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Article

Novel environmentally friendly approach to wastewater treatment eliminates aluminum sulfate and chlorination

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SUMMARY

Wastewater treatment uses harmful chemicals, including aluminum sulfate and chlorine. Aluminum sulfate, a common metal-based coagulant, can be toxic to both humans and aquatic life if ingested. Chlorine is used to disinfect wastewater by killing bacteria and other harmful microorganisms, and if ingested, chlorine and its byproducts can lead to respiratory irritation and cancer. The purpose of this research was to find equally efficient, environmentally safe alternatives to aluminum sulfate and chlorine water treatments. We investigated the effectiveness of zeolite, *Moringa oleifera* seed powder, and activated charcoal for wastewater filtration using common water contaminants and compared to the purification with aluminum sulfate. We tested lemon and orange peels as environmentally safe alternatives to chlorination by measuring the amount of bacterial growth suppression. We used inductively coupled plasma optical emission spectrometry (ICP-OES), colorimetric analysis (Lachat), bacteria colony counting, and scanning electron microscopy to analyze the effectiveness of these alternatives. For all the tested water contaminants and for analytical techniques used, our data demonstrated zeolite and charcoal were better or comparable to aluminum sulfate at removing the metals and nutrients tested. Lemon peels were very effective in suppressing bacteria growth, although further research is needed to compare chlorine to lemon peels in identical conditions to ensure that lemon peels could be a viable alternative to chlorination.

INTRODUCTION

Almost everything we do in our daily routine creates wastewater. The United Nations estimates that in North America alone, 18.7 trillion gallons of wastewater are produced annually (1). Out of that, 13.4 trillion gallons (75%) are treated and only 506 billion gallons (3.8%) of the treated wastewater are reused (1). Reclaimed, or reused, water can be useful for multiple purposes. According to the United States Geological Survey, reusing treated wastewater is important for two reasons: i) it provides much needed water particularly in dry environments, and ii) it relieves the pressure on our limited freshwater supply (2). In drought-prone states, reclaimed water is even being evaluated for use as potable water (3). To be reclaimed, wastewater goes through multiple treatments.

Normal wastewater treatment includes primary and secondary treatment. In primary treatment, screens and settling tanks remove most bigger solids from the wastewater. Next, the water goes into clarifiers to allow sludge and scum to be removed from the water. Primary treatment also includes the use of coagulants and flocculants, including aluminum sulfate (4). Aluminum sulfate is a commonly used metal-based coagulant, a compound that promotes the clumping of finer solids into larger floc so they can more easily be separated from the water (5). Even though aluminum sulfate is commonly used, it comes with a variety of health issues if ingested, ranging from brain changes characteristic of Alzheimer's disease to osteomalacia to hematopoietic disorders; the US Environmental Protection Agency (EPA) recommends a maximum allowable limit of 0.2 mg Al/L in drinking water (6, 7). Recently, the increased use of aluminum-based compounds, mainly aluminum sulfate, to manage phosphorus in freshwaters has received criticism. Overall, aluminum sulfate in our water sources can be dangerous and should be avoided for less hazardous alternatives (7).

For primary wastewater treatment, we tested the effectiveness of zeolite, *Moringa oleifera* seed powder, and activated charcoal compared to aluminum sulfate in removing contaminants of concern. Micronized zeolite has a unique honeycomb-like, crystalline structure that allows it to act as a filter (8). *M. oleifera* seeds act as a coagulant due to its positively charged, water-soluble proteins which bind to negatively charged particles creating flocs, which can be filtered out (9). Activated charcoal and charcoal have been used for centuries as a method of water purification. All three of these treatment materials are environmentally friendly and sustainable.

Secondary wastewater treatment uses bacteria to digest organic pollutants by forcefully mixing bacteria, wastewater, and oxygen, which helps the bacteria digest the pollutants faster. This treatment also removes about 90% of coliform bacteria in wastewater. To eliminate the last 10%, the wastewater goes through a process called chlorination (10). Chlorination is usually the last step in wastewater treatment before the treated water is released into waterways, such as rivers and streams. It is also the last step before treated wastewater is used for irrigation. Chlorination, a chemical disinfection method, uses chlorine to oxidize and disinfect wastewater. Chlorine, or chlorine-containing compounds, inactivate microorganisms by damaging their cell membranes.

Chlorine enters the cell and disrupts cellular respiration and DNA activity. This treatment method is common in wastewater treatment because it is inexpensive and efficient (8). However, chlorine is toxic not only to microorganisms but humans as well and can act as a respiratory and nasal irritant. Additional evidence suggests that there is a long-term cancer risk to drinking chlorinated water due to the trihalomethanes and other disinfection by-products of chlorination (10, 11). The ingestion of chlorinated water has been associated with increased risk of bladder, colon, and rectal cancers (11). In Europe, many communities have discontinued the use of chlorine due to these concerns (10).

Lemon peel and orange peel suppress bacterial growth because the peels contain vitamin C and citric acid (12). Vitamin C aids in the killing of bacteria through the Fenton reaction where ferrous iron reacts with hydrogen peroxide to generate ferric iron and an antibacterial reactive oxygen species (13). Citric acid also kills bacteria by acidifying the bacteria's environment and preventing the bacteria from absorbing essential nutrients, eventually leading to death (14). To find an environmentally safe alternative to chlorination, we tested the ability of lemon peel and orange peel at suppressing bacterial growth.

We hypothesized that these environmentally friendly and sustainable alternatives would be as, or more, efficient as aluminum sulfate and chlorine. To test the effectiveness of the alternatives, we compared their ability to treat and remove metals and compounds from wastewater. To simulate household wastewater, we used four common wastewater contaminants: laundry detergent, body wash, dish soap, and manure. Manure is not a common household contaminant but was chosen since it contains a significant amount of nitrogen. Ensuring that our proposed alternatives could remove nitrogen was crucial because of eutrophication. The eutrophication process occurs when there is too much nitrogen or phosphorus in water. These excess nutrients lead to dense algal blooms which block sunlight from reaching lower depths in natural waterways. Eventually, the algae die and the microbial decomposition leads to severe dissolved oxygen depletion, creating a 'dead zone' where most organisms cannot survive. Additionally, algal bloom can be toxic to humans and cases have been recorded in which humans have been poisoned by the toxic cyanobacteria (15).

The goal of our project was to find environmentally safe alternatives for both aluminum sulfate and chlorine. Zeolite and activated charcoal typically removed more ammonia, iron, and lead than the traditional aluminum sulfate. Zeolite was the most effective in removing ammonia. Activated charcoal was most effective at removing lead and iron but contributed additional phosphorus to the treated wastewater. For the alternatives to chlorination experiments, the lemon peel treatment was more effective than the orange peel treatment, but both displayed significant bacterial growth suppression compared to an untreated control.

RESULTS

Metal and nutrient removal from wastewater

To simulate wastewater, water was contaminated with one of the four following substances: dish soap, body wash, laundry detergent, or manure. Each sample contained only one of the "waste" substances. To test the environmentally friendly alternatives to aluminum sulfate, we combined these four different common waste products with zeolite, activated charcoal, *M. oleifera* seed powder, aluminum sulfate, or a control (no treatment).

We assessed effectiveness of wastewater treatment and analyzed water properties using an inductively coupled plasma optical emission spectrometry (ICP-OES) to detect metals and colorimetric analysis for nutrients (Lachat). The ICP-OES tested the effectiveness of the alternatives at removing metals from the simulated wastewater. We also used colorimetric analysis (Lachat) to measure the concentration of the nutrient ammonia from wastewater.

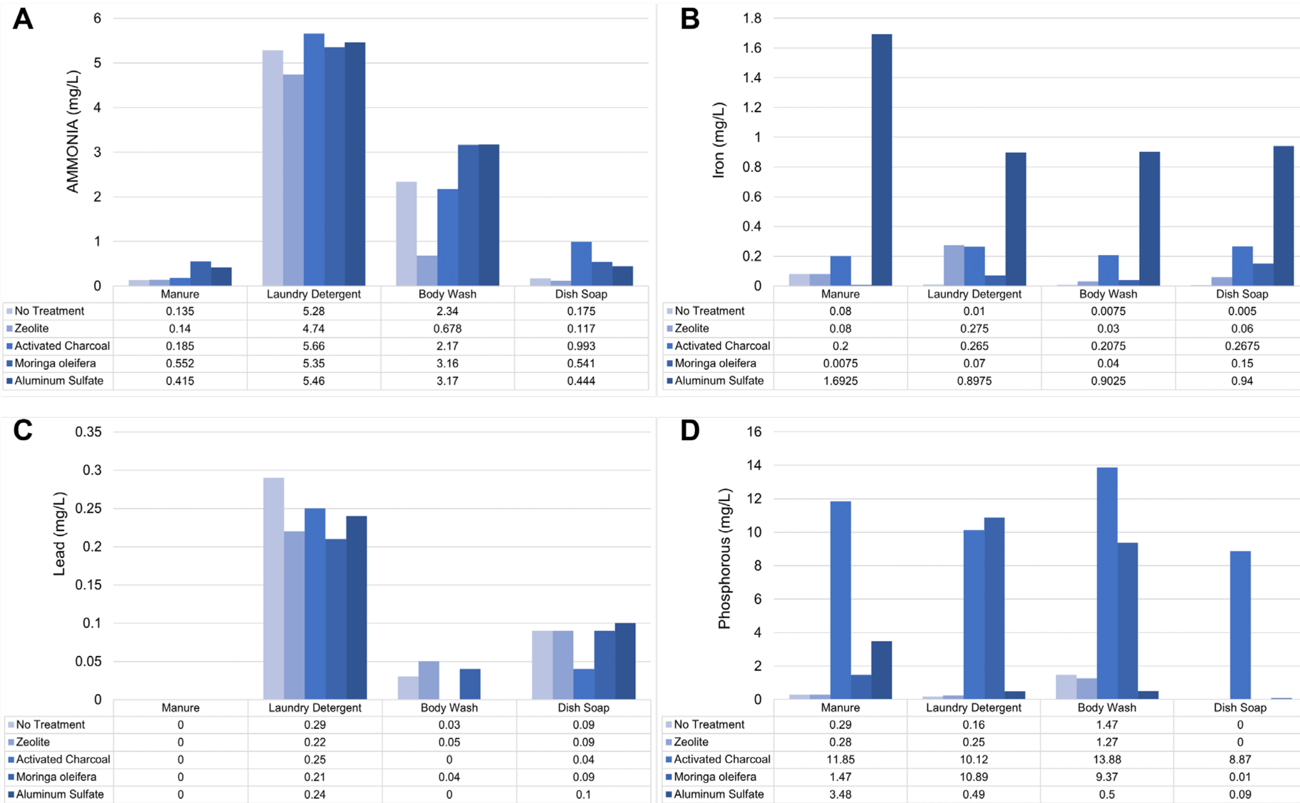
Initial experiments showed low concentrations of toxic metals, such as arsenic and lead, in the water and waste products used. To explicitly test for the ability of the proposed alternative treatments to reduce the concentration of these elements, we replicated the experiments while adding toxic elements (lead, cadmium, arsenic, selenium, zinc, copper, and silver) in levels twice that of the EPA regulatory limits to the initial starting simulated wastewater. The results of this experiment would demonstrate whether the environmentally friendly alternatives could remove toxic metals from wastewater to meet EPA standards.

The ammonia concentration of the samples treated by zeolite were lower than the concentrations of all other samples, including the no treatment and aluminum sulfate samples (**Figure 1**). The samples treated by activated charcoal, *M. oleifera*, and aluminum sulfate all had concentrations higher than those of the no treatment sample (**Figure 1A**). Iron was high in the aluminum sulfate samples, but lower in both the no treatment as well as the other treatments. The aluminum sulfate reagent used here contains iron. This contamination is visible in the laundry detergent, dish soap, and body wash condition. In the manure condition, manure was likely being broken down, leading to a higher concentration of iron than with the rest of the contaminants (**Figure 1B**). In most cases, treatments were effective at removing lead (**Figure 1C**). However, the samples treated by activated charcoal had the lowest concentrations of lead. The reduction in phosphorus across all treatments had mixed results (**Figure 1D**).

The ICP-OES also measured silver, arsenic, barium, beryllium, bismuth, cadmium, cobalt, chromium, copper, manganese, molybdenum, antinomy, selenium, thallium, and vanadium, but the concentrations of these elements in the simulated wastewater and treated water were below the detection limit for the ICP-OES.

Bacterial degradation in wastewater

To test alternatives of chlorination, in an experiment independent of the simulated waste products, we blended lemon peel or orange peel with water. We inoculated aliquots of the peel mixtures with *Escherichia coli* (*E. coli*) for 1 hour, 4 hours, 12 hours, and 18 hours. After the allocated time for each mixture, agar plates were plated with the peel and *E. coli* bacteria mixtures to incubate the *E. coli*. We utilized colony counting and scanning electron microscopy (SEM) to



(A)

	Manure	Laundry Detergent	Body Wash	Dish Soap
No Treatment	0.135	5.28	2.34	0.175
Zeolite	0.14	4.74	0.678	0.117
Activated Charcoal	0.185	5.66	2.17	0.993
Moringa oleifera	0.552	5.35	3.16	0.541
Aluminum Sulfate	0.415	5.46	3.17	0.444

(B)

	Manure	Laundry Detergent	Body Wash	Dish Soap
No Treatment	0.08	0.01	0.0075	0.005
Zeolite	0.08	0.275	0.03	0.06
Activated Charcoal	0.2	0.265	0.2075	0.2675
Moringa oleifera	0.0075	0.07	0.04	0.15
Aluminum Sulfate	1.6925	0.8975	0.9025	0.94

(C)

	Manure	Laundry Detergent	Body Wash	Dish Soap
No Treatment	0	0.29	0.03	0.09
Zeolite	0	0.22	0.05	0.09
Activated Charcoal	0	0.25	0	0.04
Moringa oleifera	0	0.21	0.04	0.09
Aluminum Sulfate	0	0.24	0	0.1

(D)

	Manure	Laundry Detergent	Body Wash	Dish Soap
No Treatment	0.29	0.16	1.47	0
Zeolite	0.28	0.25	1.27	0
Activated Charcoal	11.85	10.12	13.88	8.87
Moringa oleifera	1.47	10.89	9.37	0.01
Aluminum Sulfate	3.48	0.49	0.5	0.09

Figure 1: The concentration of ammonia, iron, lead, and phosphorous in simulated wastewater samples for different aluminum sulfate alternatives. (A) Bar graph showing mg/L of ammonia concentration in tested samples. Ammonia concentrations were measured through nutrient analysis by colorimetry buffered with sodium hydroxide to remain alkaline using a Lachat analyzer. **(B)** Bar graph showing mg/L of iron in tested samples. Iron was tested through inductively coupled plasma-optical emission spectrometry after being diluted to 0.32 M (~2% by volume) nitric acid by adding trace metal grade nitric acid. **(C)** Bar graph showing mg/L of lead in tested samples. Iron was tested through inductively coupled plasma-optical emission spectrometry after being diluted to 0.32 M (~2% by volume) nitric acid by adding trace metal grade nitric acid. **(D)** Bar graph showing mg/L of phosphorus in tested samples. Iron was tested through inductively coupled plasma-optical emission spectrometry after being diluted to 0.32 M (~2% by volume) nitric acid by adding trace metal grade nitric acid. **(A-D)** The color intensity of each bar indicates the type of aluminum sulfate alternative, while the different simulated wastewater contaminants tested are listed along the horizontal axis.

evaluate the effectiveness of the alternatives to chlorination. We counted the number of colony-forming units to determine if fewer *E. coli* bacteria grew in the treatments. We visualized how different peel treatments affected bacteria growth at a morphological level with the SEM.

The colony counts demonstrated that lemon peels are more effective than orange peels in suppressing bacterial growth. Based on SEM images, the lemon peel mixture had shorter and rounder *E. coli* cells than either the no treatment controls or the orange peel mixture (**Figure 2**). The orange peel inoculated condition had smaller cells with concave topography. The lemon peel mixture inoculated with the bacteria for one hour killed 97.3% of the bacteria relative to the untreated inoculum, while the orange peel mixture inoculated with the bacteria for one hour killed 72.3% of bacteria relative to the untreated inoculum (**Figure 3**). The bacterial growth suppression was best at one hour and became less effective over time. However, both the orange and lemon peel inoculations were always lower in the number of bacterial colonies than the untreated control. Due to limitations in time and access to instrumentation, we only visualized the morphology for the untreated control and the one-hour samples.

DISCUSSION

The results demonstrate the effectiveness of zeolite and activated charcoal as viable alternatives to aluminum sulfate. Lemon peels were more effective than orange peel but more research would be needed to ensure that lemon peel is as effective as chlorine in identical conditions.

These results support the use of zeolite as an environmentally friendly alternative to aluminum sulfate at removing metals from wastewater. Zeolite and activated charcoal are both biodegradable and were equally or more effective than aluminum sulfate in removing metal contaminants from water. However, activated charcoal added substantial amounts of phosphorus to the wastewater, which could be a barrier to its widespread implementation.

After treating the simulated wastewaters with the alternatives, we measured ammonia, lead, and iron concentrations in the treated samples to determine how well the alternatives remove chemicals and nutrients. We tested for lead because excessive amounts of lead may place adults at a higher risk for cancer, stroke, memory problems, kidney disease, and higher blood pressure (16). Ingesting lead also significantly increases the chance of brain development issues in children. We tested for iron because it is regulated

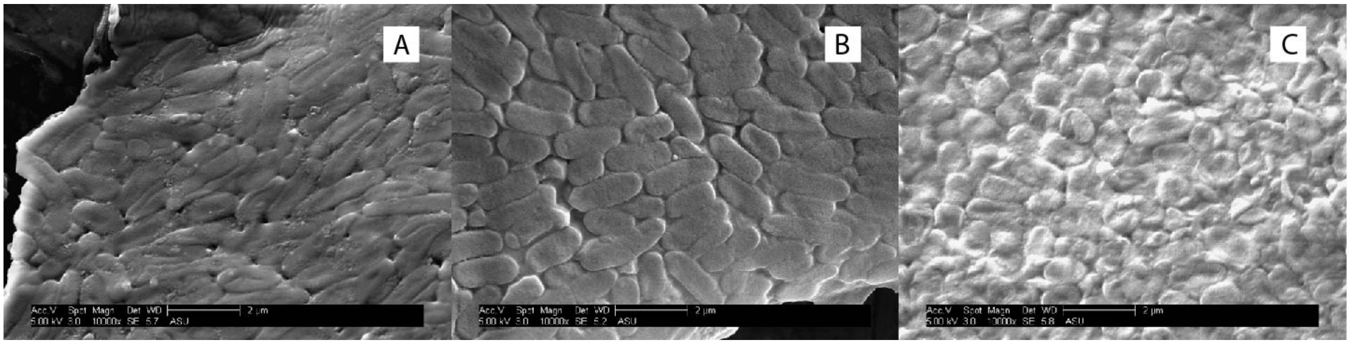
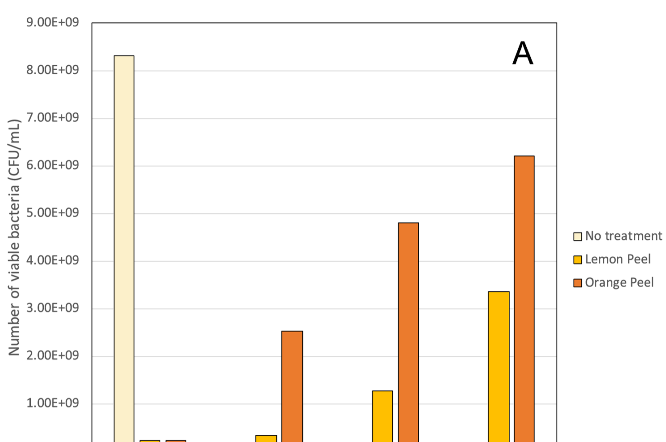


Figure 2: Morphological changes to E. coli bacteria cells with different alternate chlorination treatments. Bacteria cells were grown on agar plate. A SNE-4500M Scanning Electron Microscope was used to image the surface topography of the cultured E. coli. Cells at 10,000x magnification with 2 µm scale bar. Represented conditions include **(A)** no treatment (control), **(B)** chlorination alternative treatment (1 hour, lemon peel), and **(C)** chlorination alternative treatment (1 hour, orange peel).

as a secondary water standard and may lead to growth of bacterial biofilms in water systems (17). Phosphorus, like ammonia, is a limiting nutrient for plants and an excess of phosphorus in water released into waterways can cause eutrophication and harmful algae growth (17). In order to measure the ability of the treatments at removing metals to EPA limits, we doped the water with twice the EPA limits for lead, cadmium, arsenic, selenium, zinc, copper, and silver prior to treatment exposure.

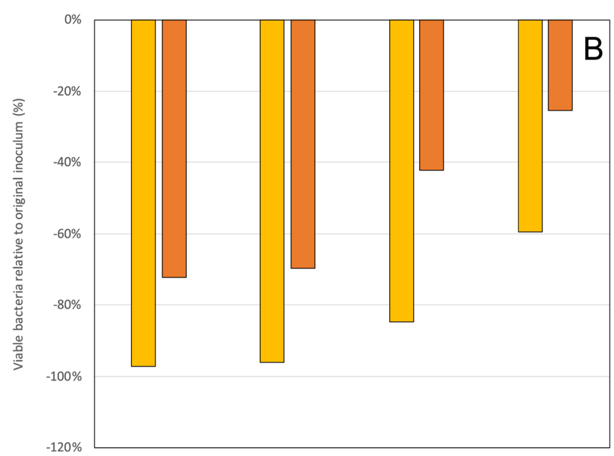
Additionally, *M. oleifera* seed powder has been researched in the past as a water filtration method (9). Although *M. oleifera* seed powder added ammonia to the samples, the ammonia added was still under the EPA limit of 17 mg/L ammonia. Some of the samples indicated that charcoal added a significant amount of ammonia and phosphorus to the sample, but the apparent addition of ammonia could be due to an analytical artifact from the opacity of the charcoal samples. Colorimetric analysis shines light through the samples and measures the relative absorbance of specific wavelengths of light. However, because charcoal is nearly opaque, light may have been absorbed by other compounds, making the ammonia results for the activated charcoal seem abnormally high. Regardless, the ammonia concentrations of all the samples treated by activated charcoal were still under EPA limits. However, the opacity of the charcoal samples did not affect the ICP-OES results since the analytical technique measures element-specific optical wavelengths emitted after ionization and excitation, suggesting that the elevated phosphorus concentrations in these samples were reflective of addition of phosphorus from the activated charcoal. In the manure samples, aluminum sulfate seemed to add a significant amount of phosphorus. This is likely because aluminum sulfate caused the manure to break down, leading to more free phosphorus in the mixture. Overall, zeolite and aluminum sulfate were the most effective at removing phosphorus from the samples.

Evaluation of environmentally more sustainable options for secondary treatment to reduce bacterial growth is an independent issue from primary treatment; we evaluated lemon and orange peels for their effectiveness in suppressing



	1	4	12	18
No treatment	8.30E+09			
Lemon Peel	2.20E+08	3.30E+08	1.27E+09	3.36E+09
Orange Peel	2.30E+08	2.52E+09	4.80E+09	6.20E+09

Hours



	1	4	12	18
Lemon Peel	-97.3%	-96.0%	-84.7%	-59.5%
Orange Peel	-72.3%	-69.6%	-42.2%	-25.3%

Hours

Figure 3: Effects of treatments on E. coli bacteria. The number of bacteria were estimated after extrapolation from measurement of colony counting in a representative unit of the agar plate. **(A)** The number of viable bacteria after the treatments and **(B)** the percent viable bacteria relative to the original inoculum control varied over time and treatment.

bacterial growth as well as any morphological deformations of the *E. coli* cells. Lemon and orange peels are known for their antibacterial properties in food and health, but they have not been tested previously as alternatives for chlorination in wastewater treatment (12). For the alternatives to chlorination experiments, we cultured bacteria after exposing them to lemon and orange peel to calculate their effectiveness on bacterial growth suppression. In the future, we recommend testing more nutrients, volatile organic compounds, and harmful compounds, as well as directly comparing the alternatives to chlorination.

Further research needs to be done to assess availability of these environmentally friendly alternatives for large scale utilization. Additional testing would also be useful to measure the effectiveness of zeolite and charcoal on other elements and volatile organic compounds. More testing would also demonstrate how to optimize the lemon peel mixture to make it even more effective. While lemon peel was extremely effective at suppressing bacteria growth under the conditions tested, additional tests need to be done with chlorine in the same conditions to ensure that lemon peel is comparable or more effective than chlorination. Finding a way to incorporate zeolite, charcoal, and lemon peels into the wastewater treatment process in a cost-effective manner would also require further research.

Overall, these results have the potential to be useful to wastewater treatment plants and local government agencies focusing on making wastewater treatment processes more environmentally friendly. The dangers of aluminum sulfate and chlorination are widely known and the use of both has been criticized. This project demonstrated alternatives to these chemicals that are environmentally friendly and effective at laboratory scale. Future testing should include testing of the conventional treatments of chlorination in similar laboratory conditions to the proposed alternatives. With additional process-scale testing, these alternatives could eventually be implemented into wastewater treatment processes.

MATERIALS AND METHODS

Metal and nutrient reduction in wastewater experiment

For the environmentally friendly alternatives to aluminum sulfate experiments, we filled 20 glasses with 1 cup of room temperature tap water each. In 5 of the cups, we added 2 teaspoons of manure as a simulated wastewater contaminant. In one of the cups with manure, we added 0.75 teaspoons of crushed zeolite powder (KMI Zeolite Inc). In another cup with manure, we added 0.75 teaspoons of activated charcoal (Relay Peak Research, LLC). We repeated this with the last three cups, adding *M. oleifera* seed powder (SoloDerma, LLC), to one of the cups, adding aluminum sulfate to another cup, and leaving the last cup without a treatment to act as a control (**Table 1**). We repeated this process with the three other simulated wastewater contaminants: laundry detergent, body wash, and dish soap (**Table 1**). Two teaspoons of contaminants and 0.75 teaspoons of each of the treatments were added similar to the manure experiments. By the end, each cup had one contaminant and one treatment, except the 5 cups that were kept as controls with no treatment (**Table 1**). These samples were incubated for 12 hours at room temperature. Then, we used a pipette to transfer the treated water into a separate cup without the settled powder or any floating waste.

Sample Number	Contaminant	Treatment
1	Dish Soap	None (Control)
2	Dish Soap	Zeolite
4	Dish Soap	Activated charcoal
5	Dish Soap	*M. oleifera* seed powder
6	Dish Soap	Aluminum Sulfate
7	Body Wash	None (Control)
8	Body Wash	Zeolite
10	Body Wash	Activated charcoal
11	Body Wash	*M. oleifera* seed powder
12	Body Wash	Aluminum Sulfate
13	Laundry Detergent	None (Control)
14	Laundry Detergent	Zeolite
16	Laundry Detergent	Activated charcoal
17	Laundry Detergent	*M. Oleifera* seed powder
18	Laundry Detergent	Aluminum sulfate
19	Manure	None (Control)
20	Manure	Zeolite
22	Manure	Activated charcoal
23	Manure	*M. Oleifera* seed powder
24	Manure	Aluminum sulfate

Table 1: Design of experiments of the metal and nutrient reduction in wastewater experiment. Table lists the sample numbers (laboratory identification numbers), contaminants, and treatments used in metal and nutrient reduction in wastewater experiment.

Metal measurement by ICP-OES

After being acidified to prevent floculation or precipitation, we diluted samples to 0.32 M (~2% by volume) nitric acid by adding trace metal grade nitric acid (catalog# A509P212, Fisher Scientific, Fair Lawn, NJ, USA). The metals were measured by ICP-OES (Agilent 5900 ICP-OES, Santa Clara, CA, USA) at the Metals, Environmental, Terrestrial Analytical Laboratory (METAL) at Arizona State University. Elemental concentrations were calibrated using a blank and four multi element ICP standards spanning the range of samples. An internal standard for instrumental drift correction of yttrium at 5 ppm was added to all blanks, standards, and samples using a Y-connection on the ICP-OES instrument. Check standards and instrumental blanks were analyzed every ten samples.

Nutrient analysis by colorimetry

Nutrient analyses for the concentration of ammonia, nitrate, and nitrite were analyzed by colorimetric measurement using a Lachat Quick Chem 8000. The colorimetric reactions were buffered with sodium hydroxide to keep the solutions alkaline. The absorbance at 630 nm is directly proportional to the abundance of ammonia in the sample. A calibration curve of a blank and seven standard solutions in the range of 0.1 to 20 mg N/L was used to quantify the amount of ammonia. A blank and continuing calibration verification (CCV) standard were analyzed every ten samples for quality control and verified that the blank remained below the detection limit.

Bacterial reduction experiment

For the environmentally friendly alternatives to chlorination experiments, we measured 50 grams of fresh lemon peel and 100 ml of water, which were then blended into a smooth paste using a Vitamix blender. We prepared four containers with 2 teaspoons of the peel-water mixture. Containers were labeled 1 hour, 4 hours, 12 hours, and 18 hours (**Table 2**). We mixed a swab of *E. coli* bacteria in each container and started a timer. After one hour, we swabbed and spread the mixture from the container labeled one hour onto an agar plate. At this point, we also spread a swab of the bacteria onto an agar plate without any treatment to act as the control to measure bacterial growth suppression against. After four hours, we swabbed and spread the mixture from the container labeled four hours onto an agar plate. We repeated this process at 12 and 18 hours with the other two containers. The colony counting was also staggered, to account for the difference in times after the mixtures were plated. Twelve hours after each mixture was plated, we counted the colonies on a portion of the agar plate. The agar plate was placed over a lined grid to divide it visually into equal subdivisions. Between 50 and 250 colonies were counted to obtain a representative number of colonies within the grid subdivisions. The number of colonies per mL was estimated from the measurement of this subsample. This process was repeated using orange peel instead of lemon peel (**Table 2**).

Scanning Electron Microscopy

We also examined the lemon and orange peel agar plates with the 1-hour mixtures and the control using SEM at the Eyring Materials Center at Arizona State University. The purpose of the imaging was to look at any morphological change or deformation of individual cells. A SNE-4500M Scanning Electron Microscope was used to image the surface topography of the cultured *E. coli* after being sputter-coated with gold to improve the conductivity of the sample. Samples were imaged at 10,000X magnification using a 5 kV accelerating voltage and a secondary electron detector.

Sample Number	Treatment	Time spent in treatment
1	Bacteria	None (Control)
2	Lemon Peel	1 hour
3	Lemon Peel	4 hours
4	Lemon Peel	12 hours
5	Lemon Peel	18 hours
6	Orange Peel	1 hour
7	Orange Peel	4 hours
8	Orange Peel	12 hours
9	Orange Peel	18 hours

Table 2: Design of experiments of the bacterial reduction experiment. Table lists the sample numbers (laboratory identification numbers), treatments, and time spent in treatment for bacterial reduction experiments.

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