrointestinal	exposure to untreated sewage; may also be waterborne
Giardia	*Giardia lamblia* protozoa	gastrointestinal	waterborne

Part of the difficulty in controlling bacterial contamination of water supplies is that it is difficult to predict how bacteria are transported from one place to another. One thing we do know is that bacteria are typically attached to a surface rather than floating in water. Surface attachment is a survival strategy. The surface not only provides a more stable environment, but it also allows the bacteria greater access to energy sources (e.g., carbon) and nutrients.

Nano2Earth Student Activity Sheet

Investigation of Bacterial Transport in Groundwater

Part 1. Particle Transport in Sand Column Activity

Purpose

The purpose of this lesson is to simulate how particles, such as bacteria, are transported through porous materials as a function of physical and chemical properties. Instead of using live bacteria, you will work with microbeads, which are similar in size to bacteria.

Background Information

Particles such as bacteria have a diameter between 10 nm and 10 µm and are so small that they cannot be seen without a microscope. Due to their small size, bacteria have a very large surface-area–to-mass ratio.

Bacteria can be removed from water by two mechanisms. They can be filtered ("strained") out of solution through porous media (like sand in Figure 4 or limestone in Figure 5). This process is a function of the differences in size between the bacteria and the mineral grains in the rock. Bacteria can also be removed by adsorption onto surfaces. When water containing bacteria passes through the ground, some bacteria become stuck to the rocks and minerals in the ground and are removed from the solution. This can slow down bacterial transport in groundwater. It is a goal of scientists and engineers to be able to predict how far bacteria will travel in groundwater and how they attach to the rocks and minerals in the ground.

The "stickiness" of bacteria to rocks and minerals depends on several factors related to forces that act on particles that are as tiny as bacteria. These forces are a function of the chemistry of the groundwater and the types of particles and minerals that are present. Many bacteria and minerals have electrostatic charges on their surfaces that can sometimes be used to determine if the bacteria will stick to each other.

This experiment is a laboratory scale study of a real-life system. In this study, there is a release of bacteria onto a sandy soil and you need to determine how many bacteria have traveled through the soil. You will also relate your findings to several other real-world situations. Environmental consultants get paid big bucks to do work just like this! Good luck and have fun!

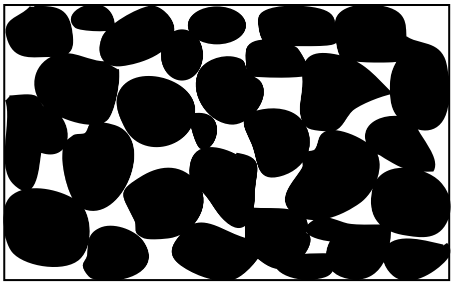


FIGURE 4: Pictured are pores between sand grains; the grains are represented as black and pores are white. If bacteria are bigger than the pore spaces, they can be filtered, or "strained."

FIGURE 5: Fractures in rocks are depicted. Depending on the width of the fracture openings, bacteria can either be transported with water or they may be strained.

Materials and Resources

(required for each group's lab setup)

- Plastic column (16 or 20 oz. bottle with bottom cut off and cap with holes)
- Column fill (very coarse sand approximately 3–4 mm diameter)
- 100 ml clean water
- 3 glass beakers (250 ml)
- Ring stand and clamp
- Flashlight (a tightly focused penlight flashlight works best)
- CBL light-sensor probe
- Masking tape
- Microbeads (2 µm fluorescent carboxylated beads; 0.1 ml will be needed per lab group)
- Graph paper
- Container to collect water from column

Procedure

1. Fill the plastic column with very coarse sand and mount on the ring stand as shown in Figure 6.

2. Fill a 250 ml glass beaker with 100 ml of clean water.

3. Set up the CBL light-sensor probe, flashlight, and beaker with water as shown in Figure 7. Use the tape provided to secure the position of the probe and flashlight. Place a sheet of paper underneath the apparatus and outline the position of the glass beaker with a pen.

4. Turn on the flashlight and align the beam from the flashlight into the light sensor probe.

5. Be sure the probe is connected to a data collector (i.e., computer or TI graphing calculator).

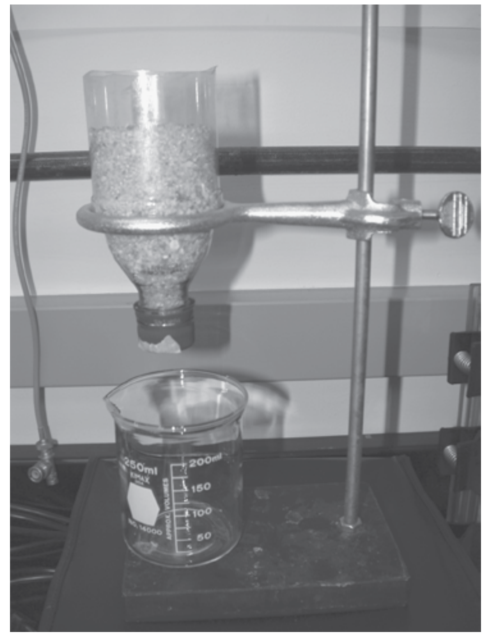


FIGURE 6: An example of a column experiment equipment setup is shown. It is not necessary to use the upper reservoir.

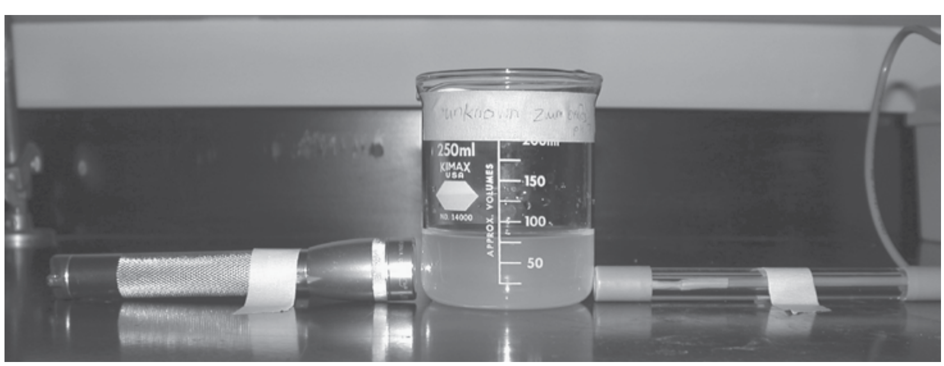


Figure 7: Setup of flashlight, beaker, and CBL light-sensor probe.

6. Measure the intensity of light as it passes from the flashlight through the beaker of water and into the light sensor probe. Collect light intensity (lux) for 10 continuous seconds. Record the average value in your science notebook. Leave the flashlight on and do not adjust the intensity coming from the flashlight.

7. Fill the column with CLEAN water to saturate the sand. Collect any water that drains from the column in a large container and dispose in sink. Do not let the column drain completely. When the column starts to drip more slowly, put tape over the bottom (on the cap) to keep the column saturated.

8. Empty the water from the 250 ml beaker into the sink and dry the beaker.

9. Label the clean, dry beaker "Unknown Sample Part 1" and place it under the column. See Figure 6.

10. Retrieve 100 ml of microbead solution from your teacher in a 250 ml glass beaker. Label the beaker "Known Sample." *Note:* Your teacher has already diluted 0.5 ml of 4.5×10^{11} beads/ml solution into 500 ml. The known microbead concentration is 4.5×10^{8} beads/ml.

11. Measure the intensity of light as it passes from the flashlight through the beaker containing the known sample and into the light sensor probe. Collect light intensity for 10 continuous seconds. Record the average value in your science notebook.

12. Pour known sample of fluorescent microbead solution into column slowly.

13. Effluent will be collected in beaker labeled "Unknown Sample."

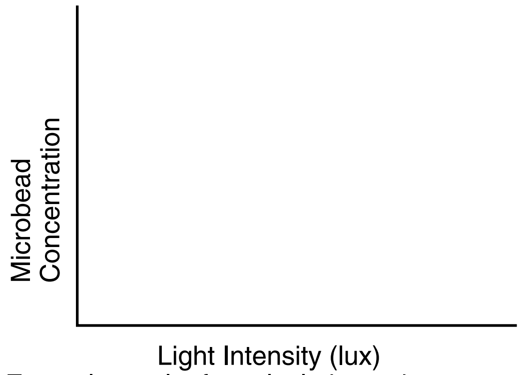
14. When approximately 100 ml of solution has collected in the beaker, remove the beaker from under the column and place in position to measure light intensity. Refer to Figure 7.

15. Collect light intensity for 10 continuous seconds. Record the average value in your science notebook.

16. Complete data table below:

	Clean Water	Known Sample	Unknown Sample
Light Intensity (lux)			
Microbead Concentration	0	4.5×10^8 beads/ml	

17. Using the above data table, plot microbead concentration (*y*-axis) versus light intensity (*x*-axis) for the clean water and known sample on a sheet of graph paper. See the following example.



Example *xy* plot for calculating unknown sample concentration.

18. Draw a straight line between the two points on your graph. Label this line the adsorption line.

19. On the *x*-axis, find the light intensity value of your "Unknown Sample Part 1."

20. Draw a vertical line from the *x*-axis to the adsorption line.

21. From the point on the adsorption line, draw a horizontal line to the *y*-axis and determine the microbead concentration.

Nano2Earth Student Activity Sheet

Investigation of Bacterial Transport in Groundwater

Questions:

1. Based on your data, would adding more microbeads to the solution increase or decrease the light intensity value?

2. Why does light intensity decrease when the microbead concentration increases?

3. Calculate the number of microbeads that remain in the column of sand.

4. Why did some of the microbeads remain in the column?

5. If a bacterium is 1–2 micrometers (1 µm = 10^{-6} m) in diameter, how does that mathematically compare to a nanoparticle? (1 nm= 1×10^{-9}m)

6. Smoke particles, viruses, and some bacteria are nanoparticles. If we used nanobeads instead of microbeads in our experiment, would you expect more or less particles to remain in the column? Why?

Nano2Earth Student Activity Sheet

Investigation of Bacterial Transport in Groundwater

Part 2. Influence of Groundwater Chemistry on Bacterial Transport

Purpose

The purpose of this lesson is to examine the influence of groundwater chemistry on bacterial transport.

Background Information

It has already been stated that groundwater chemistry has a strong effect on how particles stick to surfaces. Why does this happen? It happens because most bacteria and mineral surfaces have electrostatic charges, and these charges are a function of pH and ionic strength. pH is a measure of the concentration of hydrogen ions in solution; it determines the acidity of a solution. Ionic strength is a measure of the concentration of charged atoms, or ions, in solution. Salty water has a large amount of Na^+ and Cl^- ions in solution and, therefore, has a high ionic strength. Groundwater pH and ionic strength can change due to the types of rocks present in the ground, precipitation, evaporation, and human use. Groundwater usually has a pH that is slightly acidic (pH < 7) and has a low ionic strength.

Mineral surfaces can be negatively or positively charged, depending on their atomic structure. Sand, which is made of the mineral quartz (SiO_2), is negatively charged in water at pH 7.

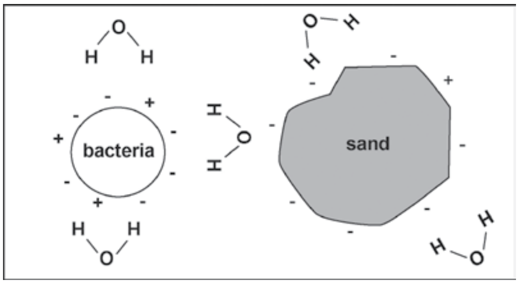


FIGURE 8: At pH 7, most bacteria and sand grains are negatively charged.

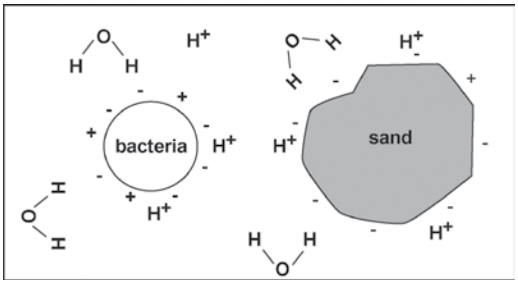


FIGURE 9: At low pH, the number of H^+ ions in solution increases and surface charges change.

The electrostatic charge of bacteria is also typically negative in water at pH 7 (Figure 8). As pH decreases (becomes more acidic) the number of H^+ ions in solution increases (Figure 9). The free H^+ ions prefer to be associated with the negative charges on bacterial and mineral surfaces. Effectively, when solution pH is lowered, the surfaces of minerals and bacteria become more positively charged. What do you think would happen to surface charge when pH is raised (becomes more basic) and fewer H^+ ions are available in solution?

Ionic strength also has an effect on surface charge. Pristine groundwater has low ionic strength. At low ionic strength, the surface charges on minerals and bacteria are loosely arranged around the surface (Figure 10) in a region called the double layer. At high ionic strength, this double layer shrinks and mineral and bacterial surface charges are more tightly arranged around the surfaces (Figure 11). When the double layer shrinks, repulsive forces become weaker and attractive forces become stronger. The charges between objects are "screened," but forces attractive under all the conditions we are studying, called van der Waals forces, are not significantly affected. As a result, bacterial and mineral surfaces become "stickier."

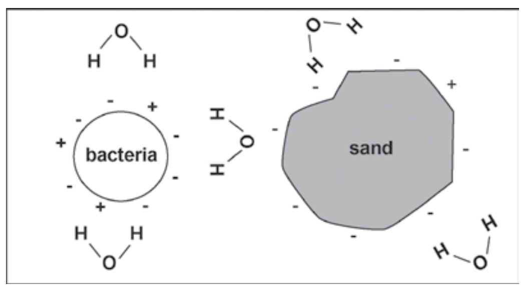


FIGURE 10: At low ionic strength, surface charges are loosely arranged around the mineral and bacterial surface. The double layer is shown in light grey.

Groundwater pH and ionic strength can vary significantly. Most groundwater is slightly acidic and has low ionic strength. Flooding, irrigation for farming, and drought can cause pH and ionic strength to change a lot. Human activities such as construction, fertilizing, and salting roads in the winter can cause drastic changes in groundwater chemistry. Imagine the effects of large-scale processes like mining!

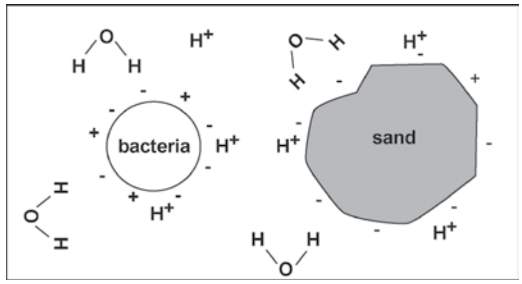


FIGURE 11: At higher ionic strength, surface charges are more tightly associated with the mineral and bacterial surfaces.

This experiment is a continuation of the column experiment that investigated the number of microbeads that passed through a column of sand. Microbeads will be used again to model the behavior of real bacteria. The microbeads and sand grains used in the study have a net negative surface charge. In this experiment, you will change solution chemistry and measure the effect the change has on microbead transport. You will relate your findings to more real-life situations and predict how bacterial transport is affected by human activities.

Materials and Resources
(required for each group's lab setup)

- Plastic column (16 or 20 oz. bottle with bottom cut off and cap with holes)
- Column fill (very course sand approximately 3–4 mm diameter)
- 100 ml clean water
- 3 glass beakers (250 ml)
- Ring stand and clamp
- Flashlight (a tightly focused penlight flashlight works best)
- CBL light-sensor probe
- Masking tape
- Microbeads (2 µm fluorescent carboxylated beads; 0.1 ml per lab group)
- Graph paper
- 10 ml of 0.1M HCl
- pH paper
- Container to collect water from column

Procedure:

1. Fill the plastic column with very coarse sand and mount on the ring stand just as you did in Part 1 (see Figure 6, p. 94).

2. Fill a 250 ml beaker with 100 ml of clean water.

3. Set up a CBL light-sensor probe, flashlight, and beaker with water as in Part 1 of this lab (see Figure 7, p. 94). Remember to use tape to secure the position of the probe and flashlight. Place a sheet of paper underneath the apparatus and outline the position of the glass beaker with a pen.

4. Turn on the flashlight and align the beam from the flashlight into the light sensor probe.

5. Be sure the probe is connected to a data collector (i.e., computer or TI graphing calculator).

6. Measure the intensity of light as it passes from the flashlight through the beaker of water and into the light sensor probe. Collect light intensity (lux) for 10 continuous seconds. Record the average value. Leave the flashlight on and do not adjust the intensity coming from the flashlight.

7. Fill the column with the 100 ml of CLEAN water to saturate the sand. Collect any water that drains from the column in a large container and dispose in sink. Do not let the column drain completely. When the column starts to drip more slowly, put tape over the bottom (on the cap) to keep the column saturated.

8. Empty any remaining water from the beaker into the sink. Dry the beaker.

9. Label the clean, dry beaker "Unknown Sample Part 2" and place under column.

10. Retrieve 90 ml of microbead solution from the teacher in a 250 ml glass beaker. Label the beaker "Known Sample."

11. Add 10 ml of weak HCl acid to the 90 ml microbead solution.

12. The known sample concentration is 4.5×10^8 beads/ml × 90ml/100ml = 4.05×10^8 beads/ml.

13. Measure the intensity of light as it passes from the flashlight through the beaker containing the known sample and into the light sensor probe. Collect light intensity for 10 continuous seconds. Record the average value.

14. Pour the known sample of fluorescent microbead solution into the column slowly.

15. Effluent will be collected in beaker labeled "Unknown Sample Part 2."

Nano2Earth Student Activity Sheet

Investigation of Bacterial Transport in Groundwater

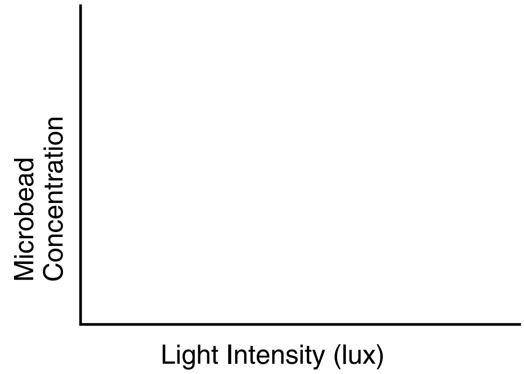
16. When approximately 100 ml of solution has collected in the beaker, remove the beaker from under the column and place it in position to measure light intensity.

17. Collect light intensity for 10 continuous seconds. Record the average value in your science notebook.

18. Complete the data table below:

	Clean Water	Known Sample	Unknown Sample
Light Intensity (lux)			
Microbead Concentration	0	4.5×10^8 beads/ml	

19. Using the data table above, plot microbead concentration (y-axis) versus light intensity (x-axis) for the clean water and known sample on a sheet of graph paper. See the following example.



Example *xy* plot for calculating unknown sample concentration.

20. Draw a straight line between the two points on your graph. Label this line the adsorption line.

21. On the *x*-axis, find the light intensity value of your "Unknown Sample Part 2."

22. Draw a vertical line from the *x*-axis to the adsorption line.

23. From the point on the adsorption line, draw a horizontal line to the *y*-axis and determine the microbead concentration.

Questions:

1. Does adding 10 ml of HCl to the microbead solution raise or lower the pH?

2. What happens to the surface charges on the sand and microbeads when pH is lowered? What happens to the surface charges when the pH is raised?

3. If you add a teaspoon of salt to the microbead solution, would you expect the surfaces to become more or less "sticky"?

4. Calculate the number of microbeads that remained in the column of sand.

5. Do you think live bacteria would behave differently than the microbeads? Why? *Hint: Are ALL bacteria the same size and shape?

6. Do you think sand accurately represents the rocks around your house and school? Explain.

7. List two differences between the sand column and the real rocks around your region.

8. When storms occur and flooding is evident, there are often concerns about drinking water purity. Why?

Lesson 5

Nanoforces in Nature:

Using Atomic Force Microscopy to Explore Microbe-Mineral Interactions

Purpose

If you did the Winogradsky column exercises (Lesson 3), you learned that it has been proposed that bacteria are using iron minerals for respiration. The disappearance of the iron mineral is related to chemical or microbial reactions. It is possible that the microbes are using the iron mineral for respiration—they are "breathing" the mineral!

In the bacterial transport experiments in lesson 4, you saw that not all of the particles introduced to the column were transported through. Nanoscale interactions between the bacteria and the minerals in the sand were causing some of the bacteria to stick.

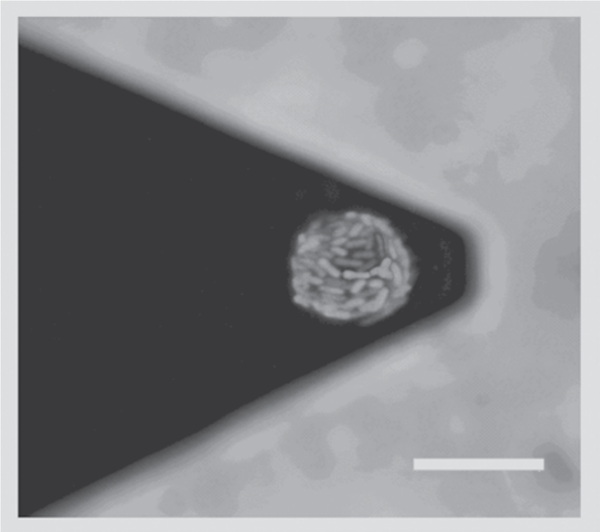


PHOTO COURTESY OF STEVEN LOWER

FIGURE 1: Confocal laser scanning microscopy image of an Atomic Force Microscope (AFM) tip with a bacteria-coated bead attached. The scale bar is 2 μm, or 2,000 nm. The bacteria are fluorescing green due to the presence of a special molecule that was genetically engineered. The green fluorescence is stimulated by the laser in the microscope. Similar bacteria-coated beads were used in force-distance measurements described in this lesson.

For either case, can the students think of any tools they can use from their lab to determine what is happening between the mineral and the bacteria? These interactions are occurring at an extremely small scale: at the nanoscale. We are going to have to use tools from nanotechnology to answer some of the questions. This lesson uses computer simulations of an atomic force microscope to investigate bacteria-mineral forces of interaction on the order of nanonewtons over nanoscale distances of interaction. In this lesson, students

- learn about the atomic force microscope (AFM), one of the most important tools used in nanoscale science and technology;
- see how the AFM is a versatile tool that can be used to study many things, including both organic and inorganic systems;
- discover how the AFM can be used to measure the forces of interaction among individual molecules, bacteria, and minerals;
- construct a model AFM;
- use the model AFM to generate data relating magnetic force to distance;
- plot actual research data collected from the AFM to generate a force curve; and
- relate changes in AFM force curves to changing conditions in the column experiments.

Possible extensions for this lesson include

- fitting the data with a mathematical model and discussing the differences between a model and empirical data, and
- integrating the force curves to look at the energies of interaction of the bacteria and minerals under different conditions.

Background Information on Atomic Force Microscopy

The atomic force microscope is one of a family of instruments known as scanning probe microscopes. The first scanning probe technique, called scanning tunneling microscopy (STM), was invented in 1982 by two scientists, Gerd Binning and Heinrich Rohrer, at the IBM Research Laboratory in Zurich, Switzerland. In 1986, they received the Nobel Prize in Physics for their invention. The STM works by taking a thin metal wire and treating it so a very sharp point is created. A conductive sample is placed in the instrument, and the metal wire is lowered toward the sample. When the wire and sample are less than 1 nm (<~10 Ångstroms!), a phenomenon known as quantum mechanical tunneling will cause electrons to jump from the sample into the tip, or vice versa. This generates a current. The amount of current varies exponentially as a function of the distance between the sample and tip; very tiny changes in the distance cause large changes in the current. By scanning the tip across the surface and monitoring the current, an image of the surface can be obtained. Under many conditions, individual atoms on the surface can be imaged!

Figure 2 shows an STM image of the mineral pyrite (FeS_2), an important mineral in environmental processes such as acid mine drainage. The bumps on the surface are individual iron atoms, and the "oxidized patches" are areas where the mineral has reacted in a similar way to how it would react in the environment, generating acid. This type of research helps us understand the fundamental processes behind environmental

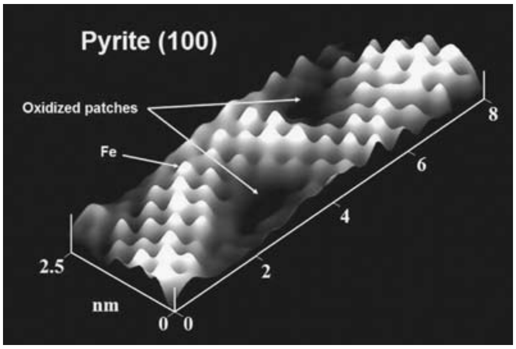


FIGURE 2: A scanning tunneling microscope (STM) image of the mineral pyrite.

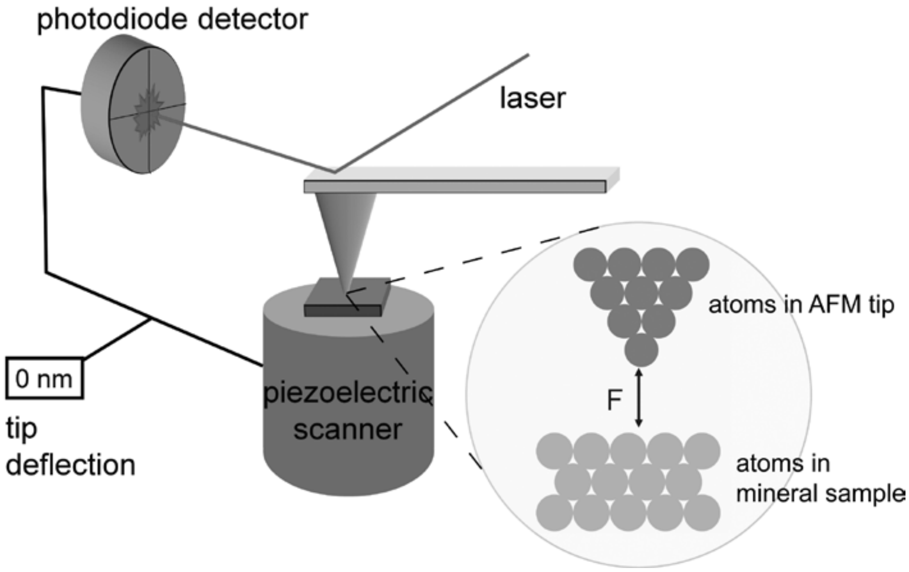


FIGURE 3: How an AFM works (not to scale): A sharp tip attached to a flexible cantilever interrogates a sample material. In our case, the samples will be the same minerals that are found in soils and rocks. As the tip and sample approach to a distance of a few to tens of nanometers apart, electrostatic, van der Waals, and other forces act to deflect the tip upward away from the sample (repulsive interaction) or downward toward the sample (attractive interaction). Motion of the cantilever is recorded by the movement of a laser beam bouncing off the back of the cantilever and into a position-sensitive detector. Cantilever displacement can then be converted to tip-sample interaction force if the instrument is calibrated and the cantilever spring constant is determined.

problems. For example, how does a pile of mine waste generate acid that wipes out plant life and makes nearby water unpotable?

There are many limitations to the STM; for example, it can be used only with samples that conduct electricity, and it is difficult to use with samples immersed in a solution (although it is somewhat possible). Binning and Rohrer later invented the atomic force microscope (AFM) to provide an alternative means to working with nonconductive samples and samples in water or other solutions. Instead of bringing a sharpened wire and a sample together, a spring system is used in the AFM. A sharpened tip (typically made of silicon or silicon nitride) is attached to the bottom of a flexible cantilever. The tip is placed above the sample. A laser is bounced off the cantilever (above where the tip rests) and into a detector. As the two are brought together, forces exerted between the tip and sample cause the cantilever to bend upward (repulsive force) or downward (attractive force). The detector translates this motion of the laser into an electrical signal that is recorded on a computer.

With the AFM, images of a surface can be collected similar to the STM. However, the AFM can also be used to measure extremely small forces between small objects. Instead of scanning the cantilever/tip across the surface, it can be held in one place. Cycles of bringing the sample and tip together followed by pulling them apart in one location generate information known as *force curves*. Under some conditions, AFMs are capable of recording molecular or possibly atomic scale images, and forces can be determined at the nano- and pico (10^{-12})-Newton level—approximately the gravitational force between two average people standing about 20 m apart.

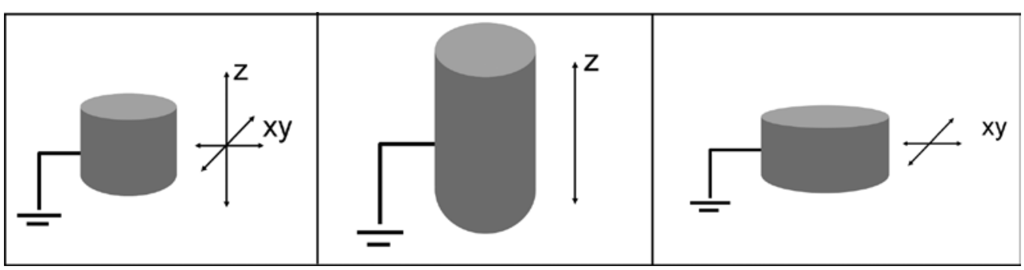


FIGURE 4: Nanoscale to sub-nanoscale control of the tip and sample distance is made possible by the piezoelectric scanner. A piezoelectric material changes its shape in response to an applied voltage. In an AFM scanner, piezoelectric crystals are embedded to allow motion in the horizontal (xy) or vertical (z) directions.

Originally, a research-grade AFM system cost over $100,000. They are continually becoming cheaper as more companies produce them and the demand increases.

For Further Information

- Cail, T. L., and M. F. Hochella, Jr. 2005. The effects of solution chemistry on the sticking efficiencies of viable *Enterococcus faecalis*: An atomic force microscopy and modeling study. *Geochimica et Cosmochimica Acta* 69: 2959–2969.
- Cail, T. L., and M. F. Hochella Jr. 2005. Experimentally derived sticking efficiencies of microparticles using atomic force microscopy. *Environmental Science & Technology* 39: 1011–1017.
- Kendall, T. A., and S. K. Lower. 2004. Forces between minerals and biological surfaces in aqueous solution. *Advances in Agronomy* 82: 1–54.
- Lower, S. K., T. J. Beveridge, and M. F. Hochella, Jr. 2001. Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and αFeOOH. *Science* 292: 1360–1363.
- Lower, S. K., C. J. Tadanier, and M. F. Hochella, Jr. 2001. Dynamics of the mineral-microbe interface: Use of biological force microscopy in biogeochemistry and geomicrobiology. *Geomicrobiology Journal* 18: 63–76.
- Maurice, P. A., and S. K. Lower. 2008. Use of atomic force microscopy to study soil particle properties and interactions. In *Methods of soil analysis, part 5: Mineralogical methods*, ed. A. L. Ulery and L. R. Drees, 299–334. Madison, WI: Soil Science Society of America.

National Science Education Standards (NSES)

Nano2Earth Lessons	NSES Standards Addressed
Nanoforces in Nature **Engage:** Introduction to the Atomic Force Microscope (AFM) **Explore:** What Happens When We Bring Bacteria and Minerals Together? **Explain:** Force Curve Computer Simulations **Elaborate:** Building a Model AFM **Evaluate:** Real-World Scenarios	Unifying Concepts • Systems • Evidence • Models • Explanations • Changes • Measurement • Form and Function Science as Inquiry • Identify Questions and Concepts That Guide Explanations • Design and Construct Scientific Investigations • Using Technology and Mathematics to Improve Communications • Formulate and Revise Scientific Explanations and Models Using Logic and Evidence • Recognize and Analyze Alternative Explanations and Models • Understanding About Scientific Inquiry Physical Science • Structures and Properties of Matter • Motions and Forces • Earth and Space Science • Geochemical Cycles • Science and Technology • Understanding About Science and Technology • Science in Personal and Social Perspectives Earth and Space Science • Natural Resources • Environmental Quality • Natural and Human-Induced Hazards • Science and Technology in Local, National, and Global Challenges History and Nature of Science • Science as a Human Endeavor • Nature of Scientific Knowledge • Historical Perspectives

Materials and Resources

- Computer simulation (Flash-based; will work on Mac or PC)
- "Using the AFM to Study Interactions Between Bacterial and Minerals: A Student's Guide" activity sheet (p. 122)
- "What Happens When We Bring the Bacteria and Minerals Together?" student activity sheet (p. 126)
- "An Introduction to Force Curves: Using Computer Simulation" student activity sheet (p. 127)
- "Building a Model AFM" student activity sheet (p. 130)
- "Scenario: Arsenic Poisoning in Bangladesh" (p. 138)
- "Scenario: Bacterial Transport in Groundwater" (p. 145)

Science Safety

The optional model AFM activity, Force Curves 2: Going Further (p. 132), requires the use of a laser pointer. Students should not shine the laser in each other's eyes. The laser pointer should be labeled with "DANGER: laser radiation" for Class 3a lasers or "CAUTION: laser radiation" for Class 2 lasers. Class 3b or Class 4 products should not be used. The wavelength should be between 630 and 680 nm and have a maximum output of less than 5 mW.

Engage: Introduction to the Atomic Force Microscope (AFM)

(Estimated time: 40 minutes)

1. Begin the lesson by asking students if they can think of any tools they can use to determine what is happening between the minerals and bacteria in the bacterial transport or Winogradsky column labs. Make a list of students' ideas on the board or overhead. Explain to the students that these interactions occur at an extremely small scale—the nanoscale; therefore, they are going to have to use tools from nanotechnology to answer some of the questions. (5 minutes)

2. To learn more about nanoscience, read the background information contained in this lesson plan. This information may be shared with students to read, along with the student activity sheet Using the AFM to Study Interactions Between Bacteria and Minerals: A Student's Guide. (20 minutes)
3. After reading the background information, arrange the students in groups to discuss the readings. The students should record their understandings of the two scenarios and create a list of questions they have about the AFM. (15 minutes)

Explore: What Happens When We Bring Bacteria and Minerals Together?

(Estimated time: 40 minutes)

- Pass out the student activity sheet "What Happens When We Bring Bacteria and Minerals Together?" In their groups, students examine the diagrams and discuss possible answers to the questions. Stress that the goal is to think about what would happen to the cantilever in response to attractive and repulsive forces, irrespective of what *type* of force it is (e.g., electrostatic, magnetic) (10 minutes)

  In the second diagram, the cantilever bends downward in response to a repulsive force. It could be electrostatic, but it is not necessary to define what the type of force is. The important concept is that the cantilever bends upward and away from the mineral in response to a repulsive interaction. The third diagram shows the cantilever bending downward toward the mineral in response to an attractive force.

- Students should conduct the computer simulation An Introduction to Force Curves using the accompanying student activity sheet as a guide (30 minutes). Preload the NanoSim executable Flash program. No installation is required; just copy the Flash executable file to whichever computer you wish to run the simulation. As long as the computer has Flash Player (freely available) loaded onto the computer, the simulation will run independently.

Students should answer and discuss questions 4–8 on pages 128–129 in An Introduction to Force Curves. (Teacher set with answers to those questions 4–8 is below.)

4. What are the initial force and distance?

   The initial force is zero and the distance is 140 nm. At 140 nm distance, the AFM does not measure any interaction between the bacteria and mineral.

5. At which distance does the interaction force become nonzero and is the initial force attractive or repulsive?

   At approximately 40 nm there is an initial repulsive force.

6. At some point, the bacteria will begin to snap down to touch the mineral. This is called the "jump to contact." At which distance does this occur? Is this an attractive or repulsive interaction?

   At approximately 15–20 nm separation distance, the force starts to become increasingly attractive along a linear trend. This is the signature of the jump to contact.

7. The mineral reaches its maximum height and the approach curve is completed. Now the retraction curve begins. Click the button marked "Begin retraction" and observe what happens. Do the bacteria pull away from the mineral at the same distance as the "jump to contact" on the approach curve (question 6)? What is happening?

   No, the bacteria remain stuck to the mineral for some distance as the mineral retracts. A minimum force, called the adhesion force, must be overcome to separate them.

8. The maximum force between the bacteria and mineral, measured during the retraction curve, is known as the adhesion force. What is the adhesion force between the bacteria and mineral in this simulation? Is it attractive or repulsive?

   The adhesion force is approximately 0.2 nN, and it is always attractive. Although the force on the graph is -0.2 nN, the absolute value is used when discussing the adhesion force.

Explain: Force Curve Computer Simulations

(Estimated time: 40 minutes)

1. Students explore how force curves can be collected with various bacteria, minerals, and solutions using the computer simulation Force Curves 2: Going Further and accompanying activity sheet (40 minutes). The Force Curves 2 data tables and graphs (pp. 134–137) are given only as reference materials; do not allow students to see these before they attempt the simulations.

Students should answer and discuss the questions found on the "Force Curves 2: Going Further" student activity sheet.

Bacteria	Mineral	Solution pH	Initial interaction (attractive or repulsive)	Jump to contact distance (nm)	Adhesion force (nN)
A	quartz	7	repulsive	18	0.20
B	quartz	7	repulsive	25	0.32
A	quartz	4	repulsive	15	0.35
B	quartz	4	attractive	50	0.54
A	iron oxide	7	attractive	40	0.39
B	iron oxide	7	attractive	30	0.43
A	iron oxide	4	attractive	15	0.31
B	iron oxide	4	repulsive	20	0.58

2. Often, the type of initial interaction can be related to the electrostatic forces between the bacteria and mineral. Acidity, or pH, is a measurement of how many H^+ ions are in solution. A lower pH is equivalent to more H^+ ions, or greater acidity. That is why minerals and bacteria become positively charged at lower pH: Some of the extra H^+ ions stick to the surfaces. Given that quartz is negatively charged for solution

pH >2, what are the charges (positive or negative) of Bacteria A, Bacteria B, and the iron oxide mineral at pH 4 and 7? Use information from the table on page 114 to help you.

At pH 4, Bacteria A are negatively charged. Bacteria B and the iron oxide are positively charged. At pH 7, Bacteria A and Bacteria B are negatively charged. Iron oxide is positively charged.

3. If Bacteria A were transported through sediment containing quartz and iron oxide, to which mineral is it more likely to adhere? Is it more likely to adhere at pH 4 or 7?

   Bacteria A are more likely to adhere to iron oxide than quartz due to the sign of the initial interaction. The bacteria are more likely to adhere at pH 4; the jump to contact distance is much greater.

4. Bacteria B are disease-causing (pathogenic) bacteria that are adhered to sediments where your drinking water is supplied. You can drill a well and inject a solution to flush the bacteria. Should you use pH 4 or pH 7 water?

   This question addresses the adhesion force. Larger adhesion forces imply the bacteria are more strongly adhered. Thus, pH 7 water should be used to flush out the bacteria because the adhesion force is lower in magnitude (a less negative number) with both quartz and iron oxide than with either of them at pH 4.

Elaborate: Building a Model AFM
(Estimated time: 20–70 minutes)

To extend their understandings of the atomic force microscope (AFM), students may construct a model of the AFM using the "Building a Model AFM" student activity sheet. This may be done in collaboration with your Industrial Arts Department. (20 minutes)

After the AFM is built, students can experiment with the model and follow the instructions on the "Building a Model AFM" activity sheet to generate a force curve. Students should also compare the model AFM with the actual AFM as described in previous activity sheets and figures of the AFM cantilever. (50 minutes)

Evaluate: Real-World Scenarios

(Estimated time: 20–70 minutes)

The scenarios are designed to correspond to concepts and problems encountered in Lessons 3 and 4. If your class completed the Winogradsky column labs (Lesson 3), the appropriate scenario is "Arsenic Poisoning in Bangladesh," (p. 138). If you completed the bacterial transport labs (Lesson 4), have your class try the "Bacterial Transport in Groundwater" scenario, (p. 145).

These scenarios use data that was actually collected in the NanoGeoscience and Technology Laboratory in the Department of Geosciences at Virginia Tech, although their application to these problems is simulated. The computer simulations are designed so that the student will record all of the data from an approach and retraction curve in a data table on the computer screen, plot the data points on the graph within the simulation, and compare their results with the correct answers, as given by the simulation.

Answer Key to "Arsenic Poisoning in Bangladesh" Scenario

("Comparing Force Curves" student questions on pp. 142–143.)

1. Look at the retraction curves under aerobic conditions. Remember, the negative numbers represent an attractive force. Which mineral do the bacteria stick to more, the iron oxide or the aluminum oxide? Do the bacteria have a purpose or advantage associated with this stronger attraction?

   The bacteria adhere more strongly to the aluminum oxide under aerobic conditions. An advantage is not clear; consider any reasonable answers. This is an example of where science has not yet provided a definitive reason.

2. Look at the retraction curves under anaerobic conditions. Which mineral do the bacteria stick to more? Use information from the graphs to support your conclusions.

   The bacteria adhere more strongly to the iron oxide mineral under aerobic conditions, as can be determined by the adhesion force values.

3. The curve for aluminum oxide was identical when comparing aerobic and anaerobic bacteria. Is there significance to this effect? What, if any, is the significance?

The bacteria cannot use the aluminum oxide mineral for respiration, so the lack of oxygen will not trigger an attraction. There is no advantage for the bacteria to be attached to the aluminum oxide under aerobic vs. anaerobic conditions.

FIGURE 5

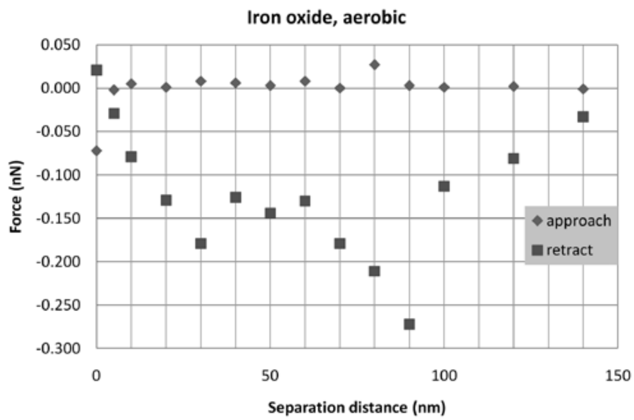


FIGURE 6

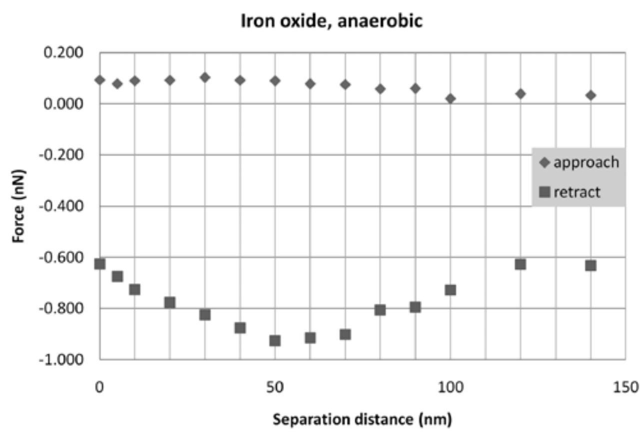
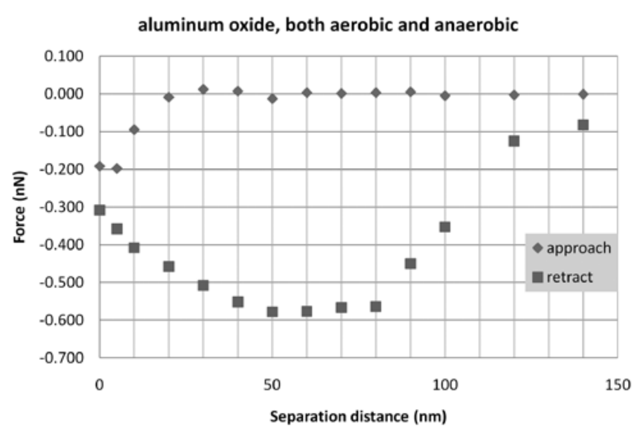


FIGURE 7



4. Under which conditions do the bacteria have the strongest interaction with the iron oxides? Do they have a purpose or advantage with this attraction?

   The bacteria have the strongest interactions with the iron oxide under anaerobic conditions. This is an example of an adaptation to survive; attaching to the iron oxide allows them to respire anaerobically.

5. Which environmental conditions could lead to release of arsenic resulting in higher levels of arsenic contamination in the wells? Are there any ways to avoid these environmental conditions?

   The arsenic is likely released during anaerobic conditions as the iron oxide minerals are reduced. Students might suggest ways to eliminate anaerobic conditions, such as removal of standing water or pumping of oxygen into the groundwater. However, these solutions are not practical due to the widespread distribution of arsenic throughout such a large area. The arsenic contamination problem still exists today.

Answer Key to Bacterial Transport Scenario

(Student questions on p. 145)

1. What are the potential sources of the *E. coli*?

   The most likely sources of **E. coli** *are sewer line leakage/overflow or transport from animal fecal matter into the water source areas for the town. E. coli live in the gastrointestinal tracts of the animals (as they do with humans).*

2. Based on this information, can you now be more specific about the source of contamination?

   Improvements in the sewer line seemed to solve the problem, indicating that the sewer line was the source of the problem.

3. Based on the bacterial transport column experiments, what do you hypothesize caused the release of bacteria to groundwater?

   A change in the chemistry of the water after the rainstorms caused bacteria that had previously been adsorbed in the agricultural fields to be transported instead.

***

FIGURE 8

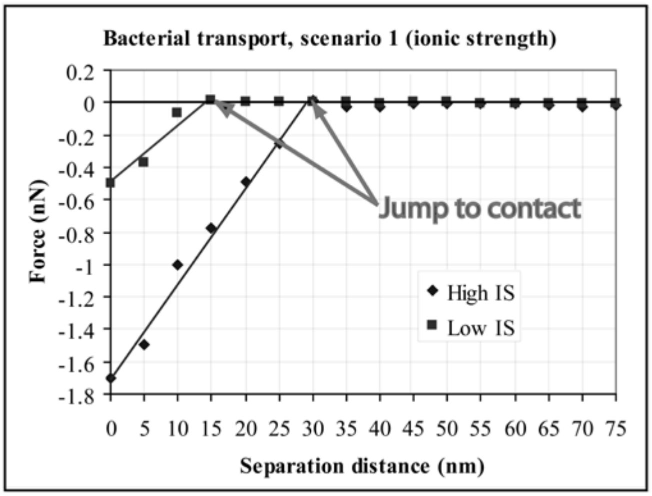


FIGURE 9

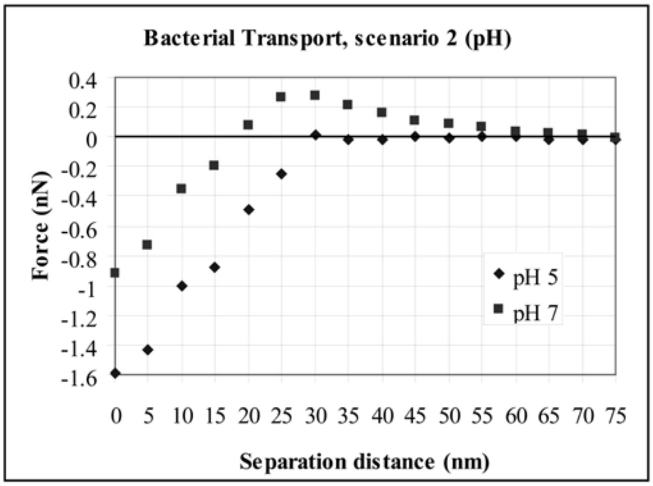
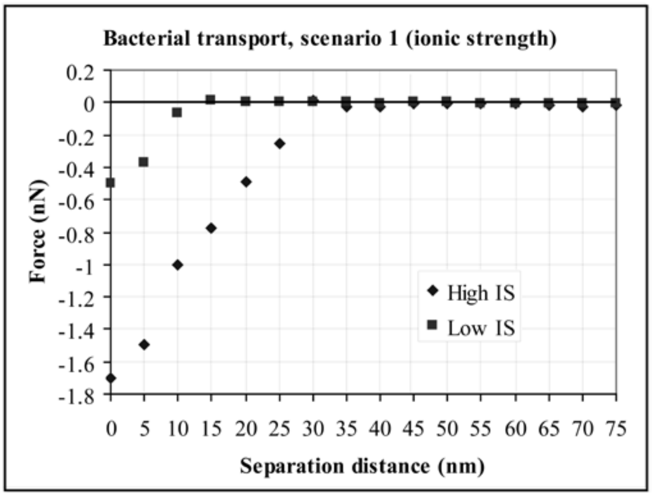


FIGURE 10



(Student questions on p. 147)

1. At what approximate distance does the approach curve move away from zero? Is the initial force repulsive or attractive?

   The approach curves are significantly different from zero for the high and low ionic strengths cases at approximately 25 nm and 10 nm, respectively. In both cases, the initial force is attractive. The convention is that a negative force indicates an attractive interaction.

2. At what approximate distance is the "jump to contact" shown in the approach curves?

   The "jump to contact" is used operationally here to describe the point at which the force between the surface and tip/bacteria begins to snap down together. This is observed as a linear trend toward increasingly attractive as the separation distance decreases. Figure XX illustrates how this can be determined for simulation 1. The jump to contact distances for the high and low ionic strength cases are 30 nm and 15 nm, respectively.

3. What does the difference in "jump to contact" distances tell you about the difference in interaction between *E. coli* and the mineral at these two values of ionic strength?

   The **E. coli** *are much more likely to stick to the minerals in the sediment at high ionic strength than low ionic strength. Although there are attractive interactions at both ionic strengths, the attractive interactions occur at much larger separation distances at high ionic strength.*

***

Sample 2—values of pH
(Student questions on pp. 148–149)

1. For each value of pH, at what approximate distance does the approach curve move away from zero? Is the initial force repulsive or attractive?

   At pH 7, the curve indicates a repulsive interaction at approximately 55 nm separation distance. At pH 5, the first interaction is attractive, which occurs at approximately 25 nm separation distance.

2. Is the repulsive force greater at pH 5 or pH 7?

   The repusive force is much greater at pH 7.

3. Based on your answers to the questions above, would you predict that the bacteria adhere (stick) to the surfaces better at pH 5 or pH 7? Does this agree with what you observed in the bacterial transport column experiments?

   At pH 7, the curve indicates a repulsive interaction at approximately 55 nm separation distance. At pH 5, the first interaction is attractive, which occurs at approximately 25 nm separation distance. This is likely to have been the same result as the column experiments if relatively pure quartz or silica sand was used.

4. Based on the sticking coefficient data, do bacteria stick to surfaces better at lower or at higher pH? Why? Does this answer agree with the AFM data?

   A higher sticking efficiency indicates the bacteria are more sticky, so they appear to stick to the sediment better at lower pH. This must have to do with the type of minerals in the sediments. Based on the previous exercises, this is consistent with the sediment having a significant amount of quartz. Quartz and **E. coli** *have strong negative surface charges at pH 7, but are less strongly negative at pH 5 where there are more protons in the solution to screen the charge. This does agree with the AFM data.*

5. Do bacteria stick to surfaces better at lower or higher ionic strength? Why? Does your answer agree with the AFM data?

   The bacteria stick to surfaces better at higher ionic strength. There are more ions in solution to screen the opposing charges. This is in agreement with the AFM data.

6. Which affects the sticking efficiency of bacteria more: ionic strength or pH?

   The solution pH seems to have a stronger effect than ionic strength.

7. If you were the town engineer, could you prevent the contamination problem from reoccurring? If so, what would you do?

   There may be many possible ways to improve the situation. One way may be to try and coat the sediment with iron oxide, which has a positive surface charge at neutral pH, or build structures that prevent water from seeping through animal pens and into the town's water supply areas.

Nano2Earth Student Activity Sheet

Using the AFM to Study Interactions Between Bacteria and Minerals: A Student´s Guide

The atomic force microscope is a very versatile instrument that has been extremely important in the development of nanoscale science and technology. It allows people to manipulate objects at a nanometer scale. One nanometer is extremely small—a typical human hair is approximately 60,000 nm across! Individual atoms are about 0.1 nm across; one water molecule has dimensions of about 0.3 nm. The AFM is capable of measuring nanoNewton forces (10^{-9} N)! How much is a nanoNewton? Imagine that you are sitting at a desk looking at a computer monitor. One nanoNewton is approximately the same amount as the gravitational force between you and a computer monitor sitting in front of you!

The AFM is so effective in part because it uses a very sensitive spring system called a cantilever. The cantilever is similar to a miniaturized plastic ruler. It is small enough and flexible enough that it will bend upward or downward in response to very small forces. At the end of the cantilever is a small sharp tip. A sample is placed on the microscope, underneath the cantilever's tip. As the sample and the cantilever are brought together, the forces of interaction between the sample and tip push the end of the cantilever up or down. The forces are recorded.

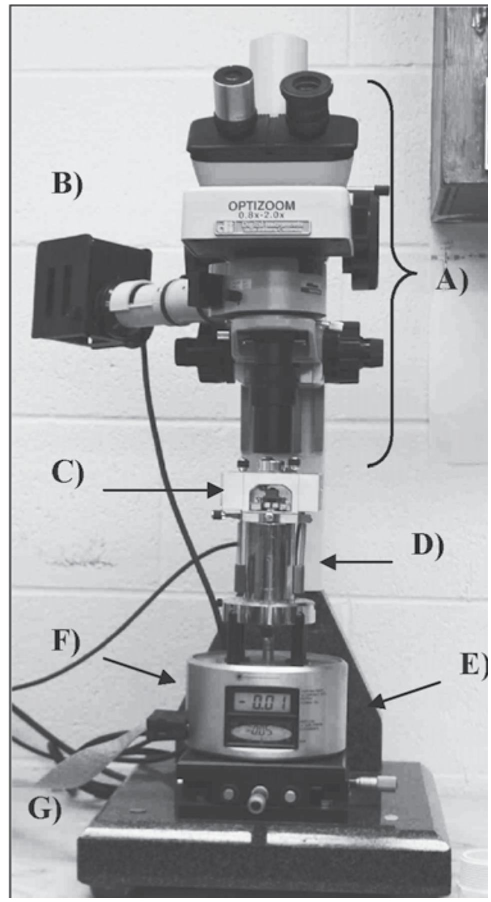


FIGURE 11: Atomic force microscope (AFM).

A picture of a typical AFM is shown in Figure 11. The parts include the following:

(A) Optical microscope. The user needs this to see if the sample and cantilever are in the proper arrangement.
(B) Light source for the optical microscope.
(C) This section is called the "head." This is where the sample and cantilever go. Figure 13 shows enlarged views of this area.

(D) Called the "scanner," this device moves the sample up and down (or sideways). The amount of movement can be controlled with an accuracy of less than one nanometer.

(E) The silver electronics unit has displays that assist the operator.

(F) A cable is attached just below the arrow. This cable connects the AFM to a set of electronics that form an interface between the microscope and a computer.

(G) AFMs usually are operated on a specially designed table to reduce the amount of vibration felt by the microscope.

FIGURE 12: Adjusting the AFM.

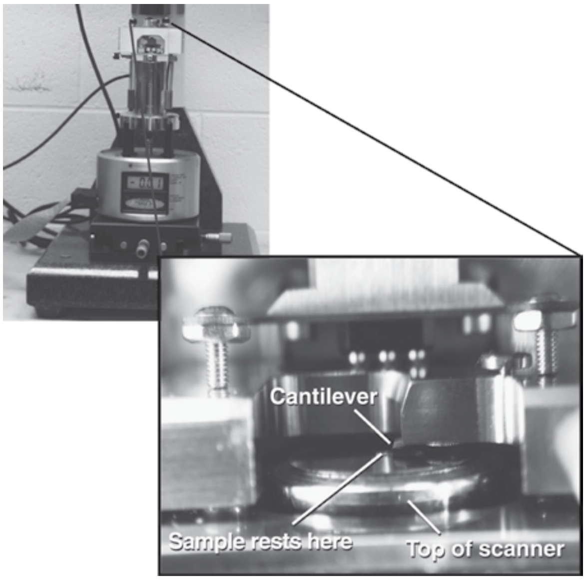


FIGURE 13: The "head" where the sample and cantilever go.

In Figure 13, you can see the AFM from Figure 11, with an enlarged image of where the sample sits. The sample sits on top of the scanner, the device that moves the sample closer and farther away from the tip. On the inset, the cantilever, sample, and the top of the scanner where the sample rests are all labeled.

Normally, a sharp tip that looks like an upside-down pyramid resides on the underside of the cantilever. Often, the tip is replaced with other things. For example, if the goal is to measure the force of interaction between bacteria and a mineral, the bacteria can be attached to the cantilever and lowered down toward the mineral. Sometimes a bead is first attached to the cantilever, and then the bacteria are attached to the bead.

Nano2Earth Student Activity Sheet

Using the Atomic Force Microscope (AFM) to Study Interactions Between Bacteria and Minerals: A Student´s Guide

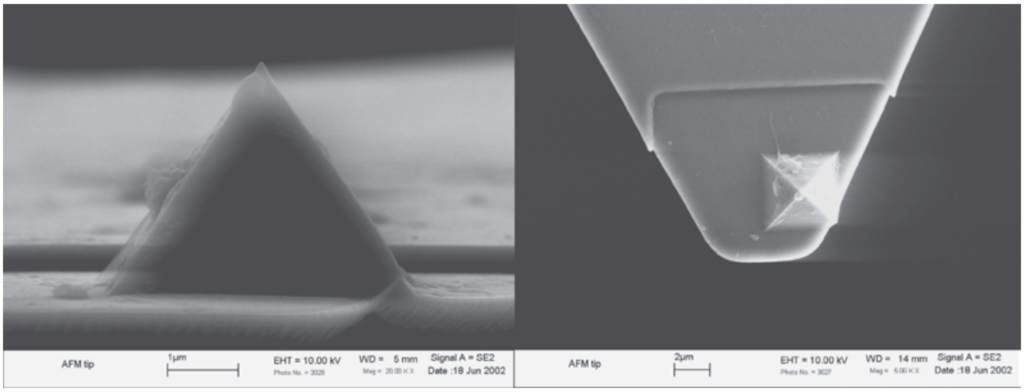


FIGURE 14: Two scanning electron micrographs of AFM tips on the end of triangular-shaped cantilevers are shown above. The tip is shaped like a triangular pyramid. Notice the scale bars are one micrometer (left) and two micrometers (right). A micrometer (µm) is 10^{-6} m, or 1,000 nm. Notice also how the tip is not centered on the cantilever in the image on the right; this is due to an error in the manufacturing process.

The experiment may look like the following: A glass bead coated with bacteria is attached to the bottom of the cantilever. The bacteria-covered bead on the end of the cantilever is put into the AFM and aligned above a mineral sample. An aqueous solution is injected into the AFM to make sure the bacteria stay alive during the experiment. The solution can be changed at any time to run experiments with variations in parameters such as dissolved oxygen, pH, or other solutes (see diagram in Figure 15).

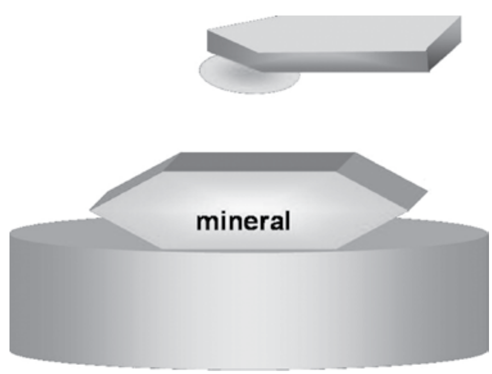


FIGURE 15: Diagram of cantilever with bacteria-coated glass bead aligned over mineral sample.

Figure 16 shows the bacteria-coated bead at the end of an AFM cantilever. The view is of the bottom side; it is upside-down. The bacteria remain attached to the bead because they are linked to it with a special molecule. They are fluorescing brightly here in response to a laser beam. The scale bar is 10 µm.

The tip (or bacteria-coated bead) and sample can be brought together and pulled apart by a computer-controlled device. With the addition of a laser and a detector, it is possible to determine the forces of interaction between the tip (or bacteria-coated bead) and the mineral. The laser is bounced off the top of the cantilever into the detector. Any motion of the cantilever will cause the laser to be displaced in the detector.

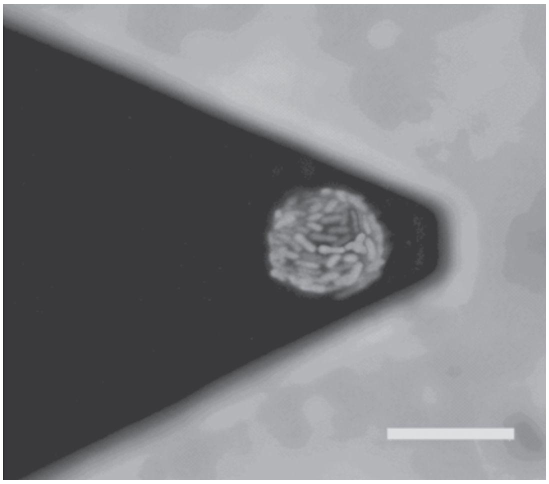
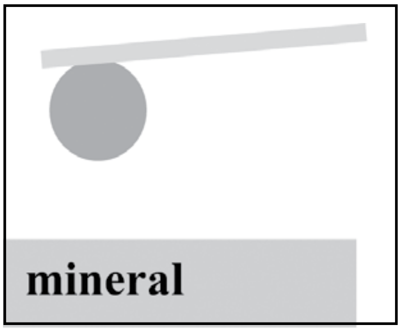


FIGURE 16: Bacteria-coated bead attached to the bottom of the cantilever.

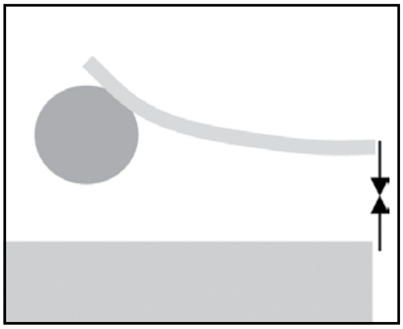
Because the cantilever can be considered a spring, the displacement of the laser beam in the detector can be converted to a force using Hooke's Law, where Force = $-kx$. The spring constant k reflects the rigidity of the cantilever, and x is the displacement of the cantilever. In practice, the cantilever deflection is measured by monitoring the position of the laser spot in the detector. The microscope can be calibrated so the cantilever deflection is precisely determined by the motion of the laser spot.

Nano2Earth Student Activity Sheet

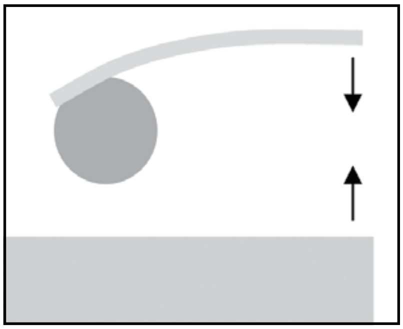
What Happens When We Bring the Bacteria and Minerals Together?



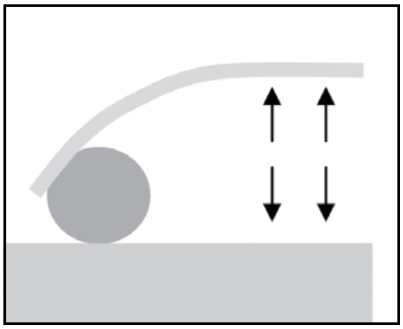
Looking at the cantilever and bacteria-covered bead from the side, you will see something like this:



As the bacteria-covered bead is lowered, the spring may deflect upward as shown in the picture to the left. What is causing it to deflect upward—an attractive or repulsive force?



Now consider some other examples. In this case, what if, as the bead and mineral are brought together, the cantilever bends downward? How would you characterize this interaction?



Eventually, regardless of what happened initially as the bead and mineral were brought together, they will stick. They will remain stuck until the force that holds them together, the *adhesion force*, is overcome.

Nano2Earth Student Activity Sheet

An Introduction to Force Curves: Using Computer Simulation

In the student activity "What Happens When We Bring thte Bacteria and Minerals Together?" you learned about what types of interactions may occur as bacteria (or bead) and mineral are brought together and then pulled apart. Scientists use the atomic force microscope to measure the forces of interaction by measuring what is known as a force curve. If the spring constant of the cantilever is known, one cycle of bringing the bacteria and mineral together and then pulling them apart generates a graph of force versus distance (a force curve). This activity simulates a force curve between Bacteria A and the mineral quartz in a solution with pH 7.

Force curves have two main parts, the approach curve (bringing the bacteria and mineral together) and the retraction curve (pulling them apart). The approach curve tells us how the bacteria and mineral attract and/or repel each other as they get closer. The retraction curve can be used to determine how strongly the bacteria and mineral stick together, a quantity called the adhesion force.

1. Load the simulation "Introduction to Force Curves" by starting NanoSim program (see p. xii of this book for download details). On the first screen, click on the "Introduction to Force Curves" button.

2. You will see a simulation with three panels. The left-hand side is a diagram of the AFM. The force curve, a graph of interaction force versus distance, will be displayed on the right-hand side, along with the table of force versus distance data points that correspond to the AFM measurements. Notice that force is the y-axis and distance is the x-axis. Notice that zero on the x-axis scale represents the bacteria and mineral first touching, while large numbers represent when they are farther apart. The bottom panel navigates between the various simulations, and between different samples within one simulation. Figure 17 (p. 128) illustrates where the various buttons and panels are located within the simulation.

   For this simulation, there is only one sample.
   Click on the "Sample 1" button.

3. In the schematic of the AFM (upper left panel), you see the laser bouncing off the back of the cantilever into the detector. The mineral sample is beneath the tip and cantilever. Click the red "Approach" button just beneath this schematic to start recording a force curve.

Nano2Earth Student Activity Sheet

An Introduction to Force Curves: Using Computer Simulation

4. The data are recorded in the data table and plotted on the force-distance graph automatically for you. What are the initial force and distance?

   Continue to advance the force curve by clicking "Approach" until the AFM and sample are only 1 nm apart. Now the "Approach" button has transformed to a "retract" button. Look at the approach curve more carefully before proceeding with the retraction curve.

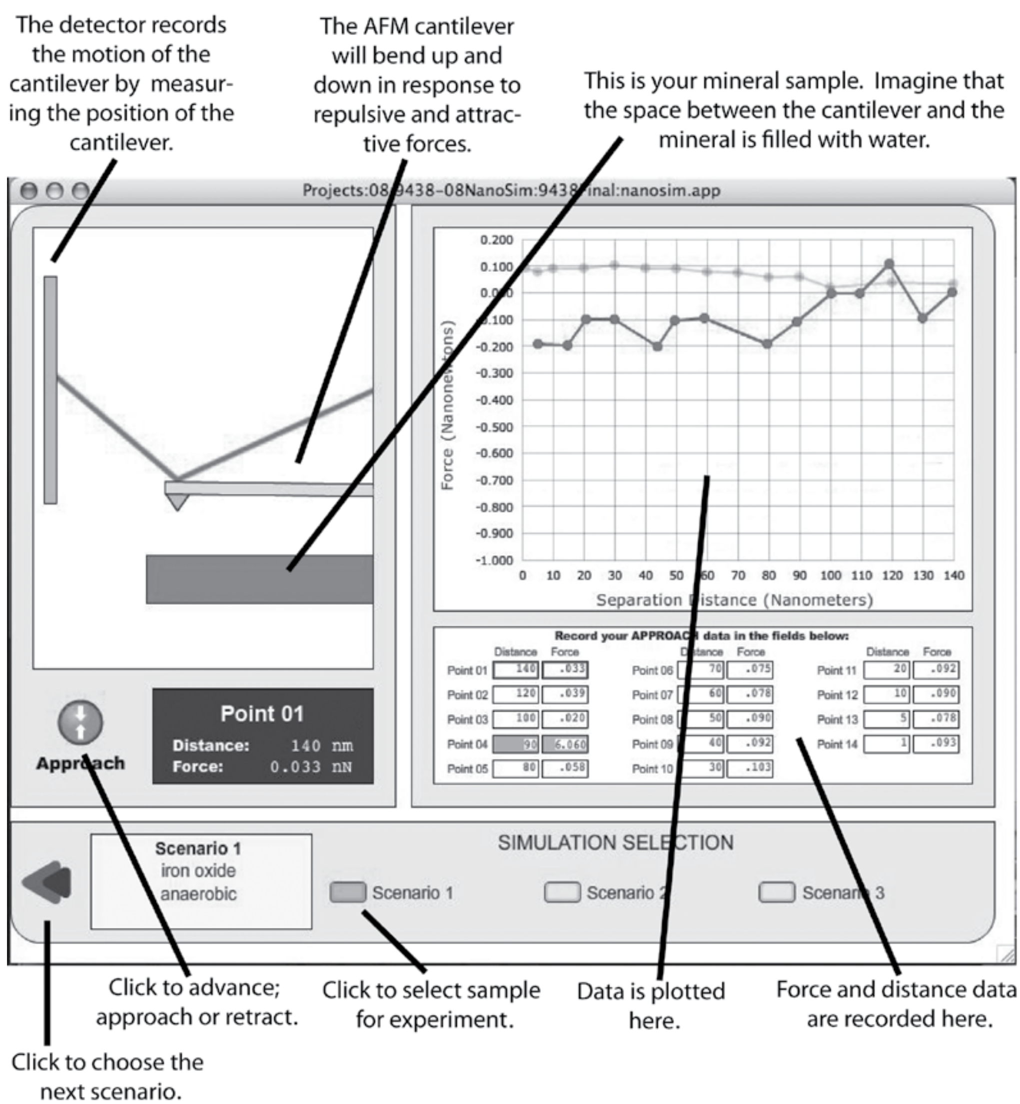


FIGURE 17: NanoSim program sample screen.

5. At which distance does the interaction force become nonzero? Is the initial force at that point attractive or repulsive?

6. At some point, the bacteria will snap down to touch the mineral. This is called the "jump to contact." At which distance does this occur? Is this an attractive or repulsive interaction?

7. The mineral reaches its maximum height and the approach curve is completed. Now the retraction curve begins. Click the button marked "Retract" and observe what happens. Do the bacteria pull away from the mineral at the same distance as the "jump to contact" on the approach curve (question 5)? What is happening?

8. The maximum force between the bacteria and mineral, measured during the retraction curve, is known as the adhesion force. What is the adhesion force between the bacteria and mineral in this simulation? Is it attractive or repulsive?

Nano2Earth Student Activity Sheet
Building a Model AFM

This is a procedure to construct a simple model that demonstrates how an AFM operates. It is important to understand that this is only a model. The magnets in this model are representations. The top magnet represents the AFM tip. Sometimes, a bacteria cell is glued onto this tip. The bottom magnet represents a mineral surface. The forces at work in an AFM are not actually magnetic.

Materials

- ¾ in. plywood, 18 × 18 in.
- 12 in. long 2×4 piece of wood
- 3 in. long 2×4 piece of wood
- Plastic or metal ruler (must bend)
- 2 magnets
- Small mirror
- Laser pointer
- Screws or nails
- Tape

!

Science Safety

Do not shine the laser into anyone's eyes; this can cause permanent damage.

Procedure

Use nails, screws, and/or tape to attach the materials according to Figure 18 (p. 131). The laser pointer must be oriented so that the laser will bounce off the mirror and hit a screen or wall. The point of the laser must be marked on the screen or wall when the model has no interaction between the magnets.

Building a Model AFM

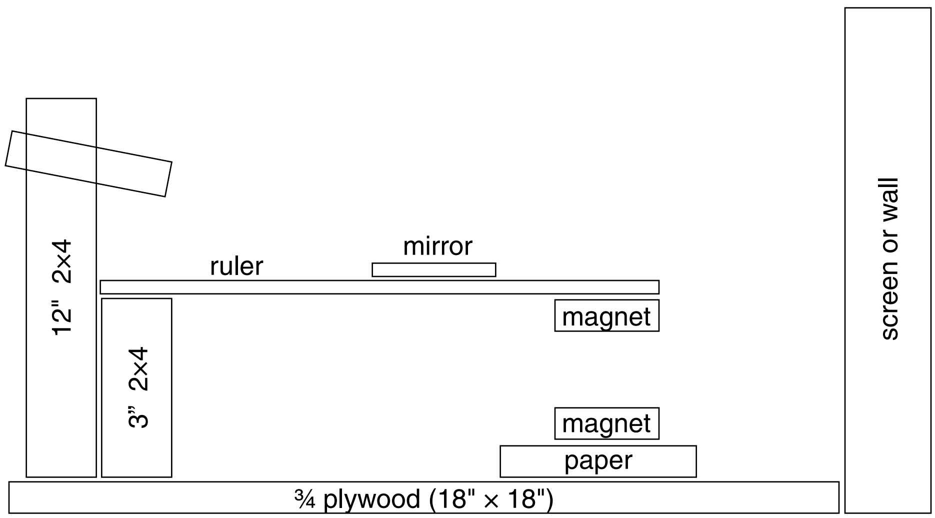


FIGURE 18: Model AFM plans.

Stacking additional paper under the magnet will raise the magnet and decrease the distance between the magnets. As the magnets are moved closer, there will be a force interaction, which bends the ruler. If the force is attractive, the ruler will bend down, and the dot on the wall will move down. If the force is repulsive, the opposite will occur, and the ruler will bend up and the dot will move up. A stronger force will move the dot more. The force is proportional to the change in distance. The x-axis on a force curve is distance. This distance is represented by the distance between the magnets. The y-axis on a force curve is force. This force is represented by the displacement of the laser dot on the wall. Movement upward represents a positive force. Movement downward represents a negative force.

Going Further Challenge* (p. 132)*: Create a force curve that displays magnetic force vs. distance using the model. The spring constant for the cantilever could be determined by hanging various weights on the ruler and monitoring the motion of the laser pointer.

Nano2Earth Student Activity Sheet
Force Curves 2: Going Further

In this computer simulation and activity, you will investigate how changing the type of bacteria, mineral, or solution pH can affect the force curves. Begin the simulation "Force Curves 2" by starting the Nano2Earth simulation program and choosing the "Force Curves 2: Going Further" option. The simulation begins with the force curve you investigated in the "An Introduction to Force Curves" student activity between Bacteria A and the mineral quartz in a solution with pH 7. In this simulation, you will be able to change the type of bacteria, the mineral, and the solution pH.

1. Fill in the data table below by completing force curves for each of the eight samples, corresponding to the various combinations of bacteria on the tip, mineral samples, and solution pH values.

Sample	Bacteria	Mineral	Solution pH	Initial Interaction (Attractive or Repulsive)	Jump to Contact Distance (nm)	Adhesion Force (nN)
1	A	quartz	7			
2	B	quartz	7			
3	A	quartz	4			
4	B	quartz	4			
5	A	iron oxide	7			
6	B	iron oxide	7			
7	A	iron oxide	4			
8	B	iron oxide	4			

Force Curves 2: Going Further

2. Often, the type of initial interaction can be related to the electrostatic forces between the bacteria and mineral. Given that quartz is negatively charged for solution pH >2, what are the charges (positive or negative) of Bacteria A, Bacteria B, and the iron oxide mineral at pH 4 and 7? Use information from the table on page 132 to help you.

3. If Bacteria A were transported through sediment containing quartz and iron oxide, to which mineral is it more likely to adhere? Is it more likely to adhere at pH 4 or pH 7?

4. Bacteria B are disease-causing (pathogenic) bacteria that are adhered to sediments where your drinking water is supplied. You can drill a well and inject a solution to flush the bacteria out. Should you use pH 4 or pH 7 water?

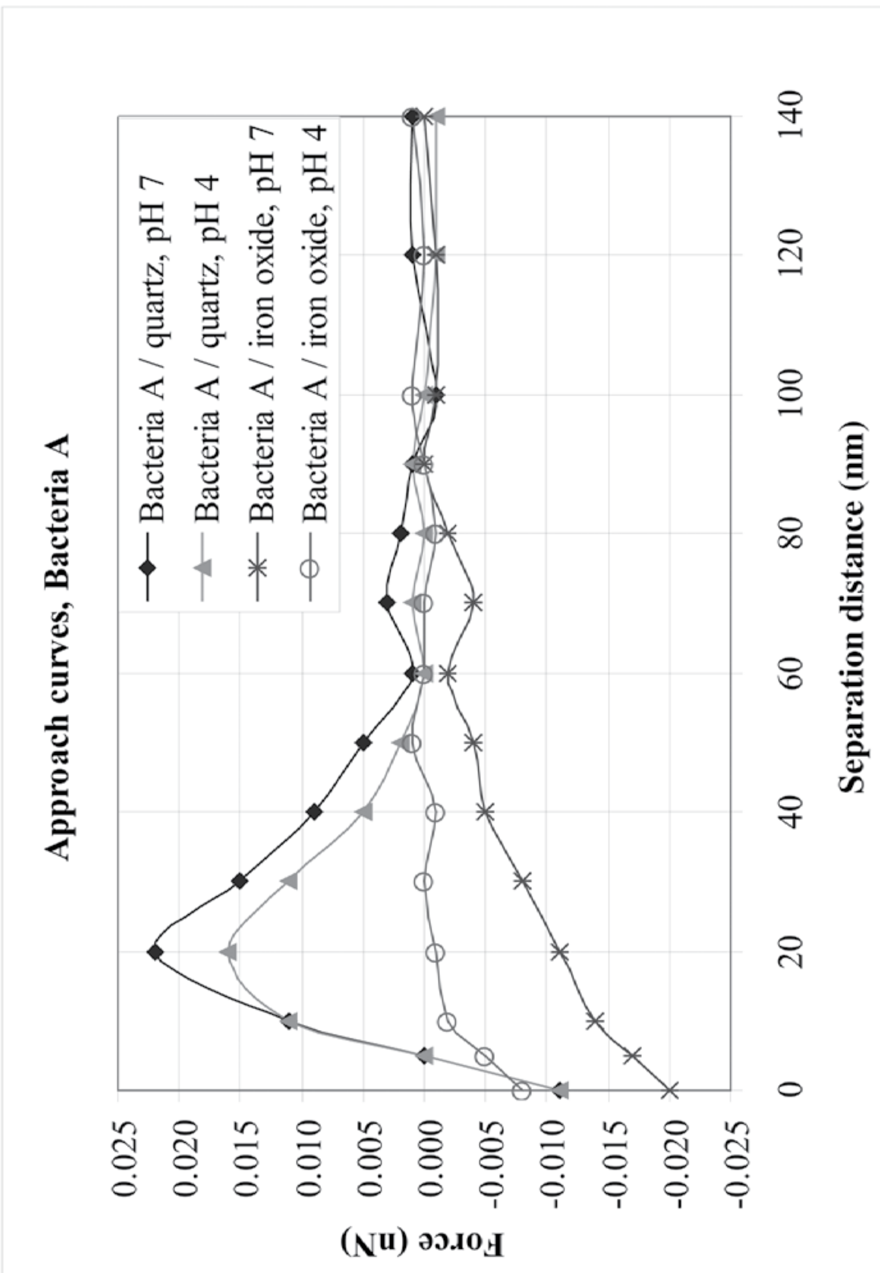
Nano2Earth Student Activity Sheet

Approach Curve Data Table for Force Curves 2: Going Further

Bacteria:	A	B	A	B	A	B	A	B
Mineral:	**Quartz**	**Quartz**	**Quartz**	**Quartz**	**Iron Oxide**	**Iron Oxide**	**Iron Oxide**	**Iron Oxide**
Distance (nm)	**pH 7**	**pH 7**	**pH 4**	**pH 4**	**pH 7**	**pH 7**	**pH 4**	**pH 4**
140	0.001	0.000	-0.001	-0.001	0.000	-0.001	0.001	0.001
120	0.001	-0.001	-0.001	-0.002	-0.001	-0.002	0.000	-0.001
100	-0.001	0.000	0.000	-0.001	-0.001	-0.002	0.001	0.000
90	0.001	-0.002	0.001	0.001	0.000	0.000	0.000	0.002
80	0.002	-0.001	0.000	0.000	-0.002	0.001	-0.001	0.001
70	0.003	0.000	0.001	-0.001	-0.004	-0.002	0.000	0.003
60	0.001	0.001	0.000	-0.003	-0.002	-0.004	0.000	0.001
50	0.005	0.002	0.002	-0.002	-0.004	-0.001	0.001	0.003
40	0.009	0.008	0.005	-0.004	-0.005	-0.004	-0.001	0.005
30	0.015	0.012	0.011	-0.006	-0.008	-0.003	0.000	0.004
20	0.022	0.005	0.016	-0.010	-0.011	-0.006	-0.001	0.006
10	0.011	-0.001	0.011	-0.013	-0.014	-0.009	-0.002	0.000
5	0.000	-0.005	0.000	-0.015	-0.017	-0.012	-0.005	-0.003
1	-0.011	-0.016	-0.011	-0.018	-0.020	-0.015	-0.008	-0.006

Nano2Earth Student Activity Sheet

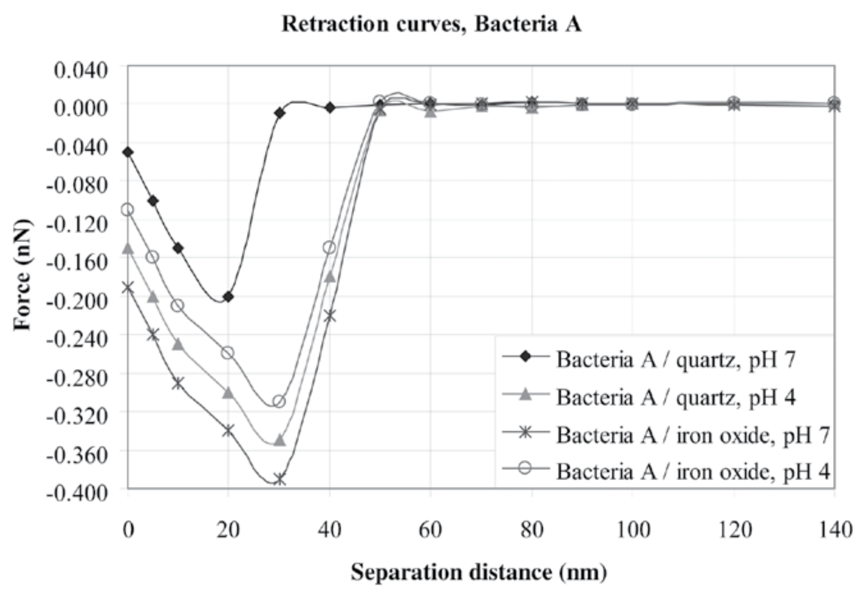
Approach Curve Data Table for Force Curves 2: Going Further



Nano2Earth Student Activity Sheet

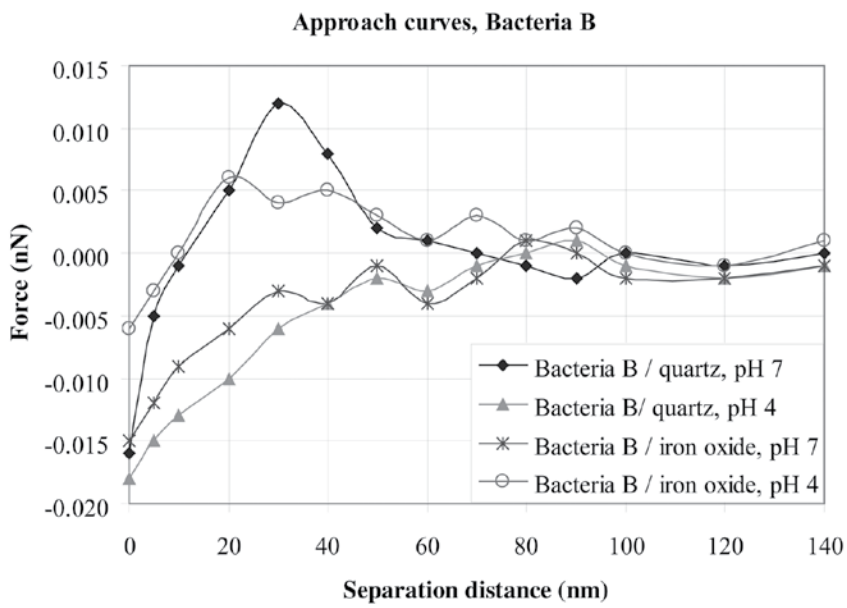
Retraction Curve Data Table for Force Curves 2: Going Further

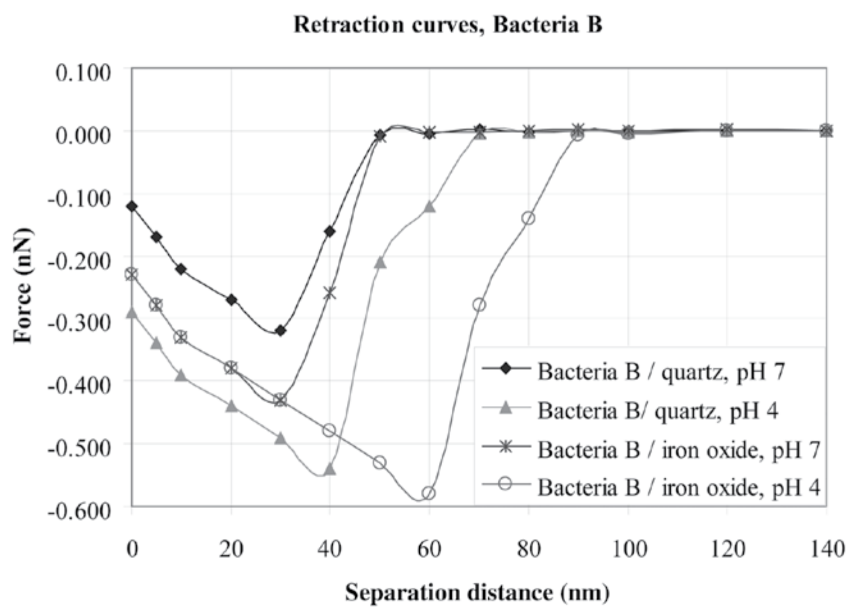
Bacteria:	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Mineral:	**Quartz**	**Quartz**	**Quartz**	**Quartz**	**Iron Oxide**	**Iron Oxide**	**Iron Oxide**	**Iron Oxide**
Distance (nm)	**pH 7**	**pH 7**	**pH 4**	**pH 4**	**pH 7**	**pH 7**	**pH 4**	**pH 4**
140	0.000	-0.001	0.000	-0.001	-0.002	-0.001	0.000	-0.001
120	0.000	0.000	0.002	-0.001	-0.001	0.001	0.000	0.000
100	0.001	-0.001	0.001	-0.003	0.001	0.000	-0.001	-0.005
90	0.000	0.000	-0.001	-0.001	0.000	0.001	-0.001	-0.007
80	0.002	-0.002	-0.003	-0.002	0.002	0.000	-0.002	-0.140
70	-0.001	0.001	-0.002	-0.004	0.001	-0.003	-0.001	-0.280
60	0.000	-0.005	-0.008	-0.120	-0.001	-0.002	0.001	-0.580
50	-0.001	-0.008	-0.006	-0.210	-0.005	-0.009	0.002	-0.530
40	-0.003	-0.160	-0.180	-0.540	-0.220	-0.260	-0.150	-0.480
30	-0.009	-0.320	-0.350	-0.490	-0.390	-0.430	-0.310	-0.430
20	-0.200	-0.270	-0.300	-0.440	-0.340	-0.380	-0.260	-0.380
10	-0.150	-0.220	-0.250	-0.390	-0.290	-0.330	-0.210	-0.330
5	-0.100	-0.170	-0.200	-0.340	-0.240	-0.280	-0.160	-0.280
1	-0.050	-0.120	-0.150	-0.290	-0.190	-0.230	-0.110	-0.230



Nano2Earth Student Activity Sheet

Approach Curve Data Table for Force Curves 2: Going Further





Nano2Earth Student Activity Sheet

Scenario: Arsenic Poisoning in Bangladesh

Read the scenario about arsenic in the groundwater in Bangladesh. Then answer the questions on pages 142–143.

The Water Quality Problem in Bangladesh

Levels of arsenic in the groundwater in Bangladesh have measured above the World Health Organization maximum of 10 parts per billion. This means that over 35 million Bangladeshis drink water from their wells that are contaminated with arsenic. This is a serious problem because arsenic has been linked to cardio-vascular and neurological diseases and is a human carcinogen.

In Bangladesh, the groundwater filters through ground that was once an ancient river delta system. The sediments that made up the river delta system contain aluminum oxides, iron oxides, and organic carbon. In the rocks of the Himalayas, mountains that surround Bangladesh, minerals can be found that contain arsenic. As the Himalayas erode due to weathering, the rocks release the arsenic, which is carried down rivers and streams and eventually finds its way into the sediments. Certain minerals in the sediments, the aluminum and iron oxides, are extremely efficient at removing arsenic from the water by trapping it on the surface through adsorption. This is shown in Figure 19.

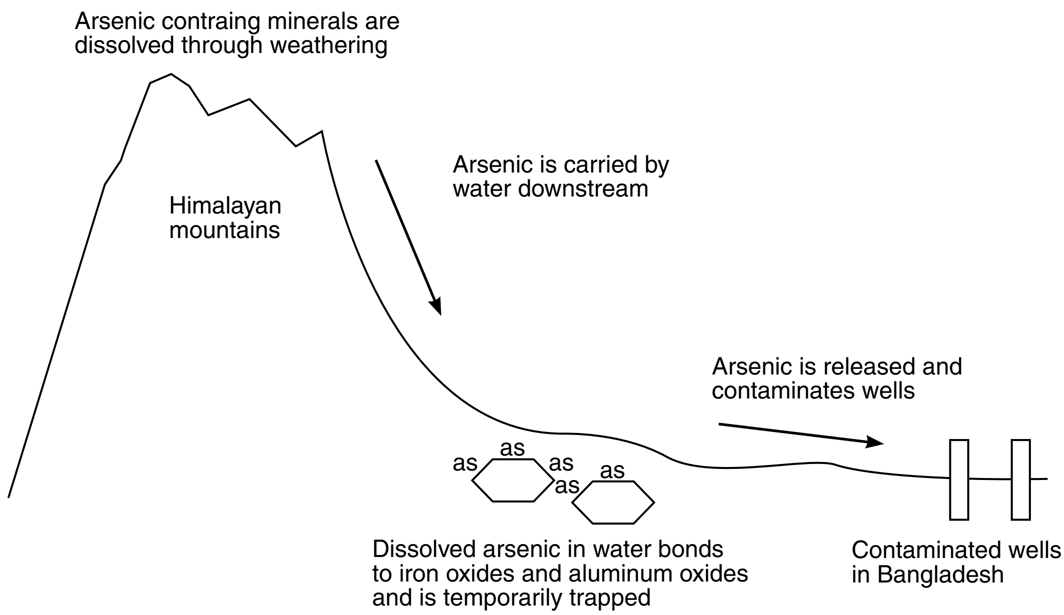


FIGURE 19: Movement of arsenic into water supply.

One current theory that addresses how the arsenic got into the groundwater and wells considers the role played by the iron oxides. Some scientists think that the iron oxides (that have arsenic attached to their surfaces) in the groundwater are being dissolved because the bacteria are reducing the iron from iron(III) to iron(II) (Figure 20). When the iron oxide dissolves, it releases the arsenic into the water. As long as the iron oxides are undissolved, they hold the arsenic to their surfaces. Once the iron oxides dissolve, however, the arsenic is also released into the water environment. Another hypothesis is that there is a change in the solution conditions that releases the arsenic mainly from the aluminum oxide surfaces.

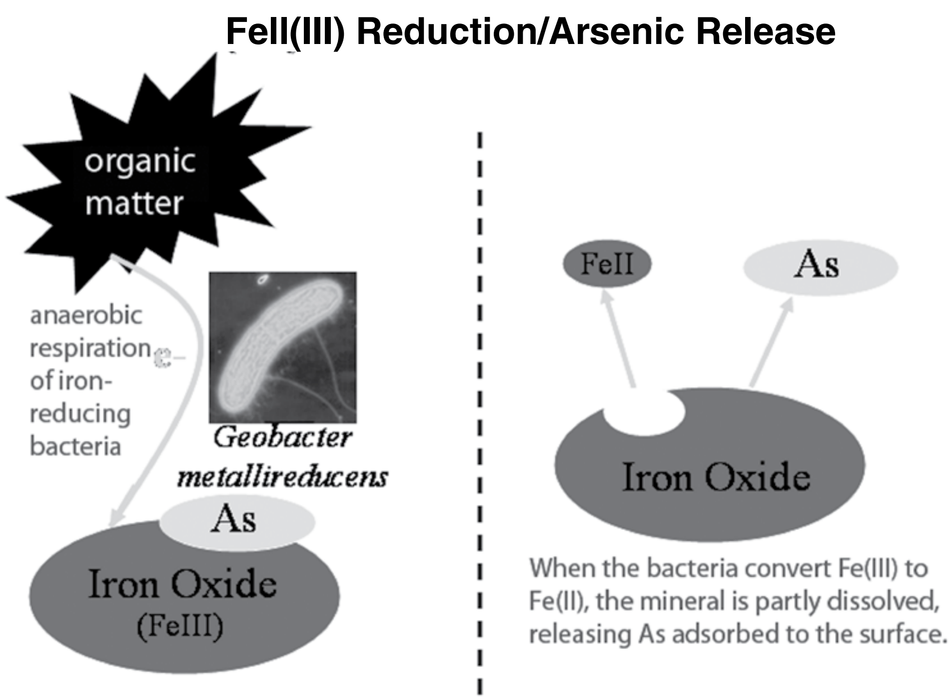


FIGURE 20: Iron(III) reduction and resulting arsenic release.

You are a scientist trying to investigate the source of the arsenic contamination in the drinking water. Your job is to come up with a way of testing one or more of the hypotheses presented above. If you completed Lesson 3, what did you conclude from the Winogradsky column about the disappearance of the red-orange color during the experiment?

Nano2Earth Student Activity Sheet

Scenario: Arsenic Poisoning in Bangladesh

To relate the interactions between the bacteria and minerals in the sediments of Bangladesh and the changes that you observed in the Winogradsky column, you have collected samples of bacteria and mineral oxides from the sediments. Next, you will collect data and create AFM force curves for four combinations of your samples:

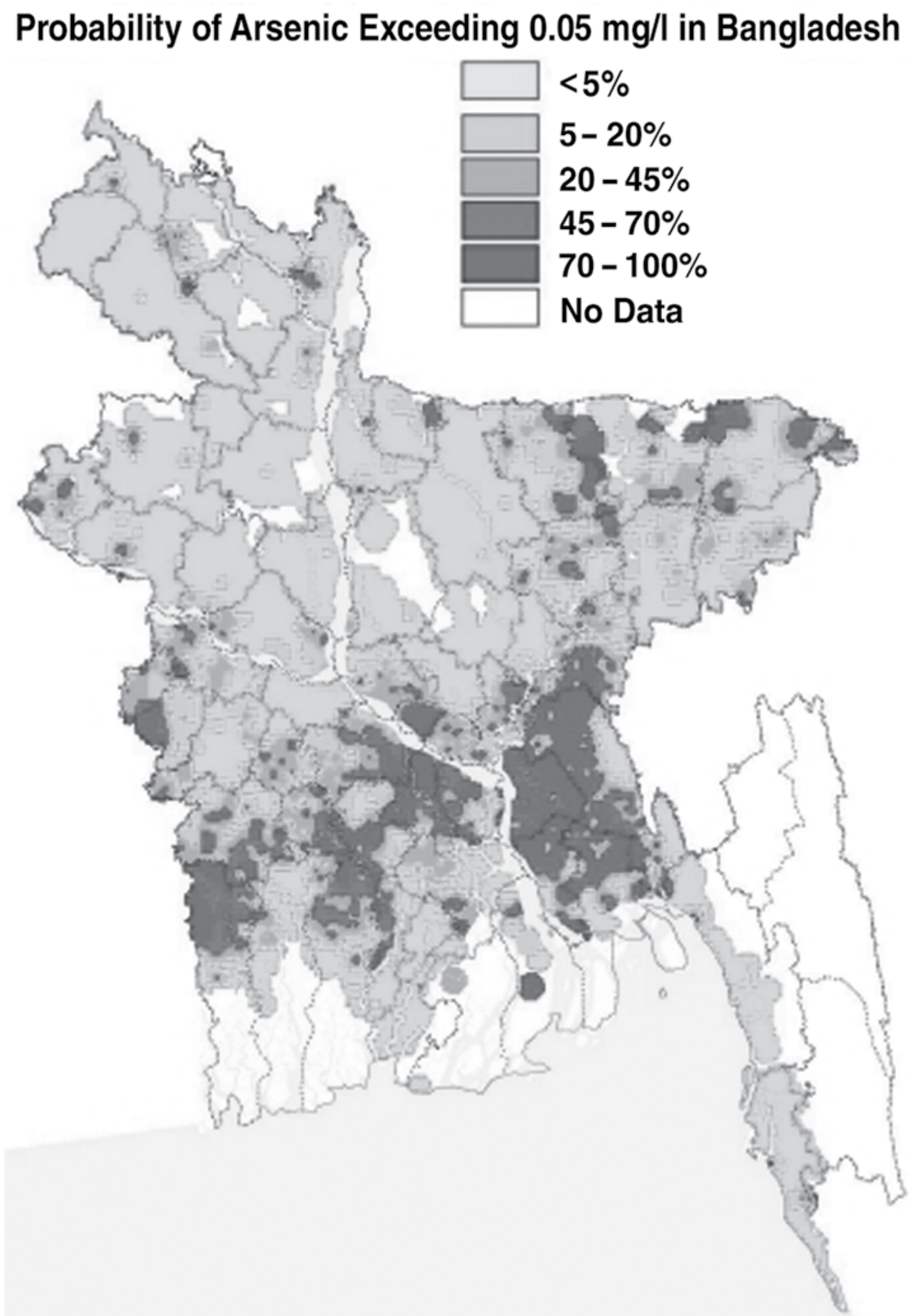
Sample 1..........................bacteria and iron oxide mineral; aerobic conditions
Sample 2..........................bacteria and iron oxide mineral; anaerobic conditions
Samples 3 and 4..............bacteria and aluminum oxide mineral; aerobic and anaerobic conditions

This data will be generated using the computer simulation "Arsenic in Groundwater." These are actual data adapted from a scientific study published in the journal *Science*. The measurements for samples 3 and 4 were essentially identical, so only plotting one force curve for bacteria and aluminum oxide will be needed.

- Start the "Arsenic in Bangladesh" simulation. For the previous simulations, the data were recorded and plotted for you by the simulation. This time, you will have to record and plot the data in the simulation yourself.
- Click on "Sample 1" at the bottom of the simulation to start collecting a force curve for Sample 1. Click the "Approach" button once. The force and distance values will appear in the gray box just next to the "Approach" button. Record those values in the data table by clicking in the empty boxes for "Point 01" and typing in the correct numbers.
- Now click the "Approach" button again, and record the values for Point 2, followed by the rest of the data points until the table is filled.
- Once all of the data have been entered into the table, a "Done" button will become active, located just after the last point in the data table.
- Now plot each of the data points you recorded on the graph. Point 01 will be plotted wherever you place the cursor and click the left mouse button. The next mouse click will plot Point 02. Proceed until all of the data points have been plotted.
- The final step for the sample is to look at how well you did. After the last point has been plotted, the simulation will check your answers. In the data table, all boxes where the data were recorded correctly will turn green; boxes with incorrectly recorded data will turn red. Similarly, data points plotted correctly will turn green. Moving the

Nano2Earth Student Activity Sheet

Scenario: Arsenic Poisoning in Bangladesh



cursor over each data point will reveal the correct answer compared with the point you plotted.

- You may want to save this plot for comparing with the other samples. One way is to copy and paste the graph into a word-processing file. First, make sure the window is maximized, then hit the "Print Screen" key on the keyboard. Open a word-processing file and paste the image there. Put a label in the word-processing file describing which sample the graph corresponds to.
- Move on to the retraction curve and then Samples 2 and 3. When all three samples are complete, compare your force curves to answer the following questions.

Questions: Comparing Force Curves

1. Look at the retraction curves under aerobic conditions. Remember, the negative numbers represent an attractive force. Which mineral do the bacteria stick to more, the iron oxide or the aluminum oxide? Do the bacteria have a purpose or advantage associated with this stronger attraction?

2. Look at the retraction curves under anaerobic conditions. Which mineral do the bacteria stick to more? Use information from the graphs (pp. 136–137) to support your conclusions.

3. The curve for aluminum oxide was identical when comparing aerobic and anaerobic bacteria. Is there significance to this effect? What, if any, is the significance?

4. Under which conditions do the bacteria have the strongest interaction with the iron oxides? Do they have a purpose or advantage with this attraction?

5. Which environmental conditions could lead to release of arsenic resulting in higher levels of arsenic contamination in the wells? Are there any ways to avoid these environmental conditions?

Nano2Earth Student Activity Sheet

Force Curve Data Table for Bangladesh Scenario

	iron oxide: anaerobic	iron oxide: anaerobic	iron oxide: aerobic	iron oxide: aerobic	Al oxide: aerobic/ anaerobic	Al oxide: aerobic/ anaerobic
Distance (nm)	approach	retract	approach	retract	approach	retract
140	0.033	-0.633	-0.001	-0.033	-0.001	-0.082
120	0.039	-0.628	0.002	-0.081	-0.003	-0.125
100	0.020	-0.728	0.001	-0.113	-0.005	-0.353
90	0.060	-0.795	0.003	-0.272	0.005	-0.450
80	0.058	-0.806	0.027	-0.211	0.003	-0.564
70	0.075	-0.902	0.000	-0.179	0.001	-0.567
60	0.078	-0.915	0.008	-0.130	0.003	-0.577
50	0.090	-0.926	0.003	-0.144	-0.013	-0.578
40	0.092	-0.876	0.006	-0.126	0.007	-0.552
30	0.103	-0.826	0.008	-0.179	0.012	-0.508
20	0.092	-0.776	0.001	-0.129	-0.009	-0.458
10	0.090	-0.726	0.005	-0.079	-0.095	-0.408
5	0.078	-0.676	-0.002	-0.029	-0.198	-0.358
1	0.093	-0.626	-0.072	0.021	-0.192	-0.308

Nano2Earth Student Activity Sheet

Scenario: Bacterial Transport in Groundwater

You live in a town located in an agricultural area. The area's economy is based on animal industry, including dairy and beef cattle, as well as poultry. The town's drinking water is supplied by one well and a small sewer line. Recently, a large subdivision was constructed in the town, but no additional wells or sewer lines were constructed to service the subdivision.

Soon after people move into the subdivision, many people in the town report stomach illnesses. The town engineer decides to analyze the well water for contamination; results show that the water contains *E. coli*, a pathogenic bacteria found in fecal matter.

1. What are the potential sources of the *E. coli*?

To prevent further contamination, the town upgrades the sewer line. After this, there are no additional reports of stomach illnesses.

2. Based on this information, can you now be more specific about the source of contamination?

The following summer, the town is hit with several large rainstorms, which dilute the groundwater. Soon after the storms, people begin to get sick again. Analysis of the water again shows contamination with *E. coli*. The engineer is perplexed, as he thought he had fixed their problem by upgrading the sewer line.

3. Based on the bacterial transport column experiments, what do you hypothesize caused the release of bacteria to groundwater?

Nano2Earth Student Activity Sheet

Scenario: Bacterial Transport in Groundwater

The engineer asks a scientist at a local university to help figure out the problem. The scientist collects some sediment samples from the aquifer, and isolates the *E. coli* from the sediment. She runs several AFM experiments to see how the bacteria attach to the sediment. She then conducts the experiments at different values of ionic strength and different values of pH to see if either of these would change the way that the bacteria attach to the surfaces.

Results for two experiments, one conducted with low ionic strength (diluted groundwater collected after rainstorm) and the other conducted with high ionic strength (normal groundwater collected before rainstorm) are shown in the computer simulation "Bacterial Transport in Groundwater." Use the simulations and the following instructions to prepare your own force curves from the scientist's data.

- Start the "Bacterial Transport in Groundwater" simulation. For the previous simulations, the data were recorded and plotted for you by the simulation. This time, you will have to record and plot the data in the simulation yourself.
- Click on "Sample 1" at the bottom of the simulation to start collecting a force curve for Sample 1. Click the "Approach" button once. The force and distance values will appear in the gray box just next to the "Approach" button. Record those values in the data table by clicking in the empty boxes for "Point 01" and typing in the correct numbers.
- Now click the "Approach" button again, and proceed recording the values for Point 2, followed by the rest of the data points until the table is filled.
- Once all of the data have been entered into the table, a "Done" button will become active, located just after the last point in the data table.
- Now you should plot each of the data points you recorded on the graph. Point 01 will be plotted wherever you place the cursor and click the left mouse button. The next mouse click will plot Point 02. Proceed until all of the data points have been plotted.
- The final step for the sample is to look at how well you did. After the last point has been plotted, the simulation will check your answers. In the data table, all boxes where the data were recorded correctly will turn green; boxes with incorrectly recorded data will turn red. Similarly, data points plotted correctly will turn green. Moving the

cursor over each data point will reveal the correct answer compared with the point you plotted.

- You may want to save this plot for comparing with the other samples. One way is to copy and paste the graph into a word-processing file. First, make sure the window is maximized, then hit the "Print Screen" key on the keyboard. Open a word-processing file and paste the image there. Be sure to put a label in the word-processing file regarding which sample the graph corresponds to.

Questions, Sample 1—Values of Ionic Strength:

1. At what approximate distance does the approach curve move away from zero? Is the initial force repulsive or attractive?

2. At what approximate distance is the "jump to contact" shown in the approach curves?

3. What does this difference in "jump to contact" distance tell you about the difference in interaction between *E. coli* and the mineral at these two values of ionic strength?

The scientist then ran some experiments where she varied the pH of the groundwater and then measured the forces between the *E. coli* and the mineral surface using the AFM. You will measure this data yourself by choosing "Sample 2."

Again, prepare your own force curves from the simulation data. Use these results and the results from the first sample to answer the following questions.

Questions, Sample 2—Values of pH:

1. For each value of pH, at what approximate distance does the approach curve move away from zero? Is the initial force repulsive or attractive?

2. Is the repulsive force greater at pH 5 or pH 7?

3. Based on your answers to the questions above, would you predict that the bacteria adhere (stick) to the surfaces better at pH 5 or pH 7? Does this agree with what you observed in the bacterial transport column experiments?

The scientist then calculated the "sticking efficiency" of the bacteria to the mineral surface under different values of ionic strength and pH. The higher the sticking efficiency, the stronger bacteria will adhere to the mineral. The table of values is below.

Sticking Efficiencies for *E. coli* and Silica at Different Values of pH and Ionic Strength

pH value	Ionic Strength	Sticking Efficiency
5	0.005	3.6×10^{-8}
5	0.050	2.6×10^{-4}
6	0.005	9.6×10^{-16}
6	0.050	5.7×10^{-33}
7	0.050	0

4. Based on the sticking efficiency data, do bacteria stick to surfaces better at lower or at higher pH? Why? Does this answer agree with the AFM data?

5. Do bacteria stick to surfaces better at lower or higher ionic strength? Why? Does your answer agree with the AFM data?

6. Which affects the sticking efficiency of bacteria more: ionic strength or pH?

7. If you were the town engineer, do you think you could prevent the contamination problem from reoccurring? If so, what would you do?

Force Curve Data Table for Bacterial Transport in Groundwater Scenario

	High Ionic Strength	Low Ionic Strength	pH 5	pH 7
Distance (nm)	approach	approach	approach	approach
75	-0.019	-0.009	-0.019	-0.008
70	-0.023	-0.007	-0.014	0.017
65	-0.014	-0.005	-0.014	0.028
60	-0.005	-0.004	0.001	0.038
55	-0.002	-0.002	-0.002	0.060
50	-0.004	0.001	-0.004	0.086
45	-0.003	0.000	-0.003	0.112
40	-0.025	-0.003	-0.022	0.162
35	-0.024	-0.001	-0.016	0.210
30	0.012	0.001	0.012	0.271
25	-0.254	0.004	-0.254	0.269
20	-0.487	0.007	-0.487	0.080
15	-0.775	0.010	-0.876	-0.194
10	-1.004	-0.062	-1.004	-0.350
5	-1.496	-0.374	-1.436	-0.736
1	-1.701	-0.502	-1.593	-0.919

Appendix 1

National Science Education Standards for Content in Grades 9-12 Addressed in the Nano2Earth Curriculum

Unifying Concepts

- *Systems:* A system is an organized group of related objects or components that form a whole. Thinking and analyzing in terms of systems will help students keep track of mass, energy, objects, organisms, and events referred to in the other content standards.
- *Evidence* consists of observations and data on which to base scientific explanations. Using evidence to understand interactions allows individuals to predict changes in natural and designed systems.
- *Models* are tentative schemes or structures that correspond to real objects, events, or classes of events, and that have explanatory power.
- *Explanations:* Scientific explanations incorporate existing scientific knowledge and new evidence from observations, experiments, or models into internally consistent, logical statements.
- *Changes* in systems can be quantified. Evidence for interactions and subsequent change and the formulation of scientific explanations are often clarified through quantitative distinctions—measurements.
- *Measurement:* Scale includes understanding that different characteristics, properties, or relationships within a system might change as its dimensions are increased or decreased.
- *Form and Function:* The form or shape of an object or system is frequently related to use, operation, or function. Function frequently relies on form. Understanding form and function applies to different levels of organization.

Science as Inquiry

- *Identify Questions and Concepts That Guide Scientific Explanations:* Students should formulate a testable hypotheses and demonstrate the logical connections between the scientific concepts guiding a hypothesis and the design of an experiment.
- *Design and Conduct Scientific Investigations:* Designing and conducting a scientific investigation requires introduction to the major concepts in the area being investigated, proper equipment, safety precautions, assistance with methodological problems, recommendations for use of technologies, clarification of ideas that guide the inquiry, and scientific knowledge obtained from sources other than the actual investigation.
- *Using Technology and Mathematics to Improve Investigations and Communications:* A variety of technologies, such as hand tools, measuring instruments, and calculators, should be an integral component of scientific investigations. The use of computers for the collection, analysis, and display of data is also a part of this standard. Mathematics plays an essential role in all aspects of inquiry. For example, measurement is used for developing explanations, and charts and graphs are used for communicating results.
- *Formulate and Revise Scientific Explanations and Models Using Logic and Evidence:* Student inquiries should culminate in formulating an explanation or model. Models should be physical, conceptual, and mathematical.
- *Recognize and Analyze Alternative Explanations and Models:* This aspect of the standard emphasizes the critical abilities of analyzing an argument by reviewing current scientific understanding, weighing the evidence, and examining the logic so as to decide which explanations and models are best.
- *Communicate and Defend a Scientific Argument:* Students should develop abilities associated with accurate and effective communication. These include writing and following procedures, expressing concepts, reviewing information, summarizing data, using language appropriately, developing diagrams and charts, explaining statistical analysis. . . .
- *Understandings About Scientific Inquiry:* Scientists rely on technology to enhance the gathering and manipulation of data. New techniques and tools provide new evidence to guide inquiry and new methods to gather data, thereby contributing to the advance of science.

Physical Science

- *Structure and Properties of Matter:* The physical properties of compounds reflect the nature of the interactions among its molecules. These interactions are determined by the structure of the molecule, including the constituent atoms and the distances and angles between them.
- *Chemical Reactions:* A large number of important reactions involve the transfer of either electrons (oxidation/reduction reactions) or hydrogen ions (acid/base reactions) between reacting ions, molecules, or atoms.
- *Motions and Forces:* The electric force is a universal force that exists between any two charged objects. Between any two charged particles, electric force is vastly greater than the gravitation force.

Life Science

- *The Interdependence of Organisms:* The atoms and molecules on the earth cycle among the living and nonliving components of the biosphere.
- *Matter, Energy, and Organization in Living Systems:* The complexity and organization of organisms accommodate the need for obtaining, transforming, transporting, releasing, and eliminating the matter and energy used to sustain the organism.

Earth and Space Science

- *Geochemical Cycles:* The earth is a system containing essentially a fixed amount of each stable chemical atom or element. Each element can exist in several different chemical reservoirs. Each element on earth moves among reservoirs in the solid earth, oceans, atmosphere, and organisms as part of geochemical cycles.

Science and Technology

- *Understanding About Science and Technology:* Science often advances with the introduction of new technologies. Solving technological problems often results in new scientific knowledge. New technologies often extend the current levels of scientific understanding and introduce new areas of research.

Science in Personal and Social Perspectives

- *Natural Resources:* Humans use many natural systems as resources. Natural systems have the capacity to reuse waste, but that capacity is limited. Natural systems can change to an extent that exceeds the limits of organisms to adapt naturally or humans to adapt technologically.
- *Environmental Quality:* Natural ecosystems provide an array of basic processes that affect humans. Those processes include maintenance of the quality of the atmosphere, generation of soils, control of the hydrologic cycle, disposal of wastes, and recycling of nutrients. Materials from human societies affect both physical and chemical cycles of the earth.
- *Natural and Human-Induced Hazards:* Natural and human-induced hazards present the need for humans to assess potential danger and risk.
- *Science and Technology in Local, National, and Global Challenges:* Individuals and society must decide on proposals involving new research and the introduction of new technologies into society.

History and Nature of Science

- *Science as a Human Endeavor:* Individuals and teams have contributed and will continue to contribute to the scientific enterprise. Science is not separate from society but rather science is a part of society.
- *Nature of Scientific Knowledge:* Because all scientific ideas depend on experimental and observational confirmation, all scientific knowledge is, in principle, subject to change as new evidence becomes available.
- *Historical Perspectives:* The historical perspective of scientific explanations demonstrates how scientific knowledge changes by evolving over time, almost always building on earlier knowledge.

Reference

National Research Council (NRC). 1996. *National science education standards.* Washington, DC: National Academies Press.

Appendix 2

Correlations Between AP Environmental Science Themes and Nano2Earth Lessons

AP Environmental Science Themes	Nano2Earth Lessons	Nano2Earth Lesson Objectives
Science is a process. • Science is a method of learning about the world. • Science constantly changes the way we understand the world.	**Lesson 1: Introduction to Nanotechnology**	Identify and compare the scale of different objects and introduce history and applications of nanoscience.
	Lesson 3: Microbe-Mineral Interactions	Investigate the reduction of iron oxide minerals in a simulated anaerobic aquatic environment.
	Lesson 4: Investigation of Bacterial Transport in Groundwater	Explore the transport of bacteria in groundwater by performing a column experiment in a simulated groundwater environment.
	Lesson 5: Nanoforces in Nature	Use evidence and mathematical modeling to interpret and graph real scientific data that has been collected using atomic force microscopes.
Energy conversions underlie all ecological processes. • Energy cannot be created; it must come from somewhere. • As energy flows through systems, at each step more of it becomes unstable.	**Lesson 3: Microbe-Mineral Interactions**	Investigate the reduction of iron oxide minerals in a simulated anaerobic aquatic environment.
	Lesson 4: Investigation of Bacterial Transport in Groundwater	Investigate how bacteria are transported through porous materials as a function of physical and chemical properties.
	Lesson 5: Nanoforces in Nature	Investigate microbe-mineral interactions at the nanoscale to learn how matter and energy cycles and flows through different physical and biological systems.

(*Cont.*)

AP Environmental Science Themes	Nano2Earth Lessons	Nano2Earth Lesson Objectives
The Earth is one interconnected system. • Natural systems change over time and space. • Biochemical systems vary in ability to recover from disturbances.	**Lesson 3: Microbe-Mineral Interactions**	Investigate changes in biogeochemical systems under laboratory conditions.
Humans alter natural systems. • Humans have had an impact on the environment for millions of years. • Technology and population growth have enabled humans to increase both the rate and scale of their impact on the environment.	**Lesson 2: Introduction to Water Pollution**	Investigate types and sources of water pollution in the local community.
	Lesson 5: Nanoforces in Nature	Examine how microbe-mineral interactions are related to metal contamination and the release of arsenic in the groundwater.
Environmental problems have a cultural and social context. • Understanding the role of cultural, social, and economic factors is vital to the development of solutions.	**Lesson 2: Introduction to Water Pollution**	Investigate types and sources of water pollution in the local community.
Human survival depends on developing practices that will achieve sustainable systems. • A suitable combination of conservation and development is required. • Management of common resources is essential.	**Lesson 3: Microbe-Mineral Interactions**	Investigate problems related to excessive nutrients entering the surface waters and what role they can play to help sustain the health of the Earth's waters.
	Lesson 5: Nanoforces in Nature	Investigate contamination of drinking well water in an agricultural area.

Glossary of Scientific and Technical Terms

Adhesion force—This is a quantitative measure of how strongly objects stick together. The higher the force, the more strongly they are bound. The adhesion force between two small objects can be measured using an atomic force microscope. The objects are brought together (approach curve) and then pulled apart (retraction curve), a process known as a force curve. The adhesion force can be determined from the lowest point on the retraction (pulling apart) curve, where the force between the two objects is the greatest. By convention, this is as a negative number, so it is necessary to take the absolute value.

Adsorption—Dissolved contents of water—for example, in rivers, lakes, oceans, and groundwater—are often attracted to the surfaces of minerals and bacteria with which they come into contact. The process of dissolved materials binding to surfaces is known as adsorption. In the environment, the dissolved materials typically contain ions such as the contaminants arsenic (AsO_4^{3-}) and lead (Pb^{2+}) along with small organic materials. The amount of adsorption is related to the chemistry of the water, including its pH and ionic strength.

Aerobic and anaerobic respiration—Organisms respire to gain energy by converting high-energy compounds such as fats and sugars into the low-energy compound carbon dioxide (CO_2). The energy is obtained by transferring electrons from an electron donor (the fats and sugars) to an electron acceptor. The most energy can be obtained by using oxygen (O_2) as an electron acceptor, a process known as aerobic respiration. When other substances are used as an electron acceptor (Fe^{3+}, $Mn^{4+}/^{3+}$, NO_3^-, SO_4^{2-}, etc.), this is known as anaerobic respiration. Typically, when oxygen is present, aerobic respiration will dominate because organisms obtain more energy by using oxygen as the electron acceptor. Humans, animals, and plants can only respire with oxygen (when your muscles are sore after exercising, that is a different phenomenon from anaerobic respiration). Some bacteria can respire both aerobically and anaerobically, while others can only respire aerobically or anaerobically.

Angstrom—This has the value of 0.1 nm, or 10^{-10} m.

Atomic force microscope (AFM)—The atomic force microscope is a tool that is very widely used in nanotechnology. Developed in 1986, it can provide detailed images of surfaces in air and solution, sometimes down to a scale where nanometer-scale features can be seen. The AFM is a form of a scanning probe microscope, where a very sharp probe (often called a tip) is rastered across the surface of interest. The tip is attached to the bottom of a cantilever, a flexible spring such as a miniature diving board. When the tip encounters features on the surface, the cantilever bends up or down. A laser reflecting off the back of the cantilever is typically used to record the signal, and an image is formed. Alternatively, an object can be put on or underneath the cantilever and forces between objects can be measured by collecting a force curve.

Cantilever—A cantilever is a flexible springlike object (you can think of a diving board as an analogy). The cantilever in an atomic force microscope flexes upward or downward depending on the attractive or repulsive forces experienced between the sample and whatever is attached to the cantilever (e.g., a tip or bacteria). The cantilever obeys Hooke's law, which means the amount of bending experienced by the cantilever is directly related to the force the cantilever is experiencing.

Critical zone—This is a place where the land meets the fluid envelopes of the Earth. In the critical zone, the geosphere, hydrosphere, biosphere, and atmosphere interact.

Cryptosporidium—This is a single-celled animal. Human ingestion of *Cryptosporidium parvumi* is one of the leading causes of waterborne disease in the United States.

E. coli—A common bacteria found in the human body, *E. coli* is used as an indicator of groundwater contaminated by sewage. *E. coli* is also one of the most studied bacteria in laboratory experiments.

Eutrophication—Eutrophication is the decay process in which overabundant algal growth uses oxygen, which decreases the dissolved oxygen available for the other aquatic life dependent on dissolved oxygen.

Force curve—A measurement made by an atomic force microscope (AFM), a force curve involves bringing the tip or other object attached to the cantilever (such as a bacteria-covered bead) in contact with a sample (approach curve) and then pulling them apart (retraction curve). The force curve starts with the greatest separation, where there are no interaction forces.

Giardia—Similar to *Cryptosporidium*, *Giardia lamblia* is a single-celled parasitic animal that is responsible for causing infection when untreated water is ingested.

Heterogeneous catalyst—A catalyst is something that causes a chemical reaction to proceed that is energetically favorable, but kinetically hindered. For example, fuel combustion by automobiles creates several gaseous compounds that are potentially harmful when released to the atmosphere. In cars today, the exhaust stream passes through a catalytic converter, which is a solid material (aluminum oxide) that causes some of these compounds to convert to more stable and less harmful gases when they come in contact with its surface.

Hooke's law—This law states that the force experienced by a spring is directly proportional to the displacement (either stretching or squishing) away from the equilibrium position. The proportionality constant is known as the force constant, k. For a one-dimensional motion of the spring, the relationship can be written as Force = $-kx$ where x is the displacement in the x direction.

Hydrophobic force—Water molecules are strongly attracted to each other because they have a distribution of positive and negative charges (a permanent dipole moment; water is "polar"). This interaction is called hydrogen bonding. Other types of molecules and solids do not have this separation of charge distribution; they are "nonpolar." Examples of nonpolar substances are oil and nonstick coatings on cooking pans. Nonpolar materials tend to be "pushed" away from water because the water molecules are more strongly attracted to each other than the nonpolar substance. This is the nature of the hydrophobic, or "water-hating," force.

Ionic strength—The dissolved constituents of a solution contribute to the properties of that solution. The ionic strength is a quantitative determination of the dissolved species, accounting for the fact that those species with a higher charge change the properties of the solution more.

Iron respiration—This is a form of anaerobic respiration, when either iron(III) is the electron acceptor or iron(II) is the electron donor.

Jump to contact—This is a feature in a force curve measured by an Atomic Force Microscope. Regardless of whether or not the initial interaction between the tip (or bacteria, etc.) and sample is attractive or repulsive, eventually they snap into contact as they are brought together (the approach curve). This is due to forces such as van der Waals forces.

Laser—This "coherent" beam of light is all at the same wavelength. We see different wavelengths in the visible spectrum as color, so lasers can be red, green, and so on.

Microbeads—Microbeads are beads that have dimensions of micrometers (otherwise known as microns). One micron is 10^{-6} m.

Molecular nanotechnology—A term coined by the Foresight Institute, this term refers to a vision of the future. Proponents of molecular nanotechnology claim that in the future, nanoscale machines will be able to synthesize almost any material from its constituent atoms.

Nano—This prefix refers to one billionth of a meter, 10^{-9} meters, or 1,000 nm.

Nanobiogeochemistry—This is the interdisciplinary study of interactions among biology, chemistry, and geology that occur over the length scale of nanometers.

Nanofilm—A nanofilm is a film that may be continuous in two dimensions but very thin (<100 nm) in the third dimension.

Nanoforce—This refers to a force on the order of a nanonewton.

Nanometer—The value of a nanometer (nm) is 1×10^{-9} or a billionth of a meter.

Nanonewton—A newton is the International System–accepted scientific unit of force. A nanonewton is 10^{-9} newtons, or a billionth of a newton. Forces between individual molecules typically fall in this range. One nanonewton is approximately equivalent to the gravitational force between someone sitting at a computer and the computer monitor, or the gravitational force between two people at opposite ends of a moderately sized room.

Nanorange—Typically this refers to a field considered to be 1–100 nanometers.

Nanoscale—Typically this size is considered to be 1–100 nanometers.

Nanoscience—This is a field of science that measures and explains the changes of the properties of substances as a function of size; these changes occur in the range of approximately 1–100 nanometers.

Nanotechnology—The National Nanotechnology Initiative of the United States defines nanotechnology as:

- Research and technology development at the atomic, molecular, or macromolecular levels, in the length scale of approximately 1–100 nanometers.
- Creating and using structures, devices, and systems that have novel properties because of their small and/or intermediate size.
- The ability to control or manipulate on the atomic scale.

National Nanotechnology Initiative (NNI)—The National Nanotechnology Initiative is a federal program designed to coordinate research and development in nanoscale science, technology, and engineering. See *www.nano.gov.*

pH—Defined as the negative logarithm of the activity of hydrogen ions in solution, this number refers to the amount of free acid (H^+) or base (OH^-) in solution. Acidic solutions have pH less than 7; basic solutions have pH greater than 7.

Probeware—This is an educational technology tool designed for the science classroom. A number of different probes (e.g., light sensor, pH meter, dissolved oxygen probe) can be linked to computers for data collection and analysis.

Radionuclides—Elements may exist with various numbers of neutrons in the nucleus, called isotopes. Isotopes with extra neutrons are sometimes unstable and spontaneously decompose with release of various forms of radiation. These unstable isotopes are known as radionuclides.

Reduction of iron—When microbial activity is involved, this usually refers to a form of anaerobic respiration, when iron(III) is the electron acceptor.

Salmonella—This is a group of bacteria that commonly live in animals, but can cause gastrointestinal disease in humans.

Scaling—Materials behave differently when scaled down in size.

Scanner—The scanner is a device on the atomic force microscope (AFM) to move the sample closer and further from the tip and/or from side to side.

Scanning Probe Microscopy (SPM)—This refers to a family of microscopes that all involve the rastering of a probe (sometimes called a tip) across a surface. Examples of scanning probe microscopes include the atomic force microscope and the original SPM, the scanning tunneling microscope (STM).

Scanning Tunneling Microscope (STM)—This is a microscope in which a metallic tip is rastered across the surface of a conducting or semiconducting material. An image is obtained by electrons that transfer (tunnel) in or out of the sample. The tunneling current depends exponentially on the distance between the sample and the tip, such that even atomic scale images have frequently been obtained.

Transmission Electron Micrograph—This is a picture taken by a transmission electron microscope (TEM). A TEM uses a beam of electrons to image extremely small features in materials, including the internal structure and chemistry. Although TEMs have been around for over 75 years, continual improvement in technology has improved them to the point that they are now capable of imaging individual atoms in some situations. Electrons are effective for imaging small features because they can be accelerated to the point that the electron beam has a wavelength approximately equal to atomic dimensions. Light microscopy is limited to features of approximately 300 nm due to the wavelength of light.

Winogradsky column—This column is designed to simulate a natural ecosystem. Mud and water collected from a lake, stream, creek, or puddle are placed into the column.

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