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Article

A Study on the Coagulating Properties of the *M. oleifera* Seed

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SUMMARY

Water is an essential part of our lives, but many people struggle to obtain enough clean water to meet their basic needs. However, the solution to this problem may be right in their backyard. *Moringa Oleifera* (*M. oleifera*) seeds contain a coagulating protein that can coagulate and remove particles in water. The purpose of this study was to analyze the coagulating efficiency of the seed and to test a new and innovative way to utilize the seed. When the part of the seed responsible for the coagulating properties was isolated and combined with sand, a new functionalized-sand (f-sand) was created. This research aimed to determine how effectively the *M. oleifera* Cationic Protein (MOCP) functionalized-sand reduces the amount of bacteria compared to other filtration techniques. We accomplished this by testing six different types of contaminated solutions before and after four different filtration techniques: plain sand, f-sand, f-sand with cilantro, and the normal moringa technique, which utilizes only moringa seed powder. We concluded that the MOCP f-sand removed the largest percent of bacteria, and when used in combination with cilantro, it was more efficient than the other techniques. This can have a large impact, especially in developing areas, as it provides a way to have clean water while also helping the economy.

INTRODUCTION

M. oleifera, otherwise known as *Moringa Oleifera*, is a fast growing, highly valued plant found in various parts of the world. It flourishes in tropical climates but can grow anywhere. The *M. oleifera* plant is native to northern India and different parts of Africa (1) . The plant is very resilient and produces leaves even during times of drought. This plant has many uses. The whole plant, including the bark, pods, leaves, nuts, seeds, oil, tubers, roots, and flowers, is edible and contains important nutrients essential to our well-being (2). The bark can be used as an appetizer, and it can also be used to treat sores, skin infections, and intestinal spasms. The pods are normally cooked in a broth, and the flesh inside is eaten. The leaves have many medicinal properties, and the whole plant has been said to have anti-inflammatory properties (3). However, the most astonishing capability of this plant is its use as a water filtration system. When the oil is pressed from the seeds of the moringa plant, seed cakes are left over as a waste product. These seed cakes can be used to filter water (4). Data collected as part of the Global Learning and Observations to Benefit the Environment (GLOBE) initiative funded by NASA shows a steady decrease in the water transparencies in the US over the last few years (5). If this trend exists in a developed country, water quality is likely an even bigger issue for developing countries as they deal with a large population and increased competition for limited natural water resources.

The utility of *M. oleifera* in water filtration may provide a sustainable solution to this issue. Moringa seeds contain a protein, the *Moringa Oleifera* cationic protein (MOCP), which has natural cationic polyelectrolytes that cause coagulation (6). Coagulation has been referred to as the most important step in water filtration, even more so than physical filtration (7). Coagulation is the reduction of electrical potential between particles in the water like microorganisms, colored particles, and clay in a way that agglomerates them to form large structures, referred to as “flocs” which then can be removed by physical filtration (8). The most common coagulants used in wastewater treatment are aluminum salts (alum), ferric and ferrous salts, lime, and cationic, anionic, and non-ionic polymers. While there are many synthetic coagulants, the chemicals found in them are not ideal, and substantial energy is required to produce them. Additionally, wide dispersal of the coagulants to areas in need has proven to be difficult. The ability of a chemical to cause coagulation is based on the charge and size of the coagulant. The coagulant in *M. oleifera*, MOCP, works with negatively charged particles, including most bacteria. Additionally, the MOCP goes a step further than the synthetic coagulants by killing the microorganisms in the water by fusing and damaging the cell membranes during coagulation (9).

M. oleifera grows in the places where people are in the most need of the advantages it offers, making it the perfect substitute for primitive and unsanitary water filtration systems. However, when moringa powder is added to the water by itself, it leaves behind organic matter in the water which encourages the growth of new bacteria, limiting long-term efficiency. This can be fixed by utilizing f-sand. When an anionic sand is mixed with an *M. oleifera* extract then rinsed, the MOCP protein adheres to the sand. The sand now has the same coagulation properties as the seed cake, but it does not leave behind the organic matter that promotes bacteria growth, making it a more efficient solution. Over the past few decades, most of the research conducted on *M. oleifera* tested its usage on the removal of turbidity (10, 11). However, there has not been a lot of research on the reduction of bacterial load in different sources of drinking water. This is specifically important as many people die due to unsanitary water.

In fact, 289,000 children under 5-years-old die each year due to diarrheal diseases caused by poor water quality and sanitation (12). Specific pathogens, including *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella*, *Campylobacter*, and *Legionella*, can cause different waterborne diseases that may be fatal, especially to people who do not have access to a nearby hospital. Due to the antimicrobial properties of the MOCP, water filtered by the f-sand also contains fewer biological contaminants. Water with fecal contamination is not deadly by itself, but the presence of fecal coliforms can indicate the presence of other pathogenic bacteria. If water contains fecal coliforms, then the water has come into contact with some form of fecal matter, either from a human or an animal. Fecal matter may also contain pathogens or viruses that can cause diseases, such as gastroenteritis, typhoid, and hepatitis A. Thus, it is important that drinking water does not contain fecal contamination because it may be a potential health risk. An additional filtering agent that we tested in this research was cilantro, which is known to have heavy-metal filtering properties. A recent study conducted by researchers in Kenya found that cilantro stems and leaves were capable of removing lead and cadmium ions from water (13). We can create a more efficient filtration system by combining both moringa and cilantro.

Based on the results of the aforementioned research, We hypothesized that the MOCP f-sand and the cilantro filter would more effectively reduce bacteria and other contaminants in water compared to plain sand or the normal-filtration technique. The impact of Moringa in developing countries is very important, as it naturally grows in these areas and can provide an easier way to clean water.

RESULTS

In this study, we gained a greater understanding of the *M. oleifera* seed and its efficiency in different forms on the reduction of bacteria. In order to ensure that the same amount of bacteria was added to each sample, the same serial dilution was conducted on each sample of bacteria. Before filtering, the water samples were thoroughly vortexed in order to ensure that the bacteria were spread evenly in the sample. Then, the same volume of each of the water samples was put through each filtering method. The bacteria strains used include *E. coli*, *Enterobacteria aerogenes*, *Citrobacter freundii*, and *Serratia* liquefaciens. In addition to testing natural water and turbidity, lake water and kaolin clay water were tested respectively. The results support the hypothesis because the MOCP f-sand and cilantro filter technique reduced the growth of the pathogenic bacteria more than any other filtration technique, with a 73.56% average reduction in bacterial colonies when compared to the water samples before filtering.

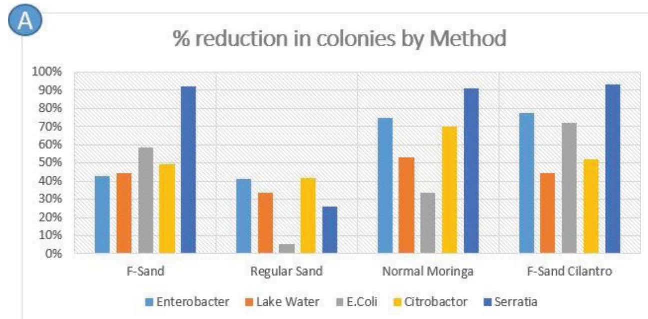

Figure 1. Bacterial Reduction Data. This graph represents the percent reduction of colonies when compared to the original bacteria mixture before it has gone through any treatments. Yellow represents *Citrobacter freundii* bactiera, light blue represents *Enterobacteria aerogenes*, gray represents *E. coli*, dark blue represents *Serratia* liquefaciens, and orange represents lake water. Labels of the x-axis represent the various water treatment methods. The f-sand cilantro technique showed the highest average percentage reduction.

With the plain sand technique, there was a 41.5% reduction of *Citrobacter* cells, 33.33% reduction of lake water bacteria, 25.76% reduction of *Serratia* cells, 41.3% reduction of *Enterobacter* cells, and 5.55% reduction of *E. coli* cells, with an average percent reduction of 34.09%. With the normal moringa technique, there was a 66.5% reduction of *Citrobacter* cells, 53.33% reduction of lake water bacteria, 91.15% reduction of *Serratia* cells, 73.04% reduction of *Enterobacter* cells, and 33.33% reduction of *E. coli* cells, with an average percent reduction of 72.66%. With the f-sand technique, there was a 49.06% reduction of *Citrobacter* cells, 44.44% reduction of lake water bacteria, 92.31% reduction of *Serratia* cells, 42.60% reduction of *Enterobacter* cells, and 38.33% reduction of *E. coli* cells, with an average percent reduction of 61.68%. With the f-sand and cilantro technique, there was a 51.88% reduction of *Citrobacter* cells, 44.44% reduction of lake water bacteria, 93.08% reduction of *Serratia* cells, 77.39% reduction of *Enterobacter* cells, and 72.22% reduction of *E. coli* cells, with an average reduction of 73.56% (**Figure 1**).

Enterobacter was more efficiently removed using the

Table 1.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
E.Coli	5	61	12.2	130.2
Serratia	5	786	157.2	13315.7
Enterobacter	5	543	108.6	5231.8
Citrobactor	5	450	90	3016
Lake Water	5	79	15.8	88.2
F-Sand	5	483	96.6	8050.8
Regular Sand	5	267	53.4	1807.3
Normal Moringa Technique	5	593	118.6	9511.8
F-Sand Cilantro	5	576	115.2	9267.2
Unfiltered	5	0	0	0

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows - Bacteria Type	77718.96	4	19429.74	8.440960275	0.000734	3.006917
Columns - Filter Type	50298.16	4	12574.54	5.462821048	0.005727	3.006917
Error	36829.44	16	2301.84			
Total	164846.56	24				

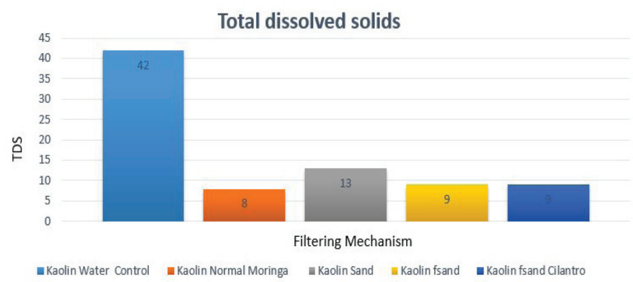

Figure 2. Total Dissolved Solids Data. This graph represents the total dissolved solids (TDS) measured in the kaolin clay water after each treatment. The legend below the graph shows what color each treatment method is correlated to. The normal moringa, f-sand, and f-sand and cilantro techniques all showed a similar reduction in TDS.

normal Moringa technique (172 colonies fewer than the non-filtered control) and f–sand cilantro (178 colonies) compared to the control sand treatment using regular sand (95 colonies), but f–sand alone (98 colonies) did not effectively remove *Enterobacter* compared to the control sand. *Citrobacter* was more efficiently removed using the normal Moringa technique (148 colonies), the f-sand and cilantro technique (110), and f–sand (104 colonies) compared to the control treatment of regular sand (88 colonies). Lake water bacteria were more efficiently removed using the normal Moringa technique (24 colonies) compared to the control treatment of regular sand (15 colonies), but f–sand alone (20 colonies) and f–sand cilantro (20 colonies) were not as effective at removing Lake Water Bacteria compared to the control sand. *E. coli* bacteria were more efficiently removed using the normal Moringa technique (12 colonies), the f-sand and cilantro technique (26 colonies), and f–Sand (21 colonies) compared to the control treatment of regular sand (2 colonies). *Serratia* bacteria were more efficiently removed using the normal Moringa technique (237 colonies), the f-sand and Cilantro Technique (242 colonies), and f–Sand (240 colonies) compared to the control treatment of regular sand (67 colonies). (**Table 1**) Also, total dissolved solids (TDS) improved across all filtering methods, with f-sand method exhibiting the highest clarity with TDS at eight points (**Figure 2**).

The data collected from this experiment resulted in a number of interesting findings. First of all, the f-sand and cilantro technique reduced the growth of bacteria better than the other techniques on average, with an average percent reduction of bacterial colonies of 73.56% (**Figure 1**). However, the normal *M. oleifera* technique performed better on the *Citrobacter* solution specifically. The sand technique removed about 41.5 %, and the f-sand technique removed about 49.06% of the colonies. The f-sand and cilantro method and the normal moringa method give similar results on average, showing that the f-sand contains the coagulant, but does not contain any organic material that might contribute to further contamination after some time. Two-factor ANOVA analysis of bacteria types and filtration methods revealed that the bacterial reduction of different bacteria types using different techniques was statistically significant (**Table 2**). MOCP f-sand does reduce the number of bacteria in water, and when used in combination with cilantro, it was more efficient than the other techniques tested.

Table 2.

Colonies Inhibited based on Day 10 Control					
Bacteria	F-Sand	Regular Sand	Normal Moringa Technique	F-Sand Cilantro	Average
E.Coli	21	2	12	26	**15**
Serratia	240	67	237	242	**197**
Enterobacter	98	95	172	178	**136**
Citrobactor	104	88	148	110	**113**
Lake Water	20	15	24	20	**20**

DISCUSSION

In this study, we investigated the effectiveness of MOCP f-sand at removing bacteria from water compared to other filtration techniques. We hypothesized that a filtration technique combining MOCP f-sand and cilantro would be the most effective at removing bacteria due to its coagulating and water-softening qualities. The results supported the hypothesis since the MOCP f-sand and cilantro filter technique reduced the growth of the pathogenic bacteria more than any other filtration technique, with an average reduction of bacterial colonies of 115.2. This result was produced because the coagulating properties of the protein was best utilized in the form of f-sand. The same properties that the *M. oleifera* powder contain are also in the f-sand, but without the negative side effect. The regular sand technique did not work as well, due to the absence of the organic coagulant. The normal moringa method performed well; however, it contained raw organic material that may have contributed to the bacterial growth after a certain time period.

For a filter created with the MOCP f-sand to be optimal for use in Third World countries, there are some issues that must be addressed. For example, the f-sand would not be able to function for long periods of time as the MOCP would get washed away. Additionally, bacteria or algae or molds might grow on the filter, especially in warm climates. The water going through the filter contains bacteria, fungi, protozoans, parasites and organic matter which could contaminate the filter, limiting the effectiveness of the filter. This means that the filter would have to go through manual cleaning periodically. However, manual cleaning of the filter would not be an obstacle for implementation in areas where sustainable water filtration is needed.

There were a few limitations experienced during this study. Due to the lack of funding, it was not possible to complete many trials in larger quantities. Additionally, since there was no access to a regulated research laboratory, many of the items required to complete a more in-depth analysis were not accessible. For this reason, the metal content in the water was not tested. Time was another constraint. Since this research was conducted in a high school lab, the only time available was a few hours after school, so it was difficult to run multiple trials. One of the errors encountered had to do with the bacterial culture. The nutrient agar slant was purchased instead of the liquid form, which created a need

Solution Type Label	Solution Description
A	100ml of water collected from Lake. After 48 hours, 0.1 ml of the water was plated on a clean agar plate using a sterile cotton swab. The zig-zag method was utilized while plating as it has been proven to be the most effective plating method. While plating, extra care was taken to ensure that the agar plate is open for the least amount of time possible as to reduce the chance of airborne contaminants landing in the agar
B	The second solution was made with kaolin clay to create a solution with a synthetically produced amount of turbidity. To create the turbidity solution, 0.5 grams of White Kaolin clay powder was added to 100 ml of the deionized water. The solution was stirred for one hour and then allow it to settle for 24 hours. Then, the supernatant was removed, and the TDS of the solution was tested. The final water sources were prepared similarly, each using a different pathogenic bacteria strain listed below
C	Solution developed as outlined in B with *E.coli*
D	Solution developed as outlined in B with *Enterobacteria Aerogene*
E	Solution developed as outlined in B with *Citrobacter Freundii*
F	Solution developed as outlined in B with *Serratia Liquefaciens*

Figure 3. Total Dissolved Solids Data. This is the list of solutions prepared for testing using different treatment methods and different bacteria types.

for an adjustment of the dilution process. If we repeated this study, a more detailed analysis of the solution, including lead content and the presence of other heavy materials, would be performed. Additionally, it would be valuable to test different types of synthetic coagulants. MOCP f-sand shows great promise for removing pathogenic bacteria from water. As an affordable, effective and natural coagulant, it is suitable for use in developing countries.

METHODS

Four different types of bacteria, *E. coli*, *Enterobacteria aerogenes*, *Citrobacter freundii*, and *Serratia* liquefaciens (Carolina Biological), were diluted and added to water individually to artificially create contaminated solutions. Then, the contaminated water was filtered in four different ways: using plain sand, the normal *M. oleifera* technique, f-sand, and f-sand with cilantro. Additionally, the ability of these filtration methods to reduce turbidity was also tested, by manually adjusting the turbidity of a solution and testing it before and after filtration.

The first step was to prepare the water mixtures. The first water source was lake water. (**Figure 3A**) 100 mL of water was collected in a sterile container from a nearby water source. After 48 hours, 0.1 mL of the water was plated on a clean agar plate using a sterile cotton swab. The second solution was made with kaolin clay to create a solution with a synthetically produced amount of turbidity (**Figure 3B**). To create the turbidity solution, 0.5 grams of white kaolin clay powder was added to 100 mL of the deionized water. The solution was stirred for one hour and then allowed to settle for 24 hours. Then the supernatant was removed and the total dissolved solids (TDS) of the solution was measured. The water sources containing bacteria were prepared similarly, each using a different pathogenic bacteria strain: *E. coli* (C), *Enterobacter*ia aerogene (D), *Citrobacter freundii* (E), and *Serratia* liquefaciens (F). To create these water solutions, a serial dilution was conducted. Using a clean, sterile, and dry pipet, 0.1 mL of the bacteria sample was added to 9.9 mL of distilled water and mixed thoroughly. Using a new pipet, 0.1 mL from Tube 1 was removed and added to Tube 2, which contained 99 mL of distilled water. Each filtration method had a sample size of five for each solution type (**Figure 3**).

The second step of the study was to design the filter. To create the filter, all of the polyvinyl chloride (PVC) pieces, the muslin cloth and the PVC adhesive were used. First, the 3-inch diameter PVC tube was cut to a length of 4.5 inches (in) using a hacksaw blade using appropriate safety measures such as gloves and goggles. The 1.5-in tube was cut to a length of 2.625 inches (2 5/8 in). Then the 3 in x 1.5 in PVC coupling adapter was added to one end of the 3-in tube using adhesive. Adhesive was used for all following steps to ensure a leakproof design. The 1.5 in female adapter was attached to the other end of the 3 in x 1.5 in PVC coupling adapter with muslin cloth in between and left aside. Then another 1.5 in female adapter was attached with muslin cloth to one side of the 1.5 in tube. On the other side of the 1.5 in tube, a 1.5 in male adapter was attached. A 1.5 in cleanout plug was attached to the male adapter on the 1.5 in tube and the 3 in tube without adhesive. This design included two separate modules. The 3-in module was used for the f-sand tests, and for the f-sand and cilantro test, both modules were attached together (**Figure 4**).

To prepare the f-sand, whole moringa seeds were crushed using a mortar and pestle. 0.25 grams of the seed powder was added to 10 mL of deionized water. The solution was agitated in a 10 mL centrifuge tube for one hour. At the end of one hour, the solution was set upright for 40 minutes. After letting it settle, the top 6 mL of the solution was removed.

2 mL of solution was added to 2 grams of sand, and the sand was incubated. After incubation at 37°C, the sand was washed 10 times with 10 mL of deionized water. The sand was stored in a sterile container with 10 mL of deionized water.

During the experiments, the different water mixtures were poured through the filter, which was filled with the corresponding agents (regular sand, f-sand, cilantro). For the normal moringa technique, 0.25 grams of the seed powder was added to 10 mL of a bacteria mixture. The mixture was shaken for 15 minutes and allowed to sit for 15 minutes. Using a muslin cloth, any remaining particles in the water were filtered out. (**Figure 5**) For the f-sand and regular sand, 6.0 grams of the corresponding sand was added to the 3 in water filter module along with 18.0 mL of the water solution. The tube was closed using an end cap. It was mixed by shaking and rolling it for up to 30 minutes then allowed to sit for 1.5 hours. For the f-sand and cilantro technique, 4 cups of cilantro was added to the 1.5 inch module. Then, the 1.5 inch module was attached to the 3 in module filled with sand by opening the cleanout plug of the 3 in module and screwing on the 1.5 in module.

The TDS of the kaolin water solution was tested using a TDS meter (HM Digital), and the reading was recorded for each treatment method. TDS is a measure of the amount of solids in water. A TDS meter measures the conductivity of a solution, and since the conductivity of water is directly related to the amount of total dissolved solids, it can derive the TDS of the water. The TDS was taken once before experimentation and after each treatment.

The petri dishes were self-poured with a nutrient-agar medium (Carolina Biological). In order to count the number of colonies grown in each petri dish, the plates were incubated at 35°C after being plated. Each colony count measurement was taken after five days of incubation. Each petri dish was split into four subsections to make it easier to count. The measurement was first taken by eye, then additional computer software was used to confirm the measurements (Promega Colony Counter). The number of colonies found in one quadrant was then multiplied by four to get the total colony count. 0.1 mL of each bacteria solution was plated on a petri dish using a sterile pipette before treatment to serve as a control, and 0.1 mL of all the bacteria solutions were plated after each treatment. The filter was cleaned in between treatments with antibacterial wipes then allowed to dry.

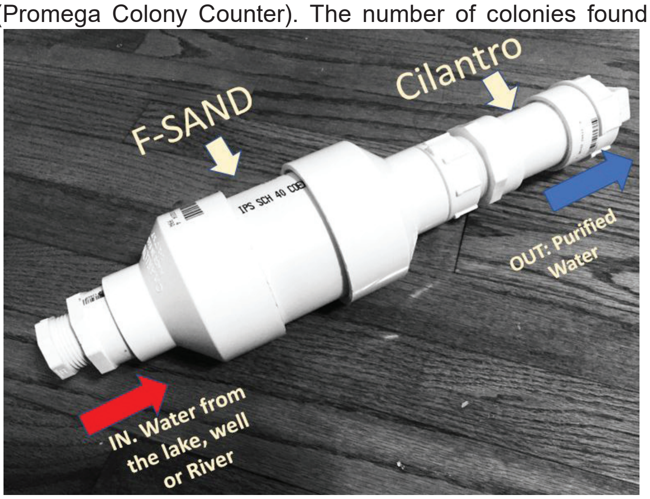

Figure 4. Filter Prototype. This is the filter prototype constructed using PVC materials there are two different components, a larger one for the f-sand and a smaller one for the cilantro.

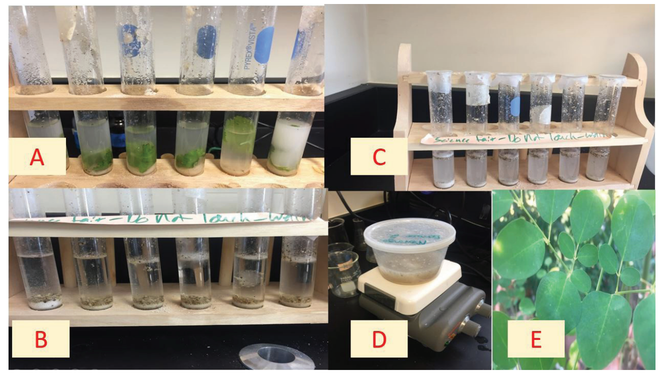

Figure 5. Experimental Images. These are a collection of images taken during experimentation. (a) This is the testing of the f-sand and cilantro method in a test tube. (b-c) This is the testing of the normal moringa technique. (d) This is the creation of the moringa serum to be added to the sand later on. (e) This is a picture of moringa leaves, which can also be eaten for nutritional value.

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REFERENCES

1. Jerri, Huda A., et al. "Antimicrobial Sand via Adsorption of Cationic *Moringa Oleifera* Protein." *Langmuir*, vol. 28, no. 4, 2011, pp. 2262–2268., doi:10.1021/la2038262.
2. Matic, Ivana, et al. "Investigation of Medicinal Plants Traditionally Used as Dietary Supplements: A Review on *Moringa Oleifera*." *J Public Health Afr*, vol. 9, no. 3, Dec. 2018. PAGEPress Publications, doi:10.4081/jphia.2018.841.
3. Minaiyan, Mohsen, et al. "Anti-Inflammatory Effect of Helichrysum Oligocephalum DC Extract on Acetic Acid - Induced Acute Colitis in Rats." *Adv Biomed Res*, vol. 3, no. 1, 2014, p. 87. Medknow, doi:10.4103/2277-9175.128000.
4. Leone, Alessandro, et al. "*Moringa Oleifera* Seeds and Oil: Characteristics and Uses for Human Health." *International Journal of Molecular Sciences*, vol. 17, no. 12, Dec. 2017, p. 2141. MDPI AG, doi:10.3390/ijms17122141.
5. Retrieve Data (ADAT) - GLOBE.Gov. June 2017, https://web.archive.org/web/20200120203737/https://www.globe.gov/globe-data/retrieve-data.
6. Vilaseca, Mercè, et al. "Valorization of Waste Obtained

from Oil Extraction in *Moringa Oleifera* Seeds: Coagulation of Reactive Dyes in Textile Effluents." Materials, vol. 7, no. 9, Sept. 2014, pp. 6569–84. *MDPI AG*, doi:10.3390/ma7096569.
7. "Water Treatment | Public Water Systems | Drinking Water | Healthy Water | CDC." *Centers for Disease Control and Prevention, Centers for Disease Control and Prevention*, 20 Jan. 2015, www.cdc.gov/healthywater/drinking/public/water_treatment.html.
8. Abebe, Lydia, et al. "Chitosan Coagulation to Improve Microbial and Turbidity Removal by Ceramic Water Filtration for Household Drinking Water Treatment." *IJERPH*, vol. 13, no. 3, Feb. 2018, p. 269. MDPI AG, doi:10.3390/ijerph13030269.
9. Shebek, Kevin, et al. "The Flocculating Cationic Polypetide from *Moringa Oleifera* Seeds Damages Bacterial Cell Membranes by Causing Membrane Fusion." *Langmuir*, vol. 31, no. 15, 2015, pp. 4496–502. American Chemical Society (ACS), doi:10.1021/acs.langmuir.5b00015.
10. Swales, Jennifer. "Researchers Study Inexpensive Process to Clean Water in Developing Nations." *Penn State News*, 9 June 2015, news.psu.edu/story/358048/2015/06/09/research/researchers-study-inexpensive-process-clean-water-developing.
11. Velegol, Stephanie Butler. "Moringa." *Stephanie Butler Velegol*, www.svelegol.com/moringa.html.
12. "Facts and Statistics: WaterAid Global." WaterAid, 2019, www.wateraid.org/facts-and-statistics.
13. Njue, Martin Kivuti. "Using cilantro leaves and stems to remove lead, cadmium and turbidity from contaminated water." *Diss. Kenyatta University*, 2017.