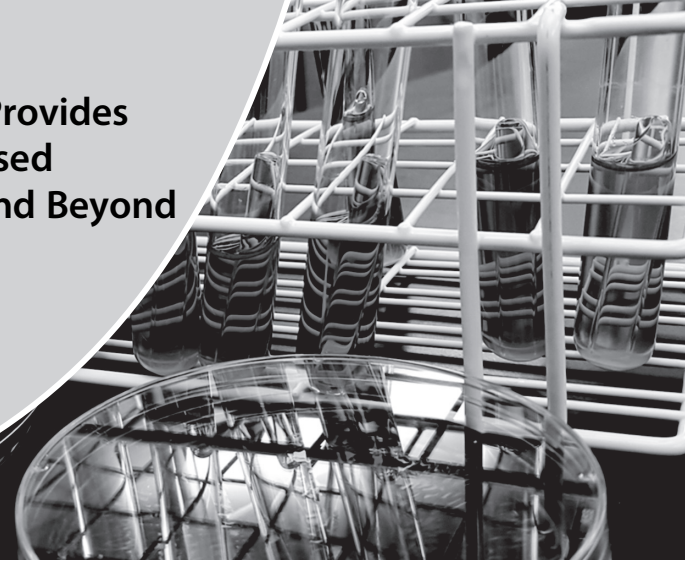
INQUIRY & INVESTIGATION

Wastewater Treatment Provides for Authentic Inquiry-Based Experiences in the Lab and Beyond



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Abstract

Efforts to reform science education focus on implementing constructivist pedagogy to engage students in scientific practices, promote critical thinking, and provide students with relevant research experiences. In this spirit, this article presents authentic, inquiry-based activities utilizing the real-world bioscience behind wastewater treatment. The activities begin with a tour of a wastewater treatment facility, followed by a guided inquiry activity in which students enumerate E. coli *levels from wastewater samples collected from different steps of the treatment process. Students then participate in an open-inquiry experiment to test a unique hypothesis. Learning about wastewater treatment introduces students to important biology content such as bioremediation, microbiology, and nutrient cycling. Additionally, students engage in science practices such as inquiry and constructing evidence-based explanations.*

Key Words: *Wastewater treatment; bioremediation; constructivism; microbiology;* E. coli*; water management; multiple tube fermentation technique; active learning; open and guided inquiry; science practices; authentic learning.*

○ Introduction

There has been much discussion in the last two decades about reforming science education (e.g., Ahlgren, 1996; Wheeler, 2006). The emerging consensus is that reform should be based on constructivist teaching practices (National Research Council, 2012). Constructivism is defined by Hartle, Baviskar, and Smith (2012) as "a theory that describes learning as taking new ideas or experiences and fitting them into a complex system that includes the learner's entire prior learning" (p. 31). Because learners must assimilate new information with old information, constructivism is an inherently active process. Research on constructivism has moved it into the forefront of science education reform where it serves as a theoretical underpinning for how people learn (Hartle et al., 2012).

The challenge that faces educators is using constructivist theory to direct pedagogy. Crowther (1999) summarizes several attempts to transform constructivist theory into practical applications for the classroom. For example, Wheatley (1991; as cited in Crowther 1999) offers a problem-centered approach that consists of three elements: tasks, groups, and sharing. As students work together on problems, they not only participate in cooperative learning, but also metacognition as they compare their problem-solving to others in the class. Better yet, Saunders (1992) provides a more elucidated and compelling strategy based on a four-part framework: 1) investigative labs, 2) active cognitive involvement, 3) stimulating work in small groups, and 4) alternative assessments. While there are numerous strategies for creating a constructivist learning environment (Crowther, 1999; Hartle et al., 2012), no single strategy is likely to work best in all situations and for all grade levels. Therefore, individual instructors must find the strategy that best suits their students and learning objectives.

Water is the single most important substance for life.

Despite the difference in opinions for how to implement constructivist theory in the classroom, many agree that inquiry, in particular, is a key component. Students who engage in inquiry display inquisitiveness, pose questions, search for answers, and seek to understand "counterintuitive phenomena" (Llewellyn, 2013, p. 6). In inquiry activities, students collect and analyze data, make evidence based-conclusions, and communicate their findings. In other words, they engage in science practices much in the same way that scientists go about studying the world and generating new knowledge (National Research Council, 2012). For these reasons, inquiry-based instruction is promoted as a leading method for teaching science (National Research Council, 2012).

There is strong evidence that inquiry and other constructivist strategies improve student achievement. For example, in a meta-analysis of 225 studies, Freeman and colleagues (2014) found that in college courses that focused on active-learning, which includes inquiry, students performed substantially better than students enrolled in conventional, lecture-based courses. In some cases, students involved in inquiry-based courses did not report significant

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increases in grades, but they did report positive increases in attitudes, the feeling that lab activities are more practically relevant and meaningful, that they have greater confidence in their skills, and that they have more positive perceptions of how their studies relate to the world around them (Beck, Butler, & Burke da Silva, 2014; Tomasik et al., 2014).

Because inquiry-based activities involve collection of data, posing questions, and designing investigations, they are a natural fit for inclusion into the laboratory portion of science courses. Unfortunately, many educators think that their lab activities are inquiry-based because they are hands-on, but these two things are not always synonymous (Llewellyn, 2103). Many common laboratory activities are deemed 'cookbook' labs because students unthinkingly follow procedures that are designed to produce a single correct answer. These do not meet the constructivist criteria mainly because students are not actively engaged or invested in their work and do little reflection upon their own learning during the activity. The false notion that any hands-on lab activity is automatically inquiry-based appears to be common, at least in the biological sciences. In a meta-analysis, Puttick, Drayton, and Cohen (2015) analyzed 111 articles published between 2007 and 2012 in this journal. Interestingly, 40% of the studies claimed that their instructional methods were inquiry-based, but only half of those studies provided any evidence to support this because few could demonstrate active student engagement in inquiry.

Inquiry-based activities exist along a spectrum of student and instructor involvement (Moyer, 2012). In the lowest levels of inquiry, much of the inquiry investigation, such as development of research questions and methodology, are determined by the instructor. This is called guided inquiry, and one meta-analysis found that it is the most common type of inquiry published in the biological sciences (Beck et al., 2014). In the highest levels, called open inquiry, nearly everything is initiated by the student. There is debate about which level of inquiry is the best (Eastwell and MacKenzie, 2009). Some argue that instructors should always strive for open inquiry, which in their view is the best type of inquiry because students have the largest degree of freedom. However, some point to the lack of evidence supporting any inherent advantages of open inquiry, and warn against overlooking the importance and effectiveness of guided inquiry practices (Eastwell and MacKenzie, 2009). As an example, in a small study, Moyer (2012) found low achievement and high uneasiness among students engaged in open inquiry compared to guided inquiry.

Inquiry-based lab activities, even guided inquiry, tend to give students considerable freedom to be active learners. Students may be given a research question or problem by the instructor, but are asked to determine the experimental methodology and conduct their own analysis (Unsworth, 2014). Or, students might be guided through an experiment by an instructor, but then asked to use their new knowledge to develop a related follow-up experiment (Ketpichainarong, Panijpan, & Ruenwongsa, 2010). Even the simple act of having students design their own data table is enough to get them to critically think about what type of data they might expect to collect and determine the best ways to organize it (Llewellyn, 2013).

With this knowledge in mind, I have developed a curriculum based on authentic, inquiry-based activities that focus on wastewater treatment. The study of wastewater treatment not only informs students about water management (Appendix A), it also introduces important biological issues such as microbiology, nutrient cycles, and bioremediation. The latter is an especially important concept in environmental biology because it involves the use of organisms to clean up waste created by humans. Bioremediation is key to wastewater treatment and is an important concept that students experience firsthand in the activities presented below.

○ Curriculum

Overview

The curriculum was originally developed for college students in an introductory biology course for non-science majors but could easily be adapted for high school students or more advanced undergraduates. These activities engage students in authentic, inquiry-based experiences. Relevance (Glynn, et al., 2011) and authenticity (Hursen, 2016) positively contribute toward motivation, and motivation is important to student achievement (Chow & Yong, 2013). Additionally, the activities meet several of the learning standards and science practices highlighted in *A Framework for K-12 Science Education* (National Research Council, 2011) and the Next Generation Science Standards (NGSS Lead States, 2013). In addition, it satisfies many of the action items in *Vision and Change in Undergraduate Biology Education* (AAAS, 2011). All of the above learning objectives are met by having students participate in data collection and analysis, providing them with authentic real-world experiences, and working in collaborative groups, to name a few. Based on the constructivist theory of learning (Hartle, et al., 2012), the curriculum requires students to be active participants as they construct their own knowledge.

The activities can be easily implemented because they do not require previous microbiology experience and because most schools are within a reasonable distance of a municipal wastewater treatment facility. Also, many of the materials are easy to acquire, such as test tubes and Petri dishes. While the lab activities require an autoclave and incubator, these items can be borrowed or constructed inexpensively. For example, an autoclave can be fashioned from a pressure cooker and a rudimentary incubator can be constructed using an insulated box and a heating pad.

Activity 1: Wastewater Treatment Facility Tour

The first activity is a field trip to a wastewater treatment facility. Any town with a centralized sewage system will have such a facility, and they are usually quite accommodating and eager to provide tours to students. A one-and-a-half-hour tour can cover the following: primary treatment to remove suspended solids, secondary treatment to remove dissolved organic material through aerobic breakdown by bacteria, and disinfection before discharging the effluent back into the environment. Some treatment facilities may include tertiary treatment, such as the biological removal of excess nutrients such as nitrogen (World Bank Group, 2015). An added benefit of the tour is that it provides a demonstration of how biology can be used in everyday life, in addition to letting students observe scientists and engineers in action.

A key feature of wastewater treatment is the use of bioremediation, in this case the use of bacteria to clean wastewater. This occurs during secondary treatment when bacteria break down dissolved organic material that would otherwise require expensive filters. Some facilities may use bioremediation in additional ways, such as anaerobic digestion of solid waste or removal of nitrogen from wastewater through microbial denitrification (Lu et al., 2014).

To promote active engagement during the tour, encourage students to take notes and to ask the tour guides at least one wastewater treatment-related question. I have found that this drastically improves student engagement. The follow-up assignment can have students reflect upon the treatment process, highlighting the specific components that utilize microbes to perform bioremediation. Instructors can ask students to relate these processes to such important concepts as chemical cycling and microbial metabolism.

It is important to collect wastewater samples during the tour so students can complete activity 2, a guided inquiry lab experiment. Sterilized containers should be used to collect samples from such locations as the primary and secondary clarifiers and at the outfall, which is the point where the fully treated wastewater is discharged back into the environment. Chill all samples immediately to prevent from overheating.

If the three aforementioned locations are chosen, it is important to understand the following: The primary clarifier allows suspended solids to settle out as wastewater becomes nearly stagnant in a large basin. Water there should contain a small amount of suspended solids, high levels of dissolved organic material, and plenty of bacteria. Meanwhile, the secondary clarifier follows the aeration basin, an area where the highly oxygenated water allows for proliferation of aerobic bacteria that feed upon and remove dissolved organic material. Much like the primary clarifier, the secondary clarifier allows suspended materials to settle out, but in this case the solids are large colonies of bacteria left over from the aeration basin. These water samples should therefore be low in dissolved organics but may still contain substantial numbers of suspended bacteria. Wastewater leaves the secondary (sometimes called "final") clarifier before being disinfected with chlorine or UV light that kills the vast majority of bacteria. After disinfection, wastewater enters the outfall and is discharged back into the environment. A sample of this water should be very low in suspended solids, dissolved organics, and bacteria.

As previously noted, wastewater treatment relies on bioremediation to remove dissolved organic material that would otherwise require costly filtration. This is done by bacteria that are naturally present in the wastewater, coming largely from human excrement. For example, bacteria can account for up to 54% of the dry weight of human feces (Rose et al., 2015). The wastewater process deliberately stimulates the growth and activity of these bacteria to help clean the water. A common species of fecal bacteria that is present in wastewater is *Escherichia coli*. This bacterium is a normal and usually benign inhabitant of the large intestines. However, certain strains, such as O157H7, can sometimes cause disease symptoms such as nausea, vomiting, kidney failure, and death (CDC, 2015). Because of the potential for illness, water quality scientists routinely test water for *E. coli*. The presence of this bacterium is an indication that the water is contaminated with human feces.

Activity 2: Guided Inquiry Laboratory Investigation

The second activity is a guided-inquiry lab investigation that quantifies *E. coli* levels in wastewater samples collected from the tour. While the scope of the experiment and its methods are determined by the instructor, students are actively involved in the inquiry process in several ways: determining the research question and hypothesis, creating their own data tables, analyzing and interpreting their data, and constructing evidence-based conclusions that they share with the class.

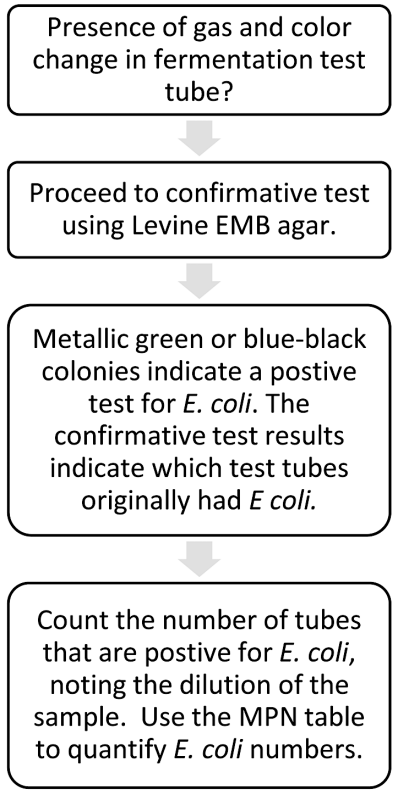

Figure 1. An overview of the multiple-tube fermentation technique (Johnson & Case, 2004).

At the beginning of this activity, organize students into small groups and inform them that they have the capacity to measure *E. coli* numbers in the wastewater samples. Knowing this, instruct each group to brainstorm potential research questions that could be tested. The question that usually comes to most peoples' minds is, "How do *E. coli* levels differ among the water samples?" With testable questions identified, groups develop their hypotheses in the form of explanatory statements. By allowing students to create research questions and hypotheses, they become active participants and stakeholders in the investigative process.

Each group of students then tests their hypothesis while gaining experience using the multiple tube fermentation technique. This technique has real-world applications and uses a presumptive test, which provides evidence for the presence of bacteria, followed by a confirmative test, which positively identifies the bacteria as *E. coli* (Figure 1). For logistical reasons, each group of students should receive wastewater from only one of the three samples. At the end of the experiment, pool data from the entire class. This allows students to work with a larger data set to test each group's hypothesis. Instructions for students are provided in Table 1, while lab preparation instructions are included in Table 2.

Background on the experimental methodology

During the presumptive test each group of students gets nine experimental test tubes plus one control, all containing phenol red lactose broth. Each test tube also contains a small inverted Durham tube. During the investigation, various amounts of wastewater are added to the experimental test tubes (see Table 1). In the absence of

Table 1. Laboratory instructions for students.

Steps	Laboratory Instructions
1	Obtain 10 prepared test tubes for your group. One of these is a control; do not add anything further to this tube. To the 3 tubes marked "1.5×" add 10 mL of your wastewater sample. To 3 other tubes add 0.1 mL of wastewater, and to the remaining 3 tubes add 1 mL of wastewater. Before you add your samples, make sure to label your tubes so that you remember the dilution of each tube. Replace the caps on your test tubes once finished, to help create an anaerobic environment.
2	Incubate your tubes at ~35°C for 24–48 hours.
3	Observe your test tubes. Those demonstrating both yellow liquid *and* gas in the Durham tube are positive results. Record your data in a table that you construct.
4	You will now begin the process of inoculating Petri dishes with bacteria from *only* the test tubes that had a double-positive (gas plus color change) result in the presumptive test. Make sure that the dishes are properly labeled to keep track of your samples (note: it is possible to divide each Petri dish into 3 or 4 subsections to conduct multiple tests, if needed). Flame sterilize an inoculation loop. Once cooled, dip it into the test tube to acquire a sample. Gently streak the sample onto the Petri dish. Repeat this step as necessary until all positive test tubes have been sampled and streaked. Make sure to flame sterilize, and then cool, your loop before obtaining each sample.
5	Place the cover on your Petri dish and store it upside down. These will now be incubated for ~24 hours at 35°C.
6	Observe your Petri dishes. Look for *E. coli* colonies, which will turn metallic green or blue-black. If a sample contains at least one *E. coli* colony, you count that as a positive result. This means that the test tube from which the sample originated contains *E. coli*. Of the 9 experimental test tubes that you originally started with, count which were positive for *E. coli*. You will use these data and the "most probable number" table to calculate the number of bacteria in your sample. Your instructor will ask each group to share their results so that data can be collated for the entire class.

Table 2. Lab materials and instructions for preparation.

Materials
• Phenol red lactose broth • Levine eosin-methylene blue (EMB) agar • Petri dishes • Pipettes • Test tubes • Test-tube caps • Test-tube racks • Durham tubes • Safety gloves • Incubator • Inoculator loops • Bunsen burner • Biohazard bag • 10% bleach solution
Instructions
Nine experimental test tubes are needed per group of students, plus one control. To reduce the amount of materials needed, 4 students per group is recommended.
Create the phenol red lactose broth using the easy-to-follow instructions on the package. Add 5 mL of the broth to 6 of the 9 experimental test tubes, plus the control. For the remaining 3, however, you want to use 5 mL of broth that is 1.5× strength. This is done to provide enough lactose for the tubes receiving 10 mL of the wastewater sample. Label these tubes as "1.5×". Thus, each group should have 7 tubes of normal strength broth and 3 tubes of 1.5× strength. An inverted Durham tube should also be placed in each test tube so that the open end of the tube is face down and the tube is at least halfway submerged in the broth. Then the test tubes should be autoclaved to sterilize them before use. The autoclave process will completely fill the Durham tubes with solution. Remove the test tubes from the autoclave and cover them with sterile test-tube caps. These caps will later be used to create an anoxic environment for anaerobic metabolism.

Table 2. Continued

Lastly, using the instructions on the package, prepare enough Levine EMB agar for 3 Petri dishes per group, plus a few backups. During the lab, ensure that students follow proper safety guidelines, including use of gloves when handling wastewater samples, proper disposal or sterilization of biohazardous waste (which includes anything that has touched wastewater; consult your institution's specific safety protocols), and disinfection of lab tables using a bleach solution.

Table 3. Most probable number (MPN) table (USDA, 2014) for different combinations of positive test tubes for sample volumes of 10 mL, 1 mL, and 0.1 mL, in that order. The MPN index provides a number of bacterial units (cells) per 100 mL. For example, if students confirmed that *E. coli* was present in 3 tubes containing 10 mL of wastewater, 2 tubes containing 1 mL of wastewater, and 1 tube containing 0.1 mL of wastewater, they would conclude that their sample has an *E. coli* density of 150 cells per 100 mL.

Combination of Positives	MPN Index per 100 mL	Combination of Positives	MPN Index per 100 mL
0-0-0	<3	2-2-0	21
0-0-1	3	2-2-1	28
0-1-0	3	2-2-2	35
0-1-1	6.1	2-3-0	29
0-2-0	6.2	2-3-1	36
0-3-0	9.4	3-0-0	23
1-0-0	3.6	3-0-1	38
1-0-1	7.2	3-0-2	64
1-0-2	11	3-1-0	43
1-1-0	7.4	3-1-1	75
1-1-1	11	3-1-2	120
1-2-0	11	3-1-3	160
1-2-1	15	3-2-0	93
1-3-0	16	3-2-1	150
2-0-0	9.2	3-2-2	210
2-0-1	14	3-2-3	290
2-0-2	2	3-3-0	240
2-1-0	15	3-3-1	460
2-1-1	20	3-3-2	1100
2-1-2	27	3-3-3	>1100

O_2, *E. coli* and similar bacteria metabolize lactose anaerobically and convert it into organic acids and gases (Leboffe and Pierce, 2011). Phenol red will change from red to yellow in the presence of such acids, whereas the gases collect in the Durham tubes. If gases are not present after 24 hours, incubate for another 24 hours. A change in color from red to yellow and the accumulation of any amount of gas in the Durham tube indicate that bacteria (potentially *E. coli*) are present. These two results, in concert, constitute a positive result for the presumptive test.

Now that students have identified bacteria that are potentially *E. coli*, they can proceed to the confirmative test. For only those test tubes that gave a positive presumptive test, students use a sterilized inoculation loop to obtain a water sample and inoculate a specially prepared Petri dish, which is then incubated (see Table 1). These dishes contain Levine eosin-methylene blue (EMB) agar, which will cause *E. coli* colonies to appear iridescent metallic green, or sometimes blue-black (Difco and BBL, 2009). This color change constitutes a positive result for the confirmative test. Other colonies of bacteria will not grow on the agar or will be a different color.

Lastly, students use the most probable number (MPN) table (Table 3) to enumerate the amount of bacteria in their samples. The MPN table is predicated on the number of test tubes that were found to have *E. coli*, not the number of colonies on the Petri dishes. Therefore, students must count the number of test tubes at each level of dilution (see Table 1) that were found to positively contain *E. coli* via both the presumptive and confirmative tests.

It is important to note that both the test tubes and Petri dishes can be refrigerated to arrest growth and activity while preserving the color changes and gas accumulation. This may be needed by instructors that do not see students daily. Also, once incubated, Petri dishes can be brought to the lecture room for analysis, as long as students safely handle them and disinfect their work spaces afterwards.

At the conclusion of this activity, students can be given an assignment to assess their understanding of bioremediation in wastewater treatment and the theoretical underpinnings of the multiple tube fermentation technique. Additionally, ask them to produce graphs of their results and to construct an evidence-based conclusion to evaluate their hypothesis.

Activity 3: Open Inquiry Laboratory Investigation

Next, students can participate in an open inquiry lab investigation using the multiple tube fermentation technique. Continuing to work in small groups, ask students to conduct a unique experiment in which they test their own water samples for the presence of *E. coli*. In addition to deciding what water sources to test, students can further personalize their experiment by evaluating other variables such as pH, turbidity, and nitrate levels. Student groups should independently pose research questions, form explanatory hypotheses, and create graphs of their predicted results. Students then plan their experimental design, which includes the location and method of extracting their water samples. Their experimental designs should be approved by the instructor, allowing that person to consider any potential safety issues, in addition to making sure that students use sterile technique. Once water samples are obtained using sterile containers, students test their hypotheses using the procedures previously described for the multiple tube fermentation technique.

With data collection concluded, student groups can be asked to give presentations. This provides students with the experience of communicating their findings to others by presenting their results, making evidence-based conclusions using their own data, consulting the scientific literature to support their conclusions, and reflecting upon the validity of their hypothesis. You can also ask students to offer suggestions for follow-up studies and to make connections to larger environmental, health, or sociopolitical issues.

Recommendations for Supplementary Activities

These activities can be followed by a tour of a municipal drinking water treatment facility. This provides an important opportunity for reflection as students compare treatment of wastewater and drinking water. It also provides another example of how science and technology impact their lives. The wastewater treatment activities can also be a springboard for further exploration into the field of bioremediation. Students can research other ways that bioremediation are used, or better yet, suggest their own novel applications.

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Appendix A: Background on Water Management

Water is the single most important substance for life. All living things rely on water for myriad purposes, including removal of wastes, transportation of nutrients, and as the solvent that enables metabolic reactions. Despite the critical importance of water to life, we as a society give little thought to how water resources are managed. A 2011 survey commissioned by The Nature Conservancy and conducted by independent polling agencies found that 77% of Americans could not correctly identify the source of their drinking water (The Nature Conservancy, 2011).

It is incorrectly assumed by some that freshwater supplies are practically inexhaustible. In reality, the overwhelming majority of water on this planet, approximately 97%, is saltwater (USGS, 2015 a) and therefore unfit for human consumption unless desalinated, an expensive and energy-intensive treatment (California Department of Water Resources, 2013). Just under 3% of water on Earth is freshwater, and of that, approximately 69% is sequestered in glaciers and ice caps (USGS, 2015 a). Therefore, of the total amount of water on Earth, only a tiny fraction is accessible and suitable for human consumption. Unfortunately, these freshwater resources are being heavily taxed by agricultural, residential, and industrial use. Most groundwater reserves, for example, are being depleted by humans due to withdrawals that exceed natural recharge rates from precipitation and infiltration through the soil (USGS, 2015 b; Famiglietti, 2014; Russo et al., 2014).

With a relatively small percentage of water on this planet fit for consumption, we must be deliberate about how we manage this limited and vital resource. This is especially true considering that freshwater reserves will continue to be stressed by a burgeoning human population that is expected to reach 9.7 billion by 2050 (UN, 2015) and by global climate change that can affect freshwater availability by increasing the duration and frequency of droughts in some locations (IPCC, 2013). For the sake of water conservation, it is imperative that people better understand how water resources are managed.