Elimination of Coliform Bacteria in Water via Filtration, Lime Juice, and Sunlight

Macy T. Lawrence[1]
Ty E. Lawrence[*2]
[1]*Canyon FFA, Canyon High School, Canyon, TX 79015*
[2]*Dept. of Agricultural Sciences, West Texas A&M University, Canyon, TX 79016*

ABSTRACT

This experiment evaluated methods to sanitize surface water with primitive supplies and minimal expense. Water samples were collected from a local park pond; initial coliform contamination of the raw source water equaled 1.43 ± 0.06 log CFU/ml. Raw water was sanitized via sand-filtration, lime juice, and sunlight in a completely randomized design using a 2 x 3 x 3 factorial treatment structure. One-half of the samples were filtered through a homemade sand filter, whereas the balance was unfiltered. Filtered and unfiltered samples were mixed with lime juice, at 0, 1, or 10% concentrations, and then exposed to two, four, or eight hours of direct sunlight. Sand-filtration did not reduce coliform counts (P = 0.39). A lime juice x sunlight interaction (P < 0.01) demonstrated poor water sanitation at the intermediate lime juice concentration (1%) and two hours of sunlight but notably improved levels of sanitation at lesser or greater lime juice concentrations and longer duration of sunlight. Raw water samples mixed with 10% lime juice or exposed to eight hours of sunlight were unable to grow colonies of coliform bacteria. This experiment demonstrates the ability to sanitize surface water using readily available fruit acid and ultraviolet light from the sun.

KEY WORDS: coliform, lime juice, sunlight, water

INTRODUCTION

Each day, in excess of a billion people struggle to find access to clean, safe drinking water (Hunter et al. 2010). Surface water sources are often contaminated with coliforms (Divya and Solomon 2016), a group of bacteria commonly defined as aerobic or facultative anaerobes that are gram-negative non-sporeforming rods with the ability to ferment lactose and produce gas and acid within 48h at 35 °C. Coliform bacteria commonly found in water typically include genera of *Citrobacter, Enterobacter, Escherichia, and Klebsiella* (An et al. 2002; Cabral and Marques 2006; Shittu et al. 2008).

Drinking water standards in the United States ensure safety of our water supply. The Safe Drinking Water Act was passed in 1974 and later amended in 1986 and 1996; this law set standards to prevent illness from our public water sources (EPA 2019). One of those standards indicates that drinking water must contain less than 1 CFU (colony forming unit) of coliform per 100 ml of water. To achieve this standard and many others, municipal water sources are commonly treated by coagulation and flocculation, sedimentation, filtration, and then disinfection (CDC 2019).

[*] Corresponding author: tlawrence@wtamu.edu

Previous research in drinking water sanitation has reported reduction of coliform bacteria biofilm in polyvinyl chloride pipes using citric acid (Tsai et al. 2003), reduction of *E. coli* in reclaimed water using chlorine and ultraviolet light (Montemayor et al. 2008), reduction of *E. coli* via hydrogen peroxide and silver (Pedahzur et al. 1995), as well as reduction of *E. coli* in dechlorinated tap water or surface water following titanium dioxide photocatalytic oxidation (Ireland et al. 1993). Sand filtration has been shown to improve water quality; Elliott et al. (2008) reported the ability of sand filtration to reduce *E. coli* by 1.9 $\log_{10}$. Limited research has been conducted using sand filtration in combination with citric acid and ultraviolet light to eliminate coliform bacteria and sanitize drinking water. Thus, the objective of this experiment was to determine if coliform-contaminated surface water could be sanitized into safe drinking water using citric acid from fresh limes and ultraviolet radiation from sunlight.

MATERIALS AND METHODS

Raw water sourcing. Two surface water sources (Southeast Park, Canyon, TX – 34°58'12.7"N 101°54'27.1"W; Ross Rogers Golf Course Lake, Amarillo, TX – 35°14'52.2"N 101°50'07.5"W) were sampled to determine which source contained greater quantity of coliform CFU per ml. Samples were collected in sterile specimen cups (item 77855, United States Plastic Corp., Lima, OH) and quantified for coliform by Food Safety Net Services (FSNS), Amarillo, TX. Coliforms were enumerated following AOAC Official Method 991.14. The initial samples from Southeast Park contained 27 CFU/ml, whereas the samples from Ross Rogers Golf Course Lake contained 11 CFU/ml. The experiment was conducted with 18 L of pond water collected from Southeast Park.

Sand filter construction. A homemade sand filter (Figure 1) was built from readily available materials. An inverted two-liter soda bottle was used as the container. Starting from the neck, two old, clean socks were placed in the bottom of the sand filter and two coffee filters were placed on top of the socks. Charcoal briquettes were crushed into a fine powder and a 5 cm layer was placed on top of the two coffee filters, and then two coffee filters were placed on top of the charcoal. A 5 cm layer of fine sand was placed on top of that, and then two more coffee filters on top of that. Lastly, a 5 cm layer of pea gravel was placed on top of that, and then two more coffee filters on top. Filtered reverse osmosis water (1.9 L) was poured through the filter to wash any existing contaminants from the pea gravel, sand, and charcoal.

Treatment application. Eighteen sterile specimen cups were filled with raw Southeast Park pond water (six with 100 ml, six with 99 ml, and six with 90 ml). Raw Southeast Park pond water (3.8 L) was poured through the sand filter. Following sand filtration, 18 sterile specimen cups were filled with filtered Southeast Park pond water (six with 100 ml, six with 99 ml, and six with 90 ml). Fresh squeezed lime juice was added to 12 raw water samples (six with 1 ml and six with 10 ml) and 12 filtered water samples (six with 1 ml and six with 10 ml) to complete each cup with 100 ml of total liquid volume. All samples were held overnight at ambient temperature (24 °C) in a sealed cardboard box to eliminate any UV light. Within each filtration by lime juice combination, two samples were exposed to two hours of direct sunlight (4-6 pm), two samples were exposed to four hours of direct sunlight (2-6 pm), and two samples were exposed to eight hours of direct sunlight (10 am-6 pm). Samples exposed to direct sunlight reached a maximum temperature of 32.4 °C.

After sunlight exposure, samples were sealed back into the dark cardboard box and were taken immediately to FSNS for coliform quantification.

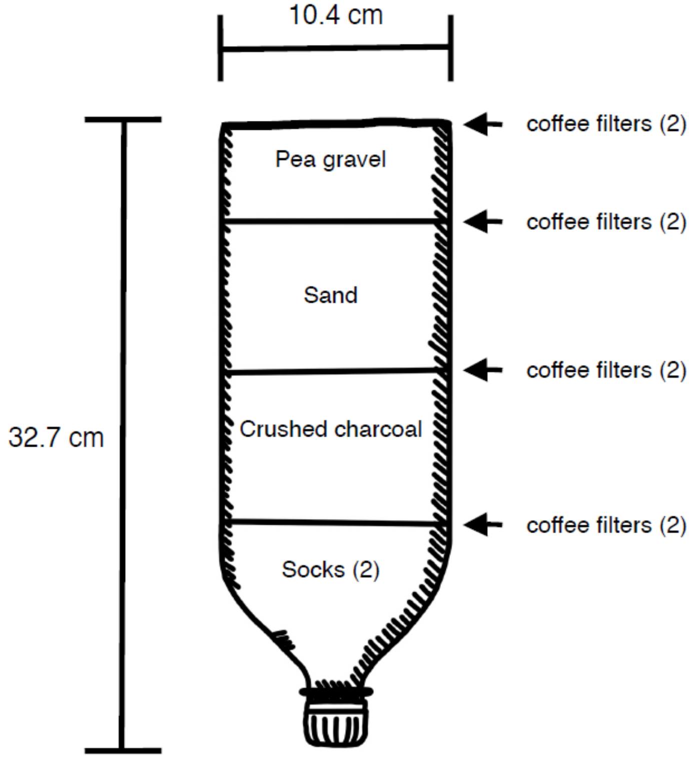

Figure 1. Artist representation of sand filter construction. Pea gravel was round-bodied and smooth in shape, approximately 3 to 9 mm in diameter. Sand was fine with a particle size of approximately 0.125 to 0.25 mm in diameter. Crushed charcoal particles were an extremely fine dust.

Statistical analysis. The experimental design was completely randomized, and the treatment structure was a 2 x 3 x 3 factorial; an individual specimen cup of water (n = 36) was the experimental unit. Coliform counts were analyzed with the GLM procedures of SAS (version 9.4; SAS Institute, Cary, NC). The fixed effects included sand filtration, lime juice concentration, and length of sunlight exposure; linear contrasts for lime juice concentration and length of sunlight exposure were also tested using the CONTRAST statement. Linear coefficients were calculated using the IML procedure.

RESULTS

The three-way interaction of sand filtration x lime juice concentration x sunlight exposure was not significant (P = 0.39, Table 1). Similarly, the two-way interactions of filtration x lime juice concentration (P = 0.35) and filtration x sunlight exposure (P = 0.47) were not significant. However, lime juice concentration x sunlight exposure displayed an interaction ($P < 0.01$; Figure 2 and Table 1). At a lime juice concentration of 0%, coliform counts were 1.75 log CFU/ml after two hours of sunlight exposure and decreased to 0.10 log CFU/ml after four hours of sunlight exposure and then were not detectable (0.00 log CFU/ml) upon eight hours of sunlight exposure. In contrast, a lime juice concentration of 1% resulted in coliform counts of 3.19 log CFU/ml after two hours of sunlight exposure which decreased to 0.00 log CFU/ml after four or eight hours of sunlight exposure. The lime juice concentration x sunlight exposure interaction is further explained by the main effect outcome of lime juice concentration. Lime juice concentration of 0% resulted in 1.28 log CFU/ml, which increased ($P < 0.01$) to 2.71 log CFU/ml at 1% lime juice concentration and then decreased ($P < 0.01$) to 0.00 log CFU/ml at 10% lime juice concentration.

Figure 2. Response of coliform bacteria in raw pond water to lime juice concentration and hours of sunlight exposure ($P < 0.01$).

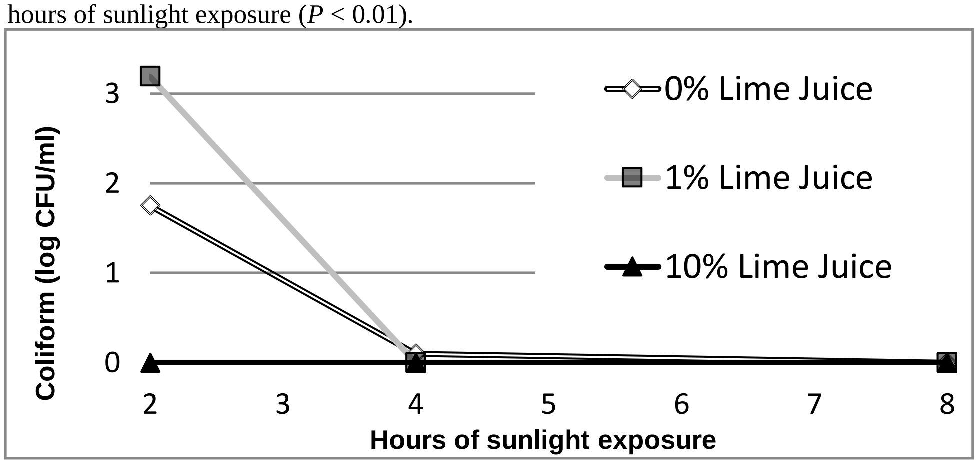

Table 1. Least squares means of coliforms (log CFU/ml) in raw pond water treated by sand-filtration, lime juice addition, and sunlight exposure.

Water treatment		**Log CFU/ml**	***P*-value**
Filtration			0.39
	Unfiltered	2.35	
	Sand filter	2.12	
Lime Juice			<0.01
	0%	1.28	
	1%	2.71	
	10%	0.00	
Sunlight Exposure			<0.01
	2 hours	2.73	
	4 hours	0.38	
	8 hours	0.00	
Filtration x Lime Juice			0.35
Filtration x Sunlight Exposure			0.47
Lime Juice x Sunlight Exposure			<0.01
Filtration x Lime Juice x Sunlight			0.39

Sunlight also reduced or eliminated ($P < 0.01$, Table 1) the coliform detection of samples. Coliform counts at two hours of sunlight exposure were 2.73 log CFU/ml, which decreased to 0.38 log CFU/ml at four hours of sunlight exposure and were eliminated to 0.00 log CFU/ml after eight hours of sunlight exposure. Sand filtration did not alter ($P = 0.39$) coliform counts (2.35 log CFU/ml for unfiltered vs 2.12 log CFU/ml for sand-filtered). Both lime juice concentration ($P = 0.05$) and hours of sunlight exposure ($P < 0.01$) were linear effects.

DISCUSSION AND CONCLUSIONS

These results demonstrate the ability to sanitize surface water using lime juice and sunlight, which is similar to results reported by Harding and Schwab (2012). Lime juice is approximately 4.9% citric acid (Penniston et al. 2008), which results in a pH of approximately 1.8 (Moutardier et al. 2015). Organic acids have the ability to inhibit bacterial growth and division via intracellular accumulation of anions (Carpenter and Broadbent 2009). In addition, sunlight is a natural source of ultraviolet light (Diffey 2002). The UV-a spectrum, which is the primary wavelength that reaches Earth's surface, is identified in the 315 to 400 nm wavelength range (WHO 2020). This wavelength range is particularly harmful to exposed skin of humans and is well known to be related to melanoma (Wang et al. 2001). The ultraviolet mechanism of action of water sanitation is via scrambling the DNA of bacterial cells, resulting in their inability to grow (Mehraj and Latha 2010).

The samples containing 1% lime juice experienced increased coliform bacteria growth, likely a result of the naturally occurring sugars found in lime juice. Lime juice is approximately 1.3% to 1.9% saccharide (summation of fructose, glucose, and sucrose (Rangel et al. 2011)). The 1% lime juice samples provided a food source for coliform bacteria, yet did not contain enough citric acid to kill the bacteria. In contrast, the 10% lime juice samples killed all coliform bacteria, demonstrating the ability of citric acid in the limes to sanitize surface water.

Because the sand filter did not reduce coliform counts, these results suggest that the sand filter was not necessary for coliform bacteria sanitation although it may have removed suspended particles in the water.

Future research should refine the minimum thresholds for concentration of lime juice and duration of sunlight needed to eliminate coliform bacteria. This could be accomplished by titrating lime juice concentrations at 1.5%, 3%, 6%, and 9% and developing a response curve. Likewise, duration of sunlight exposure could be tested in half-hour-long increments from four to eight hours and developing a similar curve. Further research should also be conducted to test the potential effectiveness of combinations of sunlight exposure and lime juice concentration against enteric viruses, protozoan parasites, and spore forming pathogens that may be present in surface water sources.

This study can positively impact those persons who have limited access to clean, safe drinking water by demonstrating that lime juice and sunlight can kill coliform bacteria and can be used as an inexpensive method to sanitize drinking water. The impact of these data could be significant for persons in developing countries, particularly those with limited access to clean drinking water.

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