Chapter 7. Biological Contaminants

Lesson 1. The idea of biological contamination.

Overview: In the U.S. and other industrialized countries, people take their water purity for granted. People who spend time outdoors may think of the pure water they take for granted when they are thirsty. They would not feel comfortable about drinking the water from lakes, rivers or streams unless they were near a pristine high altitude stream or a spring. Why the hesitation? Most likely, the reason is the biological contamination in surface water.

Biological contamination of enteric origin is of major concern. When contamination is of enteric origin, it comes from the intestines. Fecal material contains microorganisms that live in the intestines of animals, humans and waterfowl. Surface waters heavily used by humans, animals and water fowl become sinks for fecal and waste material. Such material sometimes goes directly into the water. Runoff or sewage failure may deposit it indirectly. Sometimes septic tanks break and contaminate groundwater.

Purpose: This activity consists of one part. We will explore the idea of biological contamination.

Ideas Taught:

- Pathogenic, or disease causing, organisms are of prime concern during purification of water intended for human use.

- Pathogenic organisms are of enteric (from the intestine) origin and are transmitted into surface water supplies by fecal pollution from humans, animals, waterfowl and wastewater.

- Pathogenic organism pollution in drinking water is expensive and can be difficult to detect. Rather than testing for pathogenic microorganisms, water purification testers look for coliform bacteria. These enteric, nonpathogenic organisms are abundant in fecal matter.

- Purification of pathogenic organisms from drinking water requires the practice of chemical, thermal and/or ultraviolet light treatment.

- The Environmental Protection Agency (EPA) sets strict limitations on coliform bacteria concentrations in municipal water. Private well water has no such regulations.

Materials Needed:

- 1 Water Bacterial Pollution Kit (Purchase from: Carolina Biological Supply Co., 2700 York Rd, Burlington, NC 27215 Phone: 1-800-344-5551, Item #76-6350, approx. cost $30)
- Supply of distilled water
- Paper cups
- Clean, sterilized plastic pop bottles (optional)

Procedure:

Before class, read the entire "Water Bacterial Pollution Kit Teacher's Manual." Collect water samples from five different water sources into the test tubes provided in the kit. If you wish, provide clean pop bottles and have the students collect samples. Some suggestions of sources are: the tap, a river, a lake, a spring (hot or cold), a swimming pool, and the toilet bowl.

Just before class, follow the instructions in your manual to prepare the nutrient agar petri plates. Aseptic technique is extremely important. Use it to handle your water samples and to prepare the nutrient agar plates. Microorganisms are ubiquitous. That is, they exist everywhere. Microorganisms are on your hands. They are on the surface on which you will be working. They are on sampling tubes. The inside of sample tubes and petri plates are sterilized. So are the pipettes and the nutrient agar. The latter are, therefore, bacteria free. Aseptic technique maintains the sterility of the sample tubes in which you collect water samples. It also maintains the sterility of the agar plates on which you will be inoculating your water samples. The process of aseptic technique involves washing your hands and your work surface. Use only sterile pipettes to touch the mouth of the sample tubes, the inside of the petri plates, or the nutrient agar. Keep in mind that if you touch the tip of the pipette to anything, especially your hands, you must get a new pipette.

Your students will be inoculating the petri plates with the water samples you have collected. Bacteria introduced from poor aseptic technique will produce bacterial growth not representative by the water sample.

When class begins, tell the students to imagine themselves standing beside a river. They have just been hiking for a few hours. They forgot to bring drinking water, and they are extremely thirsty. Ask the students if they would drink from the river. Discuss the topic of water contamination. Remind them that contaminants are physical, chemical or biological.

Biological contamination is the most likely hazard associated with drinking surface water. Ask the students to define a biological contaminant. It may be any contaminant that is or once was alive. Have the students give examples of biological contamination. Write the examples on the blackboard. The students should give examples of biological contamination at the microscopic level.

Tell them that some organisms that are only visible with the aid of a microscope inhabit surface waters. Some examples of such microorganisms are yeasts (the same organisms that cause bread to raise and beer to brew), molds (which inhabit stale bread), fungi, bacteria, and protozoans (the one-celled organisms that prey on bacteria). Most of the microorganisms just mentioned are harmless to humans, if humans ingest them.

Ask the students when biological contamination would be dangerous to human health. Try to clue them in on the subject of disease-causing organisms. Explain that pathogenic (or disease-causing) microorganisms are a major concern in drinking water supplies. Write the word "**pathogenic**" on the board. Most pathogenic organisms that get into surface waters originate from the intestines of humans, animals and waterfowl. Diseases such as bacillary dysentery, typhoid fever, cholera, polio, giardia and hepatitis are all caused by enteric microorganisms transmitted through drinking water.

Ask the students how a microorganism that lives in the intestine could get into water destined for drinking. Biological contamination of surface waters becomes more probable as humans, livestock, wildlife and waterfowl use of the water or the land near it increases. These animals may deposit fecal material directly into the water or indirectly through runoff. Breaks in sewage systems, failure of septic systems, improperly sealed or constructed wells, and backflow or improper pipe connections can cause biological contamination to water systems.

Detection of pathogenic microorganisms in drinking water is difficult. Remind the students that microorganisms are one-called organisms, too small to be seen with the naked eye. Tell them up to five million microbial cells can occupy the space equal to the size of a pinhead. Explain that fecal material contains many nonpathogenic microorganisms. Approximately ¼ pound of bacteria live within the human body, most of which occupy our digestive tract. They help us digest our food.

Tell the students that if they tested contaminated water for pathogenic bacteria, only one microbe out of one thousand enteric microorganisms is pathogenic. It is much more reliable to test for fecal contamination by testing for nonpathogenic intestinal bacteria. Such enteric bacteria are called coliform bacteria. Write "**coliform bacteria**" on the blackboard. Routine testing for coliform bacteria reduces transmittance of potentially dangerous diseases.

Tell the students they will be doing a test for the presence of bacteria in water. Then they will be testing specifically for coliform bacteria in their water samples. The Water Bacterial Pollution Kit provides two types of nutrient agar. One promotes the growth of a wide range of bacteria. The other promotes only the growth of coliform bacteria. Tell the students it is this type of testing which is performed daily on their municipal drinking water.

Divide the students into five groups. First, have the students wash their hands thoroughly. Then have them wipe off their work space with 5% Chlorox or with a soap solution. Give each group a water sample, two nutrient plates that promote the growth of coliform bacteria, four pipettes, and a paper cup full of distilled water. Each student should receive a Student Guide provided by the kit. The kit tests up to ten different water samples. I suggest the students do five samples twice. This is in case one of their plates gets contaminated during inoculation.

Explain to the students that bacteria are everywhere—on their skin, on their work spaces, on the outsides of the tubes and plates, etc. They washed their hands and work spaces to eliminate much of the bacteria on them. Emphasize how important it is to be as precise as possible when following their student guides. The student guide provides a procedure that keeps any outside bacteria (bacteria other than from their water samples) from contaminating their agar plates and giving false results. Tell them the pipettes are sterile (bacteria-free). The insides of sample tubes are also sterile. The only bacteria present in the tube now are those introduced in the water sample. It is, therefore, very important that they touch neither the mouth of the test tube nor the tip of the pipette! They do not want to invalidate their results by introducing bacteria from their fingers. Explain that touching the tip of the pipette to the work surface may also pick up outside bacteria.

Have one student in each group be designated as the "pipetter." He/she should practice pipetting with a practice pipette and the water from the paper cup provided to them. You may want to show how to pipette. Then the "pipetters" feel comfortable with the technique, they follow the Student Guide to inoculate the petri plates with the water samples.

After inoculation, the plates can be set on the work surface. Have the student do the chlorine test on the remainder of their water samples. Chlorine treatment causes a pale yellow color in the samples with the addition of the chlorine test solution. We will explain the purpose of chlorine treatment in a moment.

After every 24-hour period following inoculation of their plates, the students count the bacterial colonies that grow. The Teacher's Manual provided by the kit gibes a good explanation of how colony counting is done. On the back of the student guide is a worksheet to document important information about the samples. Students use this worksheet after each colony count. The growth of bacterial colonies on the agar plates suggests the number of bacteria in the samples. Students inoculated plates with one milliliter of water sample. The number of colonies that grow on their plates will be equal to the number of bacteria per milliliter. You may want to draw a table like the one below on the blackboard. Fill it in after each 24-hour period so the students can see other groups' results.

Kind of Water	General Bacteria (bacteria/ml)	Coliform Bacteria (bacteria/ml)

Remind the students that their bacteria count is not always absolute. One colony may arise from the multiplication of just one individual bacterial cell, or from a group of cells that are near each other. Many bacteria exist in pairs, chains and clusters in water. Their colonies could have come from more than one bacterium. Further, the nutrient agar may not be appropriate for other bacteria to grow. Such bacteria are missed in assessing the presence of bacteria in water supplies. Their bacterial counts are an approximate number of bacteria present in the samples.

Multiplication of microorganisms is rapid. Purification of coliform bacteria and pathogenic organisms from potential drinking water must be reliable. Remind the students how fast the colonies grew. Let's say these colonies came from one individual cell. The students could see nothing on their plates immediately after inoculation. After two or three days, the colonies became visible. Remind them that five million bacterial cells can occupy the head of a pin. How many bacterial cells are in their colonies?

Now have the students imagine that four cells/ml survive the water purification at a treatment plant. They are released for a full day with drinking water. Discuss the hazards of such an incident. Remind them that perhaps on cell in a thousand is pathogenic.

Fortunately, such an incident may not be as hazardous as the students may think. The reason is due to the fragile tolerance of enteric bacteria to temperature. Ask the students if they know their normal body temperature. If any of them have had a fecer and their mom or dad has taken their temperature, they should know right away. Remind the students that 98,5°F is the temperature at which coliform bacteria survive. Cold drinking water temperatures are about 60°F and hot water is about 118°F. Coliform bacteria and pathogenic bacteria do not survive long at these temperatures. Ask the students if temperature would be a good purification method for coliform and pathogenic bacteria. The answer is yes, but most municipal water treatment centers do not use this method. The reason is the once-celled giardia parasite.

Have the students imagine themselves dressed in a very thick, heat resistant armor. They could easily survive extreme heat or cold, as long as the armor were intact. This is exactly what the giardia parasite does. It forms a "cyst" around itself. The cyst is temperature resistant for long periods. One must boil water for at least ten minutes to completely kill the giardia cyst. Boiling large quantities of water is, however, quite impractical in water treatment plants.

Some treatment plants use ultraviolet (UV) light to kill coliform and pathogenic bacteria. UV light is the high energy wavelength of light from which we humans must protect ourselves in the sun. Ask the students why they think UV light kills coliform and pathogenic bacteria. If they need some guidance, ask them if there is UV light in their intestines. Some water treatment plants pass the water through transparent columns and shine UV light into these columns of water to kill the bacteria. Unfortunately, the giardia parasite can again protect itself with a cyst. It survives exposure to UV light if it forms a cyst. The most effective way to remove the giardia cyst is by filtration. Filtration pushes the water through a filter with holes smaller than the size of the cyst.

Ask the students what method besides boiling the water will kill coliform bacteria, pathogenic bacteria, and the giardia cyst. The topic of chemical warfare may arise. If it does not, lead students to think of what might be added to drinking water to kill these microorganisms. What might be added to a swimming pool that would kill these organisms?

Chlorine, bromine or iodine is commonly used to kill coliform bacteria, pathogenic bacteria and giardia. This method is both inexpensive and reliable. Chlorine kills microorganisms in municipal drinking water and swimming pools. Bromine can also be used in pools. Iodine is not used much, because of its ability to stain.

Ask the students if they can taste chlorine in their drinking water at home, at school, in restaurants, etc. The students did a test on their water samples to see if they were treated with chlorine. Ask the students which water samples tested positively for chlorine treatment. Would they feel comfortable drinking water from these sources? Would they drink the water that tested positively for chlorine treatment? Have the students compare their chlorine test results with their colony counts. You may want to document their findings on the board with a table like the one below.

The students should now have a very good idea about which water samples were purified for human use and which were not.

Municipal drinking water has strict regulations set by the Environmental Protection Agency (EPA) for coliform bacteria concentrations and other water quality parameters. The required EPA standard for coliform bacteria in municipal water purified for human use is no more than one coliform bacteria cell per 100 milliliters of water. Law requires water treatment plants to follow these standards. Ask the students if they get their water at home from city water or from their own private well. Explain that those students who drink city water at home can take for granted that they will always have pure drinking water coming out of their faucets. Those students who drink private well water at home should not take the purity of their water for granted. The EPA has no regulations on private well water quality. It is the well owner's responsibility to have his or her water tested regularly to assure water safety.

Kind of Water	Bacterial Count	Coliform Count	Chlorine Treatment?

Lessons Learned:

Biological contamination of drinking water from fecal material is particularly hazardous to human health. Fecal material contains pathogenic microorganisms that cause potentially fatal diseases. Examples of such diseases are typhoid fever, cholera, dysentery, giardia, and hepatitis.

Detecting pathogenic microorganisms in drinking water is expensive and difficult. Municipal water treatment plants regularly test the water intended for human use for coliform bacteria, instead. It is much easier and less costly to detect. Coliform bacteria are enteric organisms abundant in fecal material. Its detection in drinking water suggests the presence of fecal pollution, and perhaps disease causing organisms in the water.

The Environmental Protection Agency has strict regulations on concentrations of coliform bacteria and other water quality parameters in municipal water released for human consumption. No such Federal regulations apply for private well water. It is therefore the responsibility of the well owner to test his/her water regularly for coliform bacteria and other water quality parameters.

Daily treatment of municipal water includes a variety of procedures, including the use of chlorine to kill bacteria and parasites such as the giardia organism.

The lesson above was adapted from "What is Water Quality? A Resource Guide for 4-H Leaders and Teachers," 80 pages of activities and experiments related to water quality. ($5.00) Order from the Montana 4-H Program at Montana State University-Bozeman. Phone 406-994-3501.