THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

FEASIBLE CREATION METHODS AND MODELS FOR A SUSTAINABLE WATER-PURIFYING MORINGA OLEIFERA COATED SAND FILTER

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SPRING 2018

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Civil Engineering
with honors in Environmental Engineering

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ABSTRACT

Low-technology water purification methods are desirable for the millions of people who need it most. One option is the moringa-coated-sand filter. Moringa-coated-sand is created by selectively adsorbing an antimicrobial cationic protein found in the seeds of the Moringa Oleifera tree to sand. When packed into a column, this 3D media filter will allow microbes to move through the moringa-coated-sand filter, stick to the sand particles, and be removed from the water. Here the technique for creating moringa-coated sand is presented, along with analysis using total nitrogen and total organic carbon results allowing a reduction in the time of the moringa-coated sand creation process. In addition, a microscope analysis is developed, along with a low-technology version of this test, allowing the user to determine the effectiveness of moringa-coated sand before it is packed into a column. Performance was modeled mathematically based on several different parameters, and this model was tested by running E. coli influent and wastewater effluent through the filter and quantifying the coliforms removed by the moringa-coated-sand. In addition, sustainable implementation methods are discussed to ensure effective application and usage of the technology in many different cultures and societies.

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ACKNOWLEDGEMENTS

Thank you to Dr. Stephanie Velegol, Dr. Manish Kumar, Boya Xiong, and Dr. Darrell Velegol for the knowledge, guidance, and lab space for this research. Thank you also to Adam Uliana, Ariel Wang, Taylor Carlin, Bethany Piechowicz, and all of the other students I have worked with on this project.

Introduction

Billions of people around the world lack access to clean water (WHO, 2014). While the technology exists to purify water to almost 100% removal of bacteria and particles, low-technology water purification methods are more desirable for impoverished areas because of their low costs and cultural availability (Lea, 2010). One locally available flocculant for removing both sediments and bacteria can be found in seeds of the Moringa tree. Many people that live in water insecurity use Moringa seeds to create drinkable water as a part of an indigenous tradition (Santos, Argolo, Coelho, & Paiva, 2005). Plant prevalence, affordability, and cultural acceptance make water purification methods such as this very attractive solutions to the issue of water poverty (Buttice, Stroot, Lim, Stroot, & Alcantar, 2010).

Figure 1: Areas where the moringa tree is commonly found (Trees For Life).

The Moringa tree grows in areas that coincides with severe impoverishment, as shown in Figure 1. This multi-purposeful tree, shown in Figure 2, grows quickly. It has nutritional and health benefits, and can be used as a biosorbent to reduce water turbidity (Lea, 2010) and remove

dangerous substances from water (Anwar, Latif, Ashraf, & Gilani, 2007). Cleaning water using Moringa seeds, shown in Figure 3, is accomplished by crushing the seeds, stirring the powder into water, and clearing the separated waste from the water. This releases *Moringa Oleifera* Cationic Protein (MOCP), which coagulates and reduces 80-90% of turbidity, but when not used right away, this results in fouled water and increased microbial contamination as the organic matter from the seeds is a readily available food source for microbes (Liguori, 2014). Previous work has shown that selectively adsorbing the antimicrobial MOCP found in the Moringa seeds to sand particles creates a positively charged sand surface (moringa-coated-sand). Microbes will adhere, and in some cases, die, when stuck to this surface. This method can be applied to a sand filter that can be used to trap and remove microbes (Liguori, 2014). This technique increases the removal efficiency and eliminates the fouling and microbial contamination.

Figure 2: The Moringa Oleifera tree and leaves.

Figure 3: Dried moringa seeds.

The laboratory version of the moringa-coated-sand filter has been proven to be successful in removing microbes, bacteria, and particles from water. In addition, previous work has shown that MOCP is a host defense peptide protein that incorporates itself into bacterial membranes to damage and kill the cell (Shebek, Schantz, Lauser, Velegol, & Kumar, 2015). This cation targets microbes because of its positive charge (Bechtel, Riley, Tilton, Velegol, & Velegol, 2014), and it uses its helix-loop-helix structure to incorporate itself into bacteria and cause membrane fusion, acting as a "molecular knife" to kill these dangerous cells (Abubakar, B. Xiong, Velegol, Kumar, & Velegol, 2015).

Moringa-coated-sand filters show promise in being used as a highly effective water purifier in impoverished areas across the world. However, the creation of moringa-coated-sand must be altered to be both culturally and contextually appropriate to be effectively implemented in the areas that it is needed (Liguori, 2014). It currently faces several technical challenges. The most effective way to selectively adsorb the desired protein onto the sand particles and create the moringa-coated-sand filter must be determined. In addition, it must be ascertained if all moringa seeds (i.e. seeds from different locations or picked at different ripenesses) work the same way in a moringa-coated-sand filter. Before dissemination of this technology to people living in water-

scarce regions of the world, there must be a recommended moringa-coated-sand filter creation process, along with a mathematical model to determine the filter removal efficiency and lifetime (i.e. when it will clog). This can then go on to be used as an improved and sustainable water purification process for people living in water-scarce regions of the world.

Technique for Making Moringa-Coated-Sand

This is the 'standard' method for creating moringa-coated-sand, which has been proven to remove—and even destroy—bacteria and microbes in water (Liguori, 2014). This procedure was later performed and tested with various alterations to find out if other methods of making moringa-coated-sand can be as effective as this method of making 'standard' moringa-coated-sand.

Moringa serum: Approximately 15 whole, tree-dried *Moringa Oleifera* seeds were ground for about 5 minutes using a KitchenAid® BCG111 Coffee Blade Grinder, leaving no large clumps. Store unused seeds in a sealed container. Crushed seed was weighed out and added to deionized water (DI water) to create a seed solution of approximately one seed per 20 mL of solution, or a concentration of 0.01 grams of seed per mL of water. The solution was mixed for 60 minutes in a lab roller and filtered by gravity through a 7 μm filter paper to remove large seed granules. Secondary filtering followed, using a sterile filter with a polyethersulfone membrane of 0.2 μm.

Moringa-coated-sand: Desired amount of sand was measured out; standard moringa-coated-sand dictates 0.1 grams of dry sand per mL of moringa serum. Sand was rinsed 3 times by adding to a container with DI water and shaking, pouring off the supernatant after settling, and

repeating. Moringa serum was combined with the rinsed sand and mixed for 60 minutes on a lab roller or stirrer. The rinsing step was repeated three times on the serum and sand mixture.

Total Nitrogen and Total Organic Carbon Moringa-coated-sand Tests

Sixty minutes is a long amount of time to mix moringa serum for moringa-coated-sand, especially if this water purification method is recommended to those without automatic stirrers and lab equipment. Here, the objective was to determine the optimal mixing time of the moringa seed with water for creation of the moringa serum. Ideally, the mixing time of the protein in water could be reduced to less than an hour.

To determine the protein concentration of moringa serum at different mixing times, the amount of dissolved nitrogen in moringa serum was measured for moringa serum created with varying mixing times, as nitrogen is often used to measure protein concentration. The conversion factor is approximately 6.25 grams of protein per gram of nitrogen (Jones, 1941).

When moringa serum is created, it contains high amounts of organic carbon, which is a food source for many microbes and bacteria. The rinsing of the moringa-coated-sand with DI water it intended to remove the organic carbon from the moringa-coated-sand. However, mixing time may affect the amount of organic carbon that dissolves in solution, just as mixing time may affect the amount of protein that dissolves in solution. As a result, total organic carbon was measured at different mixing times as an indicator for fouling of Moringa solutions.

Ideally, total dissolved nitrogen should be maximized while total dissolved organic carbon should be minimized to find the ideal moringa-seed-and-water serum mixing time when making the moringa-coated-sand filter. This will maximize the amount of dissolved MOCP, which results in more protein coating the sand particles, and will therefore maximize the filter effectiveness. However, this will also minimize the amount of dissolved organic matter and

carbon, and this can reduce the potential for fouling and microbial contamination in the filtered water.

TOC, TN, and Total Protein Predictions and Calculations

Predictions were made for the maximum amounts of both total organic carbon and total nitrogen in the serum. These results predict the highest possible TOC and TN results if the solution is completely solubilized (which will not be the case for each of the samples).

Assuming 30% Carbon in the seeds…

$$\frac{0.5\ g\ seed}{50\ mL\ water} \times \frac{1000\ mL}{L} \times \frac{1000\ mg}{g} \times \frac{0.3\ g\ C}{g\ seed} = \mathbf{3{,}000\ \frac{mg\ C}{L}}$$

Assuming 40% protein in the seeds…

$$\frac{0.5\ g\ seed}{50\ mL\ water} \times \frac{1000\ mL}{L} \times \frac{1000\ mg}{g} \times \frac{.4\ g\ protein}{1\ g\ seed} \times \frac{1\ g\ N}{6.25\ g\ protein} = \mathbf{640\ \frac{mg\ N}{L}}$$

$$\frac{0.5\ g\ seed}{50\ mL\ water} \times \frac{1000\ mL\ water}{1\ L\ water} \times \frac{1000\ mg\ seed}{1\ g\ seed} \times \frac{0.4\ mg\ protein}{mg\ seed}$$

$$= \mathbf{4{,}000\ \frac{mg\ protein}{L}}$$

Results

TOC and TN tests were performed on each of the moringa serum samples, which were created at several different mixing times. These tests were performed at a 1:40 dilution for most accurate results, using a Shimadzu TOC-VCPH Carbon/Nitrogen Analyzer machine. Raw data of total nitrogen and total organic carbon moringa-coated-sand tests is shown in Appendix A. The maximum predicted total organic carbon was 3000 mg C per L based on earlier calculations. Results, shown in Figure 4, were not conclusive due to large error bars, but show that TOC could potentially decrease as mixing time increases.

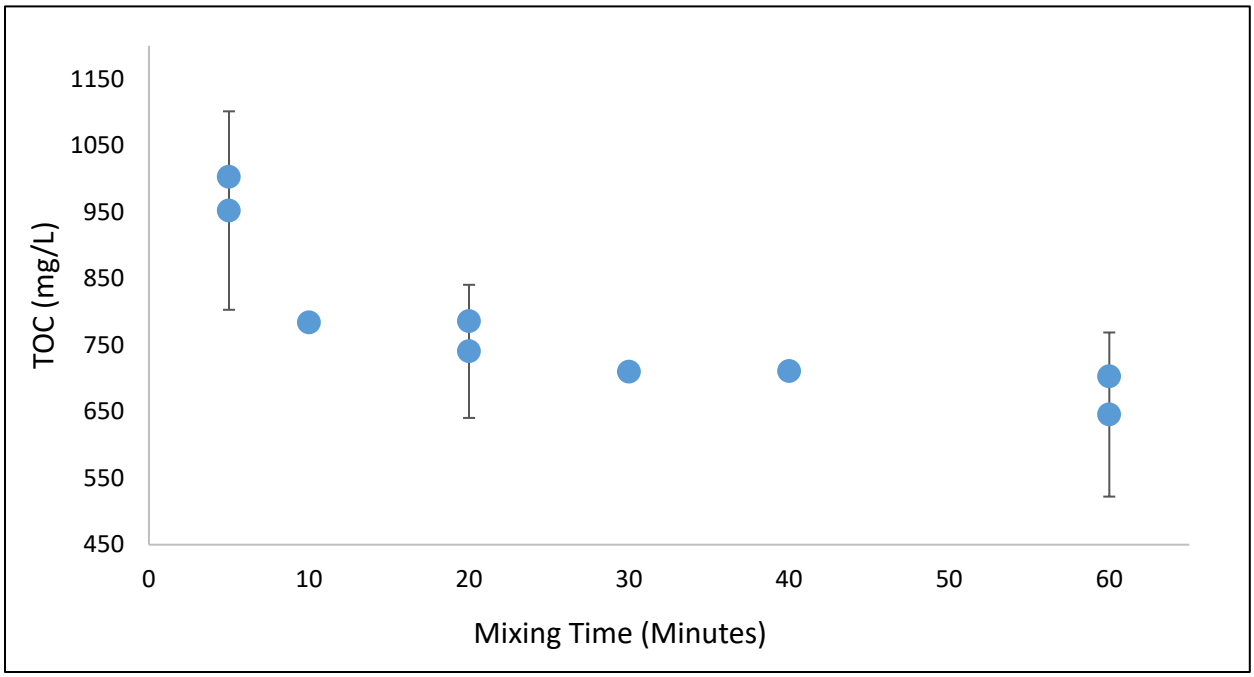

Figure 4: Total organic carbon in moringa serum at various mixing times.

Predicted maximum total nitrogen was 640 mg N per L, based on earlier calculations. This maximum amount was used to calculate the percent of dissolved protein. As shown in Figure 5, as mixing time increases, TN concentrations actually decrease, and this decrease levels off at about 40 minutes. This shows that the percent of dissolved protein decreases as mixing time increases, contrary to the initial prediction. Initially, dissolved protein was expected to

increase as mixing time increases, as one would expect more of the protein would solubilize as time goes on. These results show that the Moringa seed actually solubilizes quite quickly, and the ideal mixing time for the moringa serum is about 5 minutes.

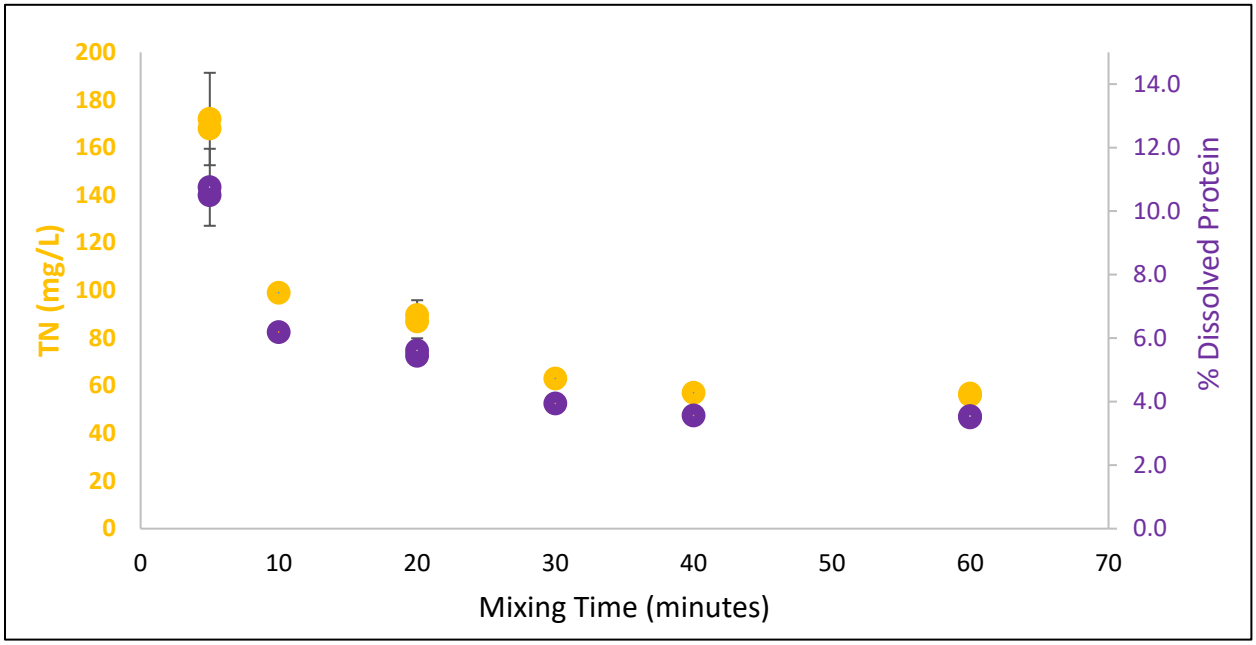

Figure 5: Total nitrogen and percent of dissolved protein in solution at various mixing times of moringa serum.

An important part of making moringa serum involves filtering the solution to remove large particles from the solution. According to quantitative analysis of the filter papers used to remove these particles, the percent of MO seed that is removed from the moringa serum actually increases as the mixing time increases, shown in Figure 6. If more solid is removed as mixing time increases, this explains why dissolved protein decreases as mixing time increases. Due to the flocculating properties of the moringa seed, as mixing time increases, this increases flocculation of the seed particles with other seed particles. This increased flocculation causes more of the seed to be removed from the solution due to the filtering process. This removes more dissolved protein from the solution as a result. Because of this phenomenon, mixing the moringa serum for smaller amounts of time will actually create a more effective moringa serum in the moringa-coated-sand process.

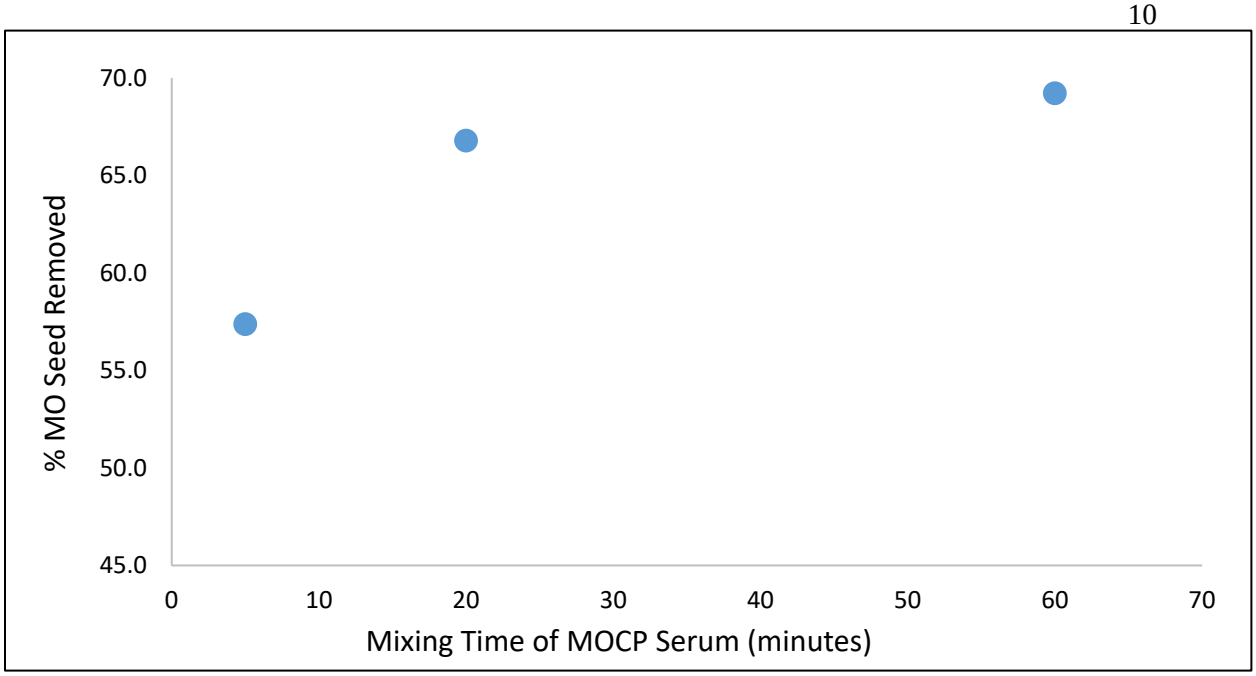

Figure 6: Amount of moringa seed removed during filtration at various mixing time of moringa serum.

This shows that the original procedures for making moringa-coated-sand, which mixed the moringa serum for 60 minutes, was actually able to achieve successful results at a low amount of dissolved protein. At about 3.5% dissolved protein, moringa-coated-sand captures and kills bacteria in water while significantly reducing turbidity (Bechtel, Riley, Tilton, Velegol, & Velegol, 2014). If the moringa-coated-sand is mixed for only 5 minutes, the amount of dissolved protein will be estimated at over 10% of dissolved protein. With even more dissolved protein, the moringa-coated-sand can be even more effective at purifying water.

Microscope Analysis

Moringa-coated-sand is proven to be effective because of the cationic protein in the moringa serum that is adsorbed onto the sand particles (Liguori, 2014). However, there is no easy way to measure the charge of a sand particle. A zeta spin test will only measure the charge of a flat plate, and a zeta pal test will not work on particles the size of the sand particles used in moringa-coated-sand (Bechtel, Riley, Tilton, Velegol, & Velegol, 2014). This microscope test was developed to measure the relative charge and effectiveness of moringa-coated-sand particles made with different mixing times and different seeds, and to compare that with that of moringa-coated-sand made by the 'standard' method, which has already been proven to be effective (Liguori, 2014).

The charge of sPSL particles is negative, and moringa-coated-sand is positive. Additionally, sand and glass beads are negatively charged. As a result of this phenomenon, sPSL particles should 'stick' to the surface of the moringa-coated-sand. On the other hand, sPSL will not 'stick' to uncoated sand. When viewed under a microscope, moringa-coated-sand particles have sPSL particles stuck to the surface, while sand particles do not. When put in a solution together, it is easy to determine which particles are the moringa-coated-sand particles, and which ones are the sand particles. By counting the number of sPSL particles 'stuck' to the surface of a specific particle, shown in Figure 7, one can quantify how effective or positive this particle may be.

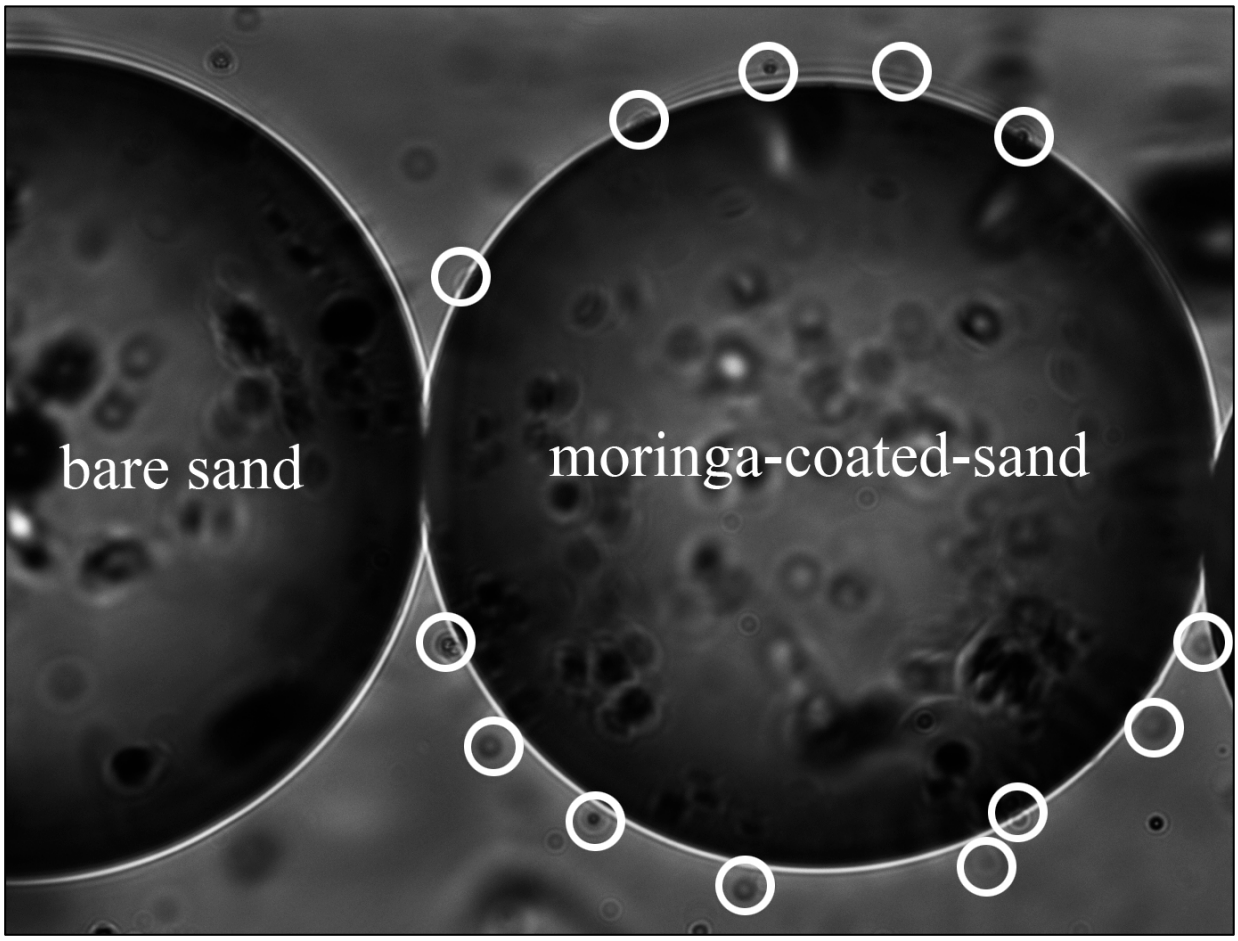

Figure 7: Both moringa-coated-sand glass beads and regular sand glass beads in solution with sPSL, with the sPSL particles that are 'stuck' to the moringa-coated-sand circled.

Materials and Methods

1.5 mL of moringa-coated-sand or regular sand in solution was combined with 0.4 µL of 8% by weight/volume 3-µm sPSL particles before mixing. These tests used moringa-coated-sand and sand solutions with variations in mixing times, seed type, glass beads vs. sand, and more changes. Vitrocom 0.900 x 0.180 wall mm glass square capillaries were filled with solution, ensuring that there are no air bubbles in the capillary, and tilted to ensure particles are scattered throughout the tube. The capillaries were then sealed with Bondic sealant to microscope slides

and viewed at 20X magnification in a Nikon TE200 Eclipse Inverted Microscope. The number of sPSL particles stuck to the glass beads or sand were then counted.

Results

Semi-quantitative analysis of this phenomenon, shown in Figure 8, has shown that moringa-coated-sand made at the mixing times of 5 minutes for the moringa serum and 5 minutes with the sand in solution is just as effective as the glass moringa-coated-sand created with mixing times of 60 minutes each for the moringa serum and for the sand in solution. Additionally, moringa-coated-sand created with seeds from Chang Mai, Nigeria, Nicaragua, and Haiti was proven to be equally effective in attracting negative particles. Moringa-coated-sand was also created from Indian seed cake, or what remains of crushed seeds after oil has been extracted, and it was determined that the seed cake moringa-coated-sand is just as effective as moringa-coated-sand created from crushed seeds. These results are very influential in modifying the moringa-coated-sand creation process. Moringa-coated-sand can be created with shorter mixing times, as already proven, and can also be made from crushed seeds or crushed seed cake. This will allow the process to be sustainably made from the waste of oil extraction processes.

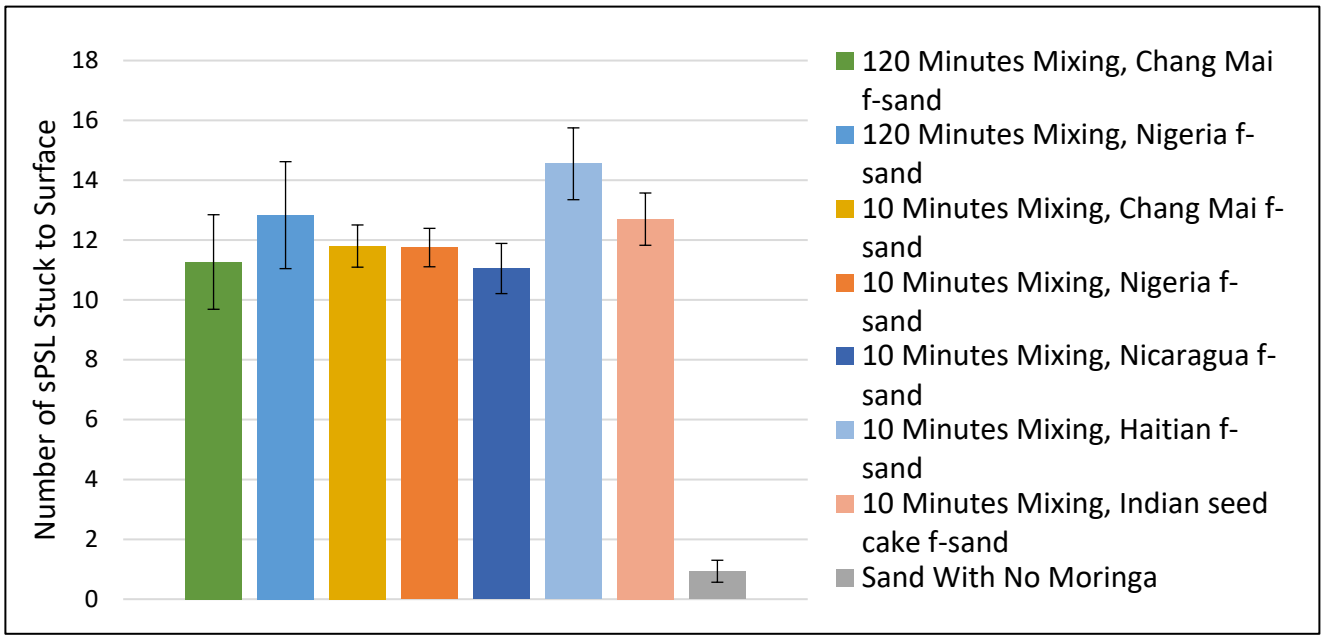

Figure 8: The effectiveness of bare sand and moringa-coated-sand with different mixing times and seed origins.

The cationic protein in the seed is what makes moringa-coated-sand work. But how much crushed *Moringa Oleifera* seeds actually needs to be added to a sand filter to make it a functional, moringa-coated-sand filter? Can one make moringa-coated-sand with one amount of Moringa seeds as effective as moringa-coated-sand with a different amount of Moringa seeds and a different mixing time? Perhaps one could make moringa-coated-sand with a lesser concentration of seed but mix it for a different amount of time and get the same effectiveness of the moringa-coated-sand. Moringa-coated-sand was made with moringa serum with no dilutions, a 10X dilution, a 100X dilution, and a 1000X dilution, all while holding the amount of glass bead particles constant. This was repeated with a moringa serum mixing time of 5 minutes, and again with a moringa serum mixing time of 60 minutes. The number of negative particles attracted and stuck to the moringa-coated-sand particles decreases as the amount of Moringa seeds is decreased. Additionally, there was virtually no difference between the moringa-coated-sand at either mixing time for each dilution of Moringa seed, shown in Figure 9. This shows that the

specific amount of *Moringa Oleifera* seeds needed to make moringa-coated-sand effective does not vary with mixing time.

To effectively implement the moringa-coated-sand filter to purify water, the amount of Moringa seed used must be the minimal amount needed to ensure clean water. This will reduce costs and resources used in the process. Based on these dilution tests, the concentration of crushed Moringa seed should be is the undiluted amount, or 0.1 grams Moringa seeds/10 mL water, regardless of the moringa serum mixing time, seen in Figure 9.

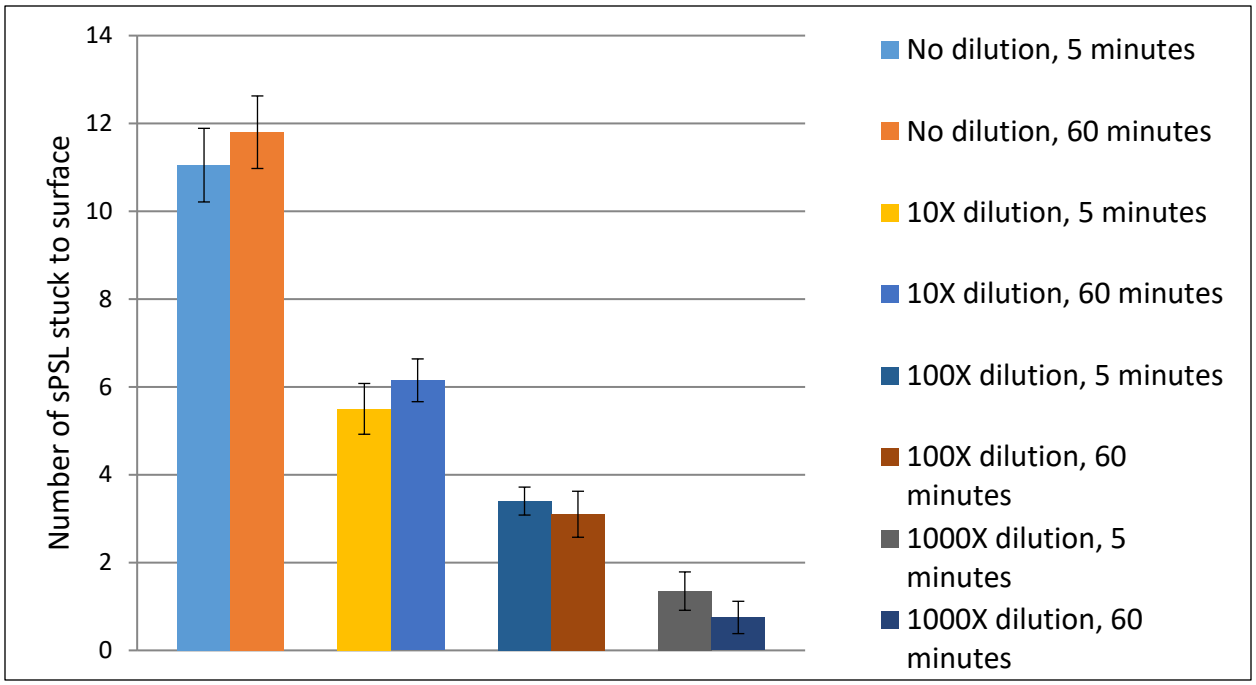

Figure 9: Effectiveness of moringa-coated-sand at varying dilutions and two different mixing times.

Does the seed harvest time matter when creating moringa-coated-sand? Nigerian seeds, both green and brown, were picked during the dry and wet seasons, with some of the seeds dried on the tree, and some of the seeds not yet dried when they were picked. Moringa-coated-sand was then created from these seeds, and all four different harvest seeds were analyzed, shown in Figure 10, according to the microscope analysis procedure outlined above. Seeds picked when they were green or brown, dried on the tree or off the tree, or picked during the rainy or dry

seasons were equally effective according to this microscope technique. This suggests that the ripeness of the seed (if it is green or brown) and the harvest time/season of the seed does not affect the effectiveness of moringa-coated-sand. Previous work has shown that the percent of MOCP does not vary among seed harvest time or type (Jerri, Adolfsen, McCullough, Velegol, & Velegol, 2011), so this makes sense that seed effectiveness is not changed by altering these parameters.

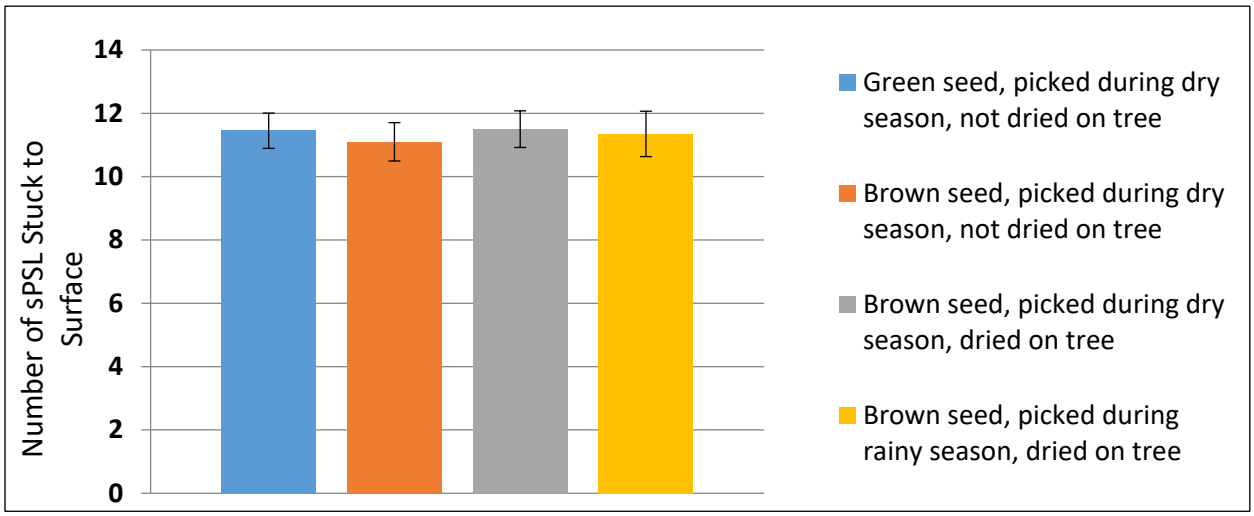

Figure 10: Effectiveness of moringa-coated-sand created with seeds from various harvest methods.

Most of the seeds that can be found on a Moringa tree are green when they are growing, and then as they dry on the tree, they turn brown. However, some Moringa seeds turn white. Figure 11 shows Moringa seeds that were all picked from the same tree at the same time in Tharaka, Kenya. Some of these dried seeds are white, and some of these dried seeds are brown with white edges (which is what the “typical” Moringa seed looks like when dried).

Figure 12: Brown and white moringa seeds from Tharaka, Kenya.

Moringa-coated-sand was created from both of these seed variations and tested with the same microscope analysis. Both seeds were equally effective at attracting negative sPSL particles when viewed in a microscope, shown in Figure 12. These results suggest that moringa-coated-sand can be made from seeds of varying colors, as well.

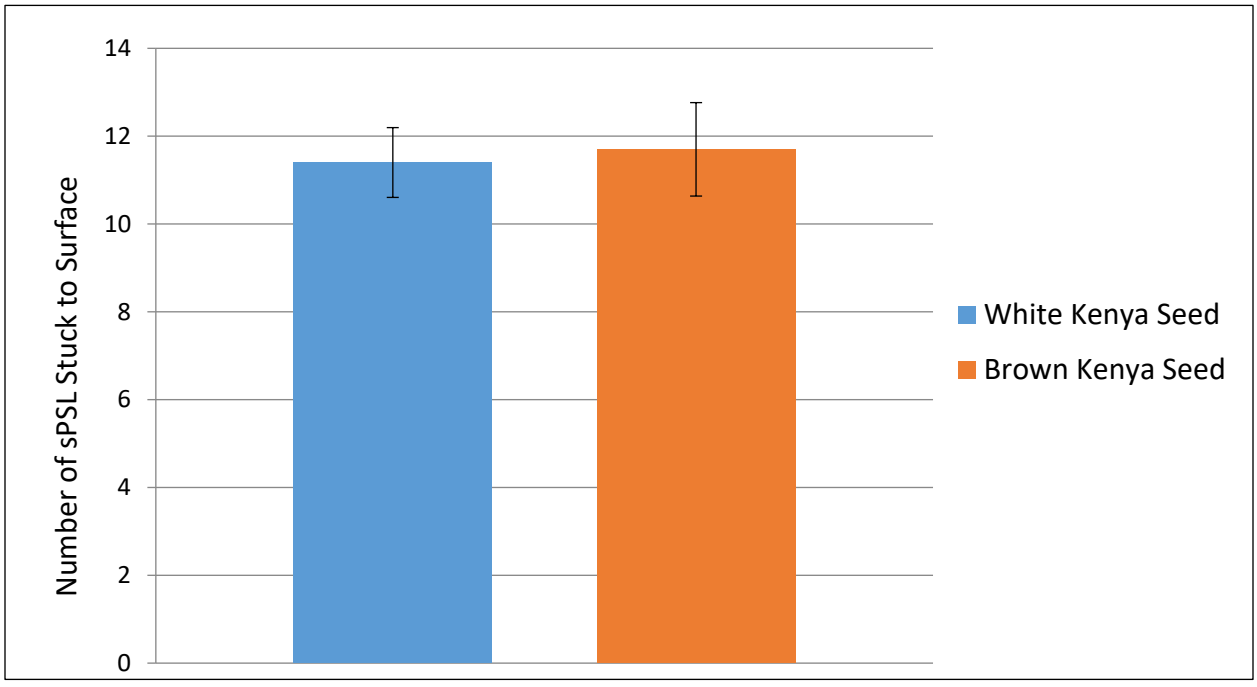

Figure 11: Effectiveness of brown and white moringa seeds from Tharaka, Kenya.

One concern with a sustainable water purification method that depends on plants such as moringa-coated-sand is that variations in the seeds could cause variations in the moringa-coated-sand effectiveness. Moringa seeds from various different countries, harvested at different times,

and white or brown seeds from the same tree have been shown to be equally effective. However, what about seeds from different trees in the same local area? Haitian moringa seeds were picked from five different trees in the same village and tested with the microscope analysis technique described above. Two of these trees were in the same yard and found adjacent to each other. The other three trees were found in three separate yards. All five of these Haitian variations in moringa-coated-sand were found to be equally effective according to the microscope analysis technique, shown in Figure 13. This suggests that moringa seeds are effective at creating moringa-coated-sand despite their location of origin, harvest time, color, or other differences.

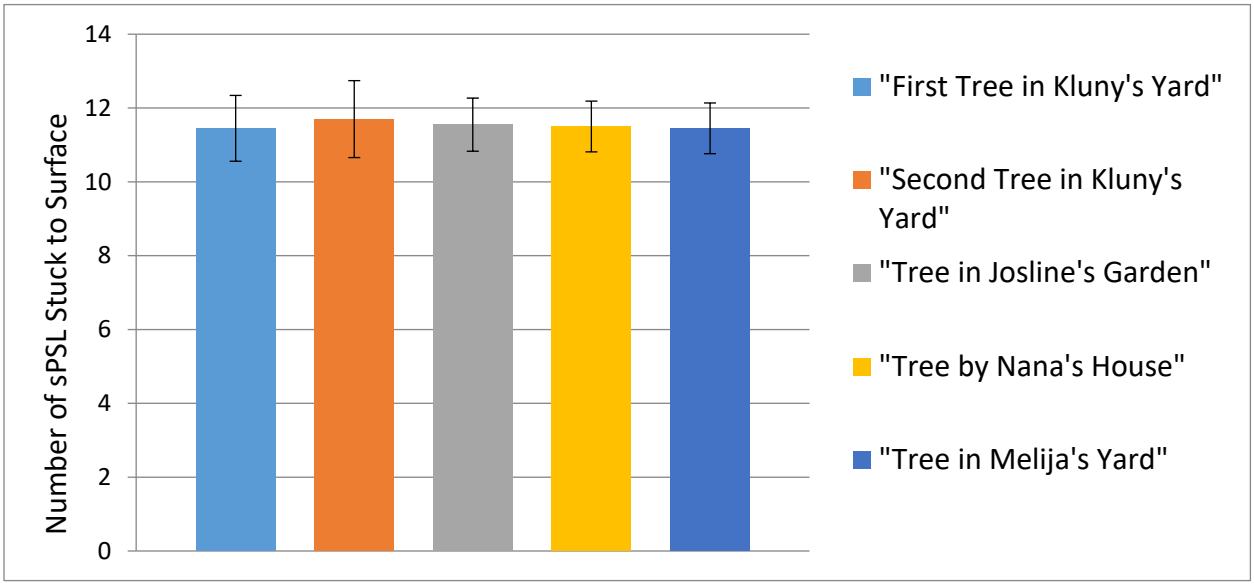

Figure 13: Effectiveness of moringa-coated-sand from seeds originating from five locations in Haiti in close proximity to each other.

Another potential problem for moringa-coated-sand implementation is the way that the moringa serum is mixed. In the lab, it is mixed on an automatic lab roller. However, this is not feasible in low-technology areas. Moringa-coated-sand was created with the known to be effective lab roller mixing method, and again with a shaking mixing method. This was accomplished by putting the moringa-coated-sand solution in a closed falcon tube, and simply shaking the tube. Both methods of mixing moringa-coated-sand were tested in the microscope

analysis test, and both methods suggested that there is no real difference in moringa-coated-sand charge or effectiveness based on the mixing method, shown in Figure 14. Therefore, the shaking method of mixing could easily be implemented in the developing world, as plastic water bottles are common waste objects even in areas with lower incomes. Moringa-coated-sand could easily be made and then mixed in a plastic water bottle where automatic laboratory stirrers or rollers are not available. This makes the moringa-coated-sand process even more sustainable, as it can be mixed not only outside of the lab, but in a common waste product found in many areas of the world.

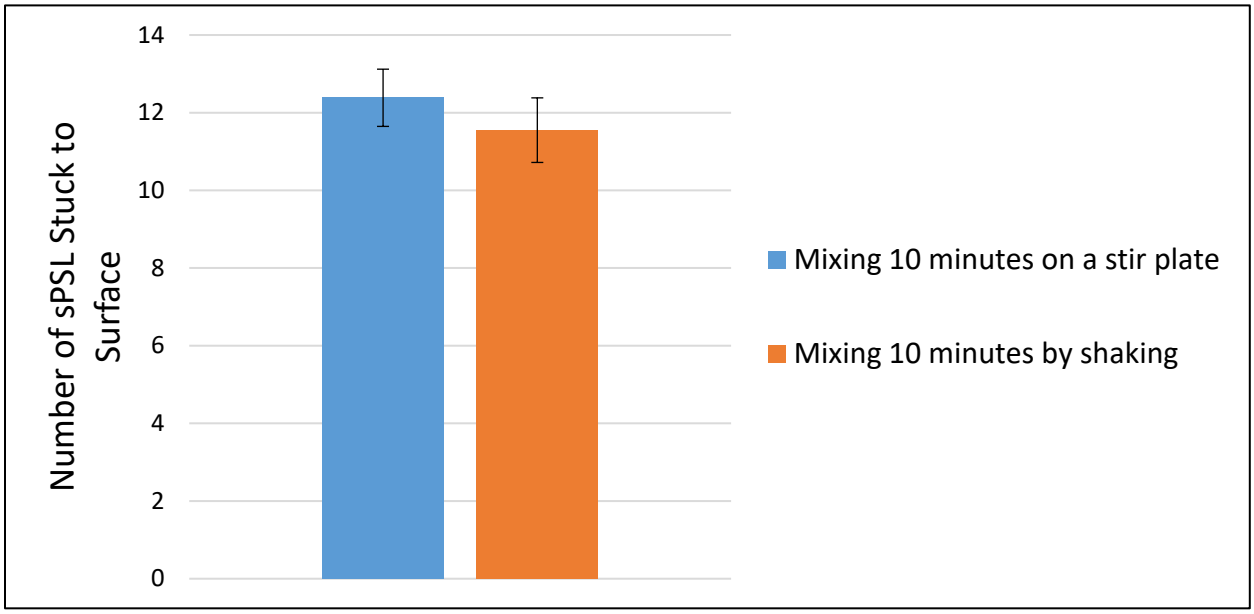

Figure 14: Effectiveness of moringa-coated-sand made with a laboratory stirrer and by shaking in a sealed tube.

Raw data showing the numbers of sPSL stuck to the surface of moringa-coated-sand particles made in all of the varying conditions in this section is shown in Appendix B.

Low Technology “Stick Test” Technique

This newly developed microscope test is great for determining the effectiveness of moringa-coated-sand in a lab. However, for the moringa-coated-sand creation process to be effective in the developing areas of the world that lack access to clean water, a simpler technique to test moringa-coated-sand must be developed. This low-technology technique is easy to explain to scientists and engineers, along with those with much less education, and is perfect for testing moringa-coated-sand for effectiveness.

Because plastic containers such as empty water bottles or falcon tubes are negatively charged, moringa-coated-sand in solution is attracted to the walls of a plastic tube, while sand with no moringa is not attracted to the walls of a plastic tube. This “stick” test, shown in Figure 15, allows one to quickly determine if moringa-coated-sand is positively charged without the use of a microscope or other equipment.

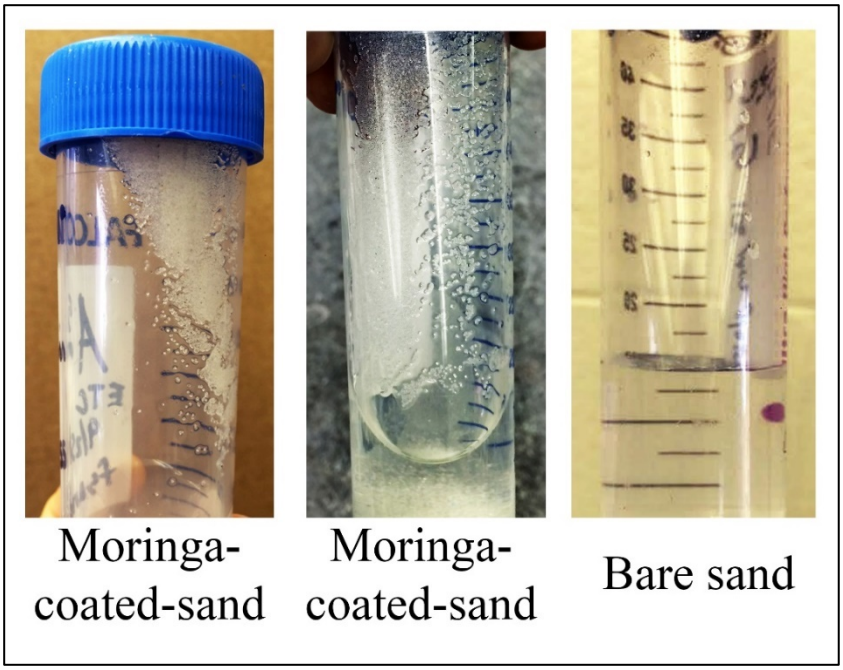

Figure 15: Moringa-coated-sand and bare sand in the "stick" test.

Here is the technique.

1. Pour the moringa-coated-sand solution into a plastic container with a volume twice as much as the volume of solution being added. Tighten the lid.
2. Turn the plastic container upside down. Positive moringa-coated-sand will stick to the walls of the tube, while negative sand will not.

Because moringa-coated-sand only works if it is positive, it should be attracted to the walls of a negative surface if it is truly functionalized moringa-coated-sand. It was predicted that all of the variations of moringa-coated-sand that successfully attracted the same range of sPSL particles would all pass the “stick” test described earlier, while regular sand in solution would not stick to the plastic surface.

Every single one of the different samples of moringa-coated-sand listed in Appendix C were tested with both the microscope analysis test and with the “stick” test that was developed. Every single one of these samples passed the microscope analysis test as effective in attracting a significant amount of negative sPSL particles to the positive moringa-coated-sand surface.

Additionally, every single one of these samples passed the low technology “stick” test and stuck to the walls of the negative falcon tubes. When regular sand in solution was tested with the “stick” test, it never stuck to the walls of a negative surface such as a falcon tube, but moringa-coated-sand consistently passed this test, proving that the “stick” test directly corresponds with the microscope test described in the last section. Therefore, this simple “stick” test can be used in low-technology areas to determine if moringa-coated-sand is effective before it is used to filter water.

Moringa Research Abroad and "Stick Test" in Rwanda

In May 2016, the Moringa research team traveled to Rwanda, in Africa, to perform Moringa research on the ground. The research group worked in collaboration with PIVOT, the first wastewater and sewage treatment plant in the country, to perform the Moringa research. PIVOT not only treats sewage but turns the waste product into useable fuel that can be burned for energy. The group also worked in collaboration with Asili Oils, a company that grows Moringa trees in Rwanda to use the seed oil in Moringa cosmetics and products sold to The Body Shop. This was the first time for many of the researchers to see a Moringa tree growing in its natural habitat. Knowing that seed cake – the byproduct left behind when the seeds are pressed for oil extraction – also contains the protein needed for Moringa to flocculate, the use of this seed cake waste product was tested for use for moringa-coated-sand processes. The standard process for making moringa-coated-sand was followed as much as could be possible in a field setting. The most educational part of this trip was learning to work in the field. The PIVOT science lab was in a repurposed truck trailer and did not have running water. The water used to make the moringa-coated-sand was rainwater, not the DI filtered water that was used in the lab at Penn State. Instead of using glass beakers cleaned with special laboratory-designed soap, plastic containers that had been rinsed by hand in rainwater were used. Samples were not kept in a refrigerator, but in closed containers at room temperature. But even with all of these changes, the moringa-coated-sand created passed the "stick" test, pictured in Figure 16.

Figure 16: Moringa-coated-sand from made in the field in Rwanda with seed cake waste product, rainwater, and natural sand found outside, passing the "stick" test.

Although the low-technology "stick" test is not quantitative, the more quantitative method of counting sPSL particles and its correlation to the "stick" test makes it more scientifically reliable in low-technology areas. Both tests have independently demonstrated the effectiveness of moringa-coated sand filters made in the following situations when compared to bare sand filters:

1. Reduced mixing time of serum and seed and of serum and sand
2. Various mixing methods of serum and seed and of serum and sand
3. High-technology and low-technology creation methods of moringa-coated-sand
4. Seeds with different origins
5. Seeds picked during different seasons and at different ripenesses
6. Seeds of different varieties (white or brown)

As a result, the "stick" test is a reliable method of determining relative effectiveness of moringa-coated-sand and should be used when these filters are made in low-technology areas.

Parameters and Modeling Performance of Moringa-coated-sand Filters

The purpose of this section is to determine parameters critical for the design of a Moringa-coated-sand filter for water purification. Moringa seeds were used to coat the surface of sand to carry a positive surface charge, and then tested to determine log removal for *E. coli* and model 1 μm particles. These results were compared to the log removal of bare sand filters, and clean bed filtration modeling was used to determine critical parameters for scale up design, validate these parameters under many different filtration conditions, predict scale up and saturation of moringa-coated-sand filters.

Materials and Methods

To validate α under various ionic strengths, collector sizes, and flow rates, filtration experiments were conducted with 1 μm particles and E. coli as the influent, varying one parameter at a time while other conditions were kept constant. These experimental results were then compared to predicted results based on model calculations (discussed in the following section) to back-calculate an experimental α.

The default conditions for 1 μm model particle removal experiments were 1 mM NaCl, 10^6/mL particle concentration as influent, a flow rate of 1.6 mL/min, and collector diameter of 106 μm.

The default conditions for E. coli removal experiments were 10 times diluted phosphate-buffered saline (PBS) buffer (0.016 M), 10^8 colony-forming unit (CFU)/mL concentration as influent, a flow rate of 1.6 mL/min, and collector diameter of 106 μm.

Breakthrough experiments were conducted with 1 μm particles, 1 mM NaCl, 10^7/mL particle concentration as influent, a flow rate of 0.7 mL/min, and collector diameter of 106 μm.

Moringa-coated sand preparation: Whole moringa seed was ground with a coffee grinder until it is of a uniform consistency and finely ground. The ground seed was mixed with DI water for five minutes to achieve desired seed concentrations. The serum was then filtered with 1.5 μm cellulose acetate filter to remove seed debris. Sigma Aldrich glass beads, acting as sand particles, (amounts depending on column size) were washed and rinsed three times before being mixed with the moringa serum for five minutes. Then the moringa-coated-sand was rinsed three times.

Column experiments: The moringa-coated sand was poured into a Bio-rad glass column of either 10 cm or 5 cm in length and either 1.5 cm or 1 cm inner diameter. The porosity of the packed sand column was gravimetrically determined to be 0.37. The columns were packed with DI water overnight and then equilibrated with the background electrolyte for 20 pore volumes before switching to appropriate influent solutions prepared in the same background electrolyte. A constant flow rate was achieved using a Cole-Parmer peristaltic pump.

Influent preparation: Influent was prepared using Life Technologies green-yellow fluorescent FluoSpheres® polystyrene particles with a diameter of 1 μm as model particulate contaminants or Escherichia coli strain TGI (E. coli) containing plasmids that express red fluorescent protein (pCA24N-rfp-lasR) to be used as model pathogens. Sterilized water and NaCl solution (for 1 μm model particle removal experiments) or 10 times diluted Phosphate buffered saline (PBS) (for E. coli removal experiments) were used as buffer while sterilized vials were used to collect effluent samples at specified pore volumes. Effluent samples were then analyzed using an Millipore Amnis FlowSight Imaging Flow Cytometer (for 1 μm model particle removal experiments) or by

counting the number of colonies grown with a conventional plate counting method to quantify colony-forming-units (for E. coli removal experiments).

Model Calculations

Experimental log removal of particles was calculated with the following equation, where N and N_0 is the effluent and influent particle concentration.

$$Log\ removal_{exp} = -log_{10}\left(\frac{N}{N_0}\right)$$

Classic clean bed filtration models are widely applied to describe the colloidal and particle deposition and transport in saturated porous media. The extent of deposition can be estimated from the collector efficiency, η_0, defined as the probability of a particle striking a collector given the specifics of the column and its hydrodynamic conditions. The correlation between log removal and collector efficiency is below.

$$\ln\left(\frac{N}{N_0}\right) = \frac{-3(1-\varepsilon)L\alpha\ \eta_0}{2d_c}$$

In this equation, d_c is the collector diameter, ε is the column porosity, and L is the column length. The model developed by Tufenkji and Elimelech (TE model) was used to calculate theoretical η_0 due to the superior agreement of the predicted values with experimental data (Tufenkji & Elimelech, 2004). This model is based on numerical solutions of convective-diffusion equations for particle transport around a single collector, considering hydrodynamic interactions and van der Waals forces. The sticking coefficient, α, describes the probability of a particle sticking to a collector upon collision and has a theoretical range from 0 to 1. The values of α for moringa-coated-sand and bare sand columns were determined using the above equations

under various column and flow conditions. Predicted log removal values were then calculated using the following equation:

$$Log\ removal_{pred} = -\log_{10}[e^{\frac{-3(1-\varepsilon)L\,\eta_0\alpha}{2d_c}}]$$

With a standard deviation of:

$$\sigma_{\log \text{removal}} = -\log_{10}(e)\,(\frac{-3(1-\varepsilon)L\,\eta_0}{2d_c})\sigma_\alpha$$

This saturation equation was used to calculate the fraction, f, of sand area occupied by particles at breakthrough:

$$f = \frac{A_s N_s}{J_p\,V_b A_p}$$

This is defined as the fractional coverage when N/N_0 exceeds 0.1. A_s is the surface area of one sand particle, N_s is the total number of sand particles in the column, J_p is the flux of influent particles, V_b is the volume filtered at breakthrough and A_p is the cross-section area of an influent particle.

Results

In previous work, enhanced flocculation by moringa-coated-sand with a two to five hour mixing time and seed dosage of 0.01 g seed/mL was shown (Jerri, Adolfsen, McCullough, Velegol, & Velegol, 2011). Here, seed mass per total surface area of sand (g/m^2) was optimized by evaluating zeta potential in 1 mM NaCl at seed concentrations ranging over four orders of magnitude (0.02 to 230 g seed/m^2, 2×10-5 to 0.2 g/ml) using 3μm silica oxide particles as sand substitutes. As shown in Figure 17, the zeta potential of sand reverses from -42 mV to +10 mV (1 mM NaCl) at a seed dosage of 1-10 g/m^2 (0.001-0.01 g seed/ml). These concentrations are

comparable to those identified by previous neutron reflectivity analyses of purified MOCP adsorption onto a silica interface (Shebek, Schantz, Lauser, Velegol, & Kumar, 2015) and can be used to determine the seed extract to sand ratio in the field to yield effective moringa coated sand materials. The fast adsorption kinetics of moringa coated sand at 0.005 g/ml (5.6 g/m^2) seed loading, shown in Figure 17, suggests that a five-minute mixing time for sand and seed extract is sufficient to enable charge reversal by MOCP (2-5 hours in previous methods (Jerri, Adolfsen, McCullough, Velegol, & Velegol, 2011)).

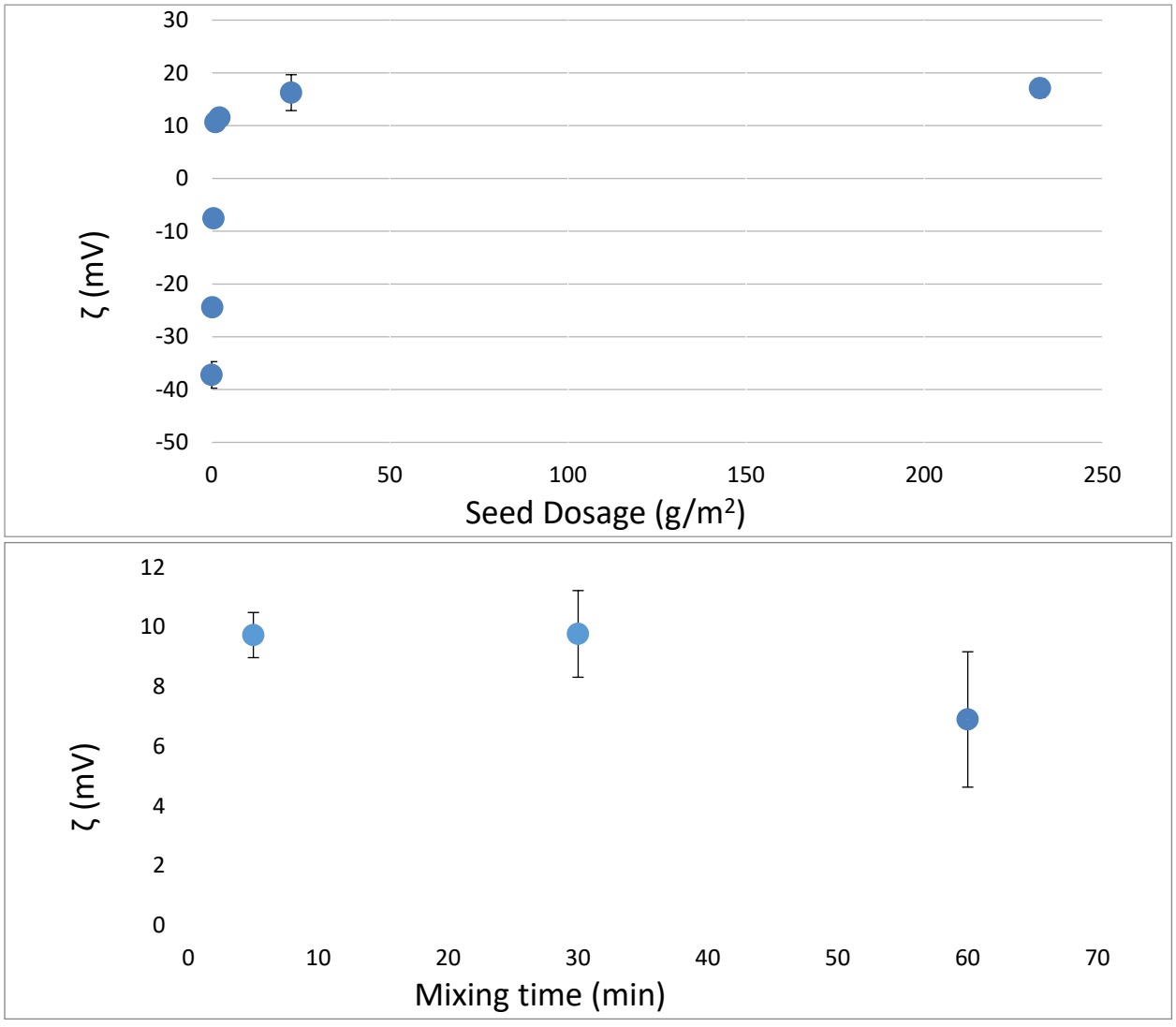

Figure 17: Top: Zeta potential measurements of moringa-coated-sand showing charge reversal with increased seed loading. Bottom: Zeta potential of glass beads at 5.6 g/m^2 moringa serum concentration at different mixing times.

The moringa coated sand filter achieved 3-4 log removal of 1 μm particles (1 mM NaCl), compared to 0.060 log removal by the bare sand filter under identical flow conditions. Particles of 1 μm size were chosen because they represents many target microbial contaminants (such as coliform bacteria) and the collector efficiency is typically the lowest at this size as predicted by CBF models (Tufenkji & Elimelech, 2004). The moringa coated sand filter performance was evaluated with the seed dosages that led to positive sand charge (1.1, 5.6 and 11.2 g seed/m^2 sand surface) using 1 μm particles at a concentration of 10^6/ml. As shown in Figure 18, removal was consistent from 2 to 10 pore volumes; values were averaged and presented for each specific seed loading in Figure 19. Increasing the seed dosage from 1.1 to 5.6 g/m^2 resulted in an increase in log removal from 2.9±0.2 to 3.9 ±0.4 (surface potential increase from +10 to +16 mV) compared to 0.060±0.008 log removal by bare sand filters. Further increases in the seed loading did not increase surface charge or particle removal; thus 5.6 g/m^2 seed loading was chosen for all subsequent filtration experiments.

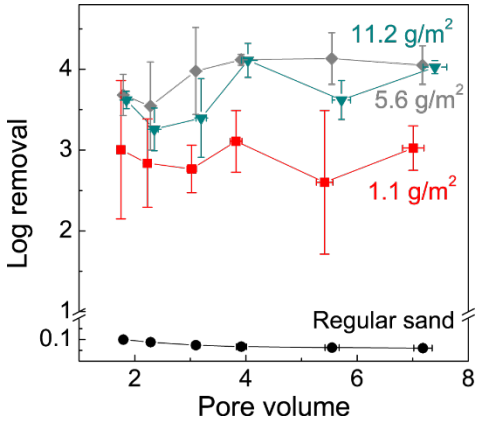

Figure 18: Log removal of moringa-coated-sand columns coated with various amounts of Moringa seed over various pore volumes.

It was further determined that the moringa coated sand filters removed >8 log red fluorescent *E.coli*, as no bacteria was detected in the effluent when the influent concentration was 108 CFU/ml. In comparison, bare sand only removed 0.05 log *E. coli* under the same flow

conditions, shown in Figure 19. Previous Baclight® stain tests have also demonstrated the loss of viability of the bacteria attached to the moringa coated sand surface, (Jerri, Adolfsen, McCullough, Velegol, & Velegol, 2011) likely due to the membrane fusion by the MOCP (Shebek, Schantz, Lauser, Velegol, & Kumar, 2015).

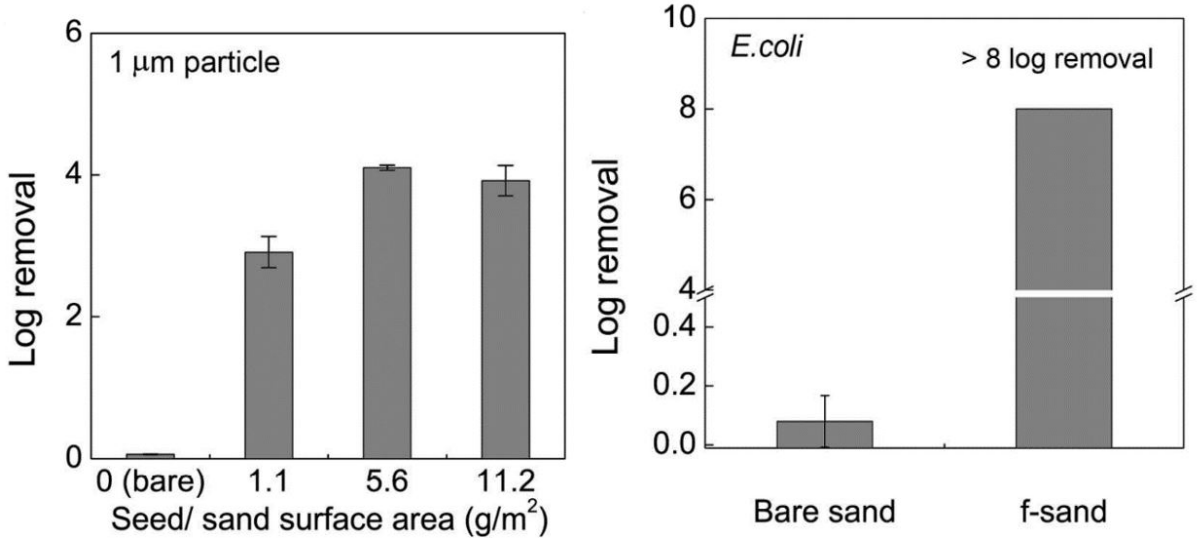

Figure 19: Moringa-coated-sand achieved 3-log to 4-log removal of 1 µm particles with various seed loadings compared to < 0.06 log removal by bare sand (left). Moringa-coated-sand filters also removed over 8-log removal of E. coli compared to 0.05 log removal by bare sand (right).

In order to confirm the role of electrostatic attractive forces in the particle removal in moringa coated sand filters, the classic Derjaguin-Landau-Verwey-Overbeek (DLVO) theory demonstrated in Figure 20 was used to calculate the total interaction energy between a spherical particle and the sand collector surface (modeled as a flat plate).

The electrostatic interaction energies were calculated using the below equation developed by Hogg *et al.* (Efstratiou, Ongerth, & Karanis, 2017):

$$\phi_{EDL} = \pi\epsilon_0\epsilon_r a_p \left\{ 2\psi_p\psi_c \ln\left[\frac{1+e^{-kh}}{1-e^{-kh}}\right] + (\psi_p^2+\psi_c^2)\ln[1-e^{-2kh}] \right\}$$

$\epsilon_0$is the dielectric permittivity in vacuum, $\epsilon_r$is the relative dielectric permittivity in water, a_p is the particle radius, ψ_p and ψ_c are the surface potential of particle and glass beads

(collector) experimentally determined as zeta potential, k is the inverse of debye length, and h is the separation distance between particle and collector. The van der Waals interaction energies were calculated using the following equation.

$$\phi_{VDW} = -\frac{Aa_p}{6h}[1 + \frac{14h}{\lambda}]^{-1}$$

Where A is the Hamaker constant and a value of 1×10^{-20}J was used for the polystyrene-water-quartz system (WHO, 2014). λ is the characteristic wavelength of the dielectric (100 nm^3).

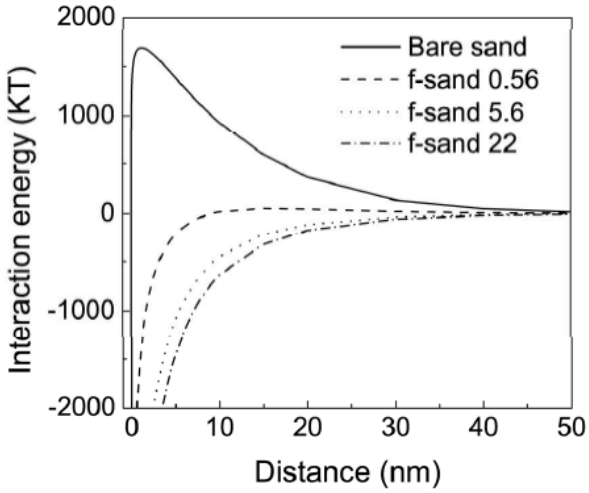

Figure 20: DLVO theory-based interaction energy calculations suggest strong attractive forces between negatively charged particles and the surface of moringa coated sand with various seed dosages, in comparison to a large energy barrier seen in bare sand.

The energy barrier between bare sand and a polystyrene spherical particle in 1 mM NaCl reaches a maximum repulsion of 1700 kT at 1.2 nm distance while moringa coated sand and the same particle results in an attractive interaction energy of -3000 kT at 1.2 nm. This strongly suggests a favorable deposition condition for negatively charged particles and bacteria on moringa coated sand, which agrees well with the observed log removal performance of moringa coated sand filters.

The electrostatic interaction energies suggest a high α for a particle and moringa coated sand (where the energy is negative) and a low α for a particle and bare sand (where the energy is

positive). The experimental data confirmed this trend: an α of 0.83 ± 0.08 was calculated for the removal of 1 μm particle by moringa coated sand (1mM NaCl, 1.6 mL/min with 106 μm glass beads) compared to an α of 0.011±0.001 for bare sand. In order to utilize the calculated values for broader design applications, the α value of moringa coated sand was validated with a series of filtration experiments at a range of ionic strengths, collector sizes, and flow rates that are typical for water treatment processes. As shown in Figure 21, the predicted log removals agree well with experimentally measured log removals under various ionic strengths (1- 10 mM) that are typical

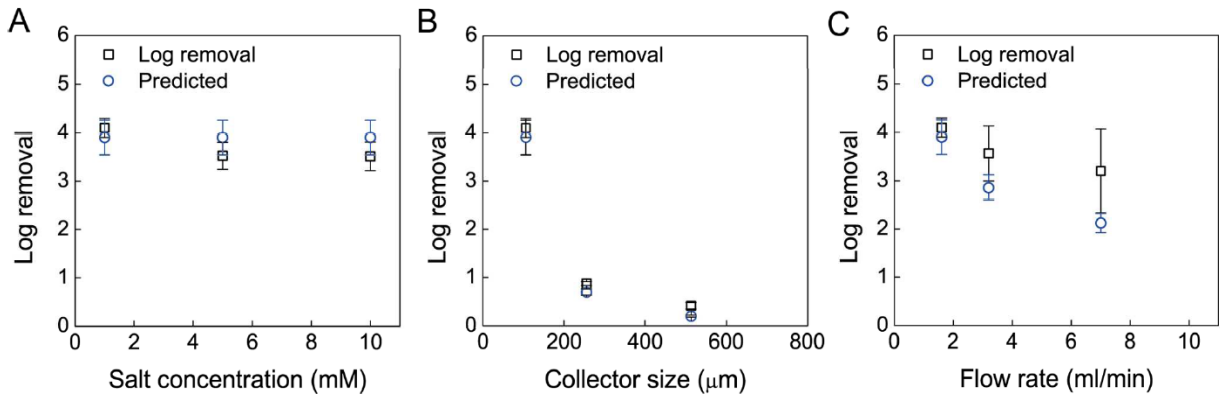

Figure 21: Experimental and predicted log removal of 1 μm particles by f-sand agrees well at various (A) NaCl concentrations, (B) collector sizes and (C) flow rates, using a sticking coefficient of 0.82.

in natural waters.

The model also agrees with experimental data at different collector sizes (106-520 μm); these collector sizes were selected after considering proper scaling between the filter diameter and the collector size from full scale sand filters based on a previous scale-down analysis of granular activated carbon filters (Crittenden, et al., 1991). The following equation determines scaling between the small and large column empty bed contact time (EBCT) from the ratio of adsorbed particle sizes.

$$\frac{\mathrm{EBCT_{sc}}}{\mathrm{EBCT_{lc}}} = [\frac{\mathrm{d_{sc}}}{\mathrm{d_{lc}}}]^2$$

$EBCT_{sc}$ and $EBCT_{lc}$ are EBCT of small and large columns, which are calculated from column volume divided by superficial velocity.Adsorbent collector particle sizes are $d_{sc}$and d_{lc}. The equation is under the assumption that the porosity, bulk densities and capacities are identical in the two scales and that intraparticle diffusivities do not change with particle size. A slow sand filter and a rapid sand filter were considered at the typical full scale, and this equation was used to perform scale-down analysis to calculate collector size given the column dimension and flow rate used. Media diameter, filter length and flow rate used to determine the calculated collector size is presented in Table 1. The scale-down collector size ranges from 0.03-0.075 mm considering a slow sand filter and ranges from 0.7-1.6 mm considering a rapid sand filter. 0.1-0.6 mm collector size was chosen for this study.

Table 1: Scale-down collector sizes calculated from the design parameters of full scale slow sand filter and rapid sand filters.

	Full scale slow sand filter (low, high)	**Small column scale-down from slow sand filter**	**Full scale rapid sand filter (low, high)**	**Small column scale-down from rapid sand filter**
Filter Length (cm)	90, 150	10	60, 180	10
Flow rate (m/h)	0.05, 0.2	0.48	5, 15	0.48
Collector size (mm)	0.3-0.45	0.03, 0.075 (low, high)	0.5, 1.2	0.7, 1.6 (low, high)

The experimental log removals at various flow rates were also within the range of predicted values, although the variation in average values is large compared to other conditions. These flow rates are similar or higher than that of a typical slow sand filter, with head losses between 0.1- 0.6 m, as shown in Table 2, which is appropriate for gravity driven household applications. Head loss or the minimal head required is calculated using the equations below (Crittenden, Trussell, Hand, Howe, & Tchobanoglous, 2012). For a flow at Darcy flow regime at Re<1, H_L (in meters) is calculated based on Poiseuille's law:

$$\frac{H_L}{L} = \frac{K_k \mu S^2 v}{\rho_W g \varepsilon^3}$$

where K_k is Kozeny coefficient (unitless) which is an empirical coefficient and assumed to be about 5 for spherical media (Carman, 1937). S is the specific surface area (calculated with the below equation if ε is column porosity, v is superficial velocity (m/s), and μ and ρ_W are viscosity and density of water, respectively.

$$S = \frac{6\,(1-\varepsilon)}{d}$$

This agreement strongly suggests that the sticking coefficient determined is robust and moringa coated sand surface sustains its ability to attract and retain particles under a range of conditions.

Table 2: Superficial velocity and head loss of the filtration experiments with various collector sizes and flow rates.

Collector size (μm)	Flow rate (ml/min)	v (m/h)	Head loss (m)
106	1.6	0.48	0.13
	3.2	0.95	0.25
	7	2.09	0.55
106	1.6	0.48	0.13
256			0.02
512			0.005

In order to model the rod-shaped *E. coli (*1.2 μm in diameter and 3.7 μm in length), a Hamaker constant of 6.5×10^{-21}J and a equivalent diameter of 1.7 μm were used (Redman, Walker, & Elimelech, 2004). The difference between the prediction and the experimental result is likely due to the larger interaction area between flexible rodshape bacteria and that of rigid 1 μm polymer spherical particles, which are calculated with the following equations (Velegol, 2016).

$$A_{\text{spherical polymer particle}} = 2\pi r\kappa^{-1}$$

$$A_{\text{rod-shape bacteria}} = 2L\sqrt{r\kappa^{-1}}$$

Where κ^{-1} (nm) is the Debye length in 1 mM NaCl. The calculation was under the assumption of the distance between particle or bacteria and sand surface is equal to the Debye length. The sand surface is considered a flat surface given the significant size difference between particle and sand.

The length of the bacteria can also enhance the collector efficiency by improved interception; the predicted log removal of a 3.7 μm sphere is calculated to be 10.49. The α of moringa coated sand determined here should be a conservative design parameter with a safety factor, when considering the design of field filters for bacteria removal.

To estimate the lifetime of the filter, a fraction (f) of the sand surface area covered by particle contaminants (projected area) was determined at an experimentally determined breakthrough point. The breakthrough point occurred when the N/N_0 increased above 0.1. The two pore volume values right before and after breakthrough were averaged and used to estimate the *f* value, which was determined with the following equation.

$$f = \frac{A_s N_s}{J_p V_b A_p}$$

where A_s is the surface area of one sand particle, N_s is the total number of sand particles in the column, J_p is the flux of influent particles, V_b is the volume filtered at breakthrough, and A_p is the cross-section area of an influent particle.

Six repeated runs, as shown in Figure 22, indicate a breakthrough pore volume of 350 ± 116, which corresponds to 400-600 ml, generating an f value of $4.1\% \pm 2\%$. This value can be directly used for designing filter scale and dimension that meet lifetime requirements.

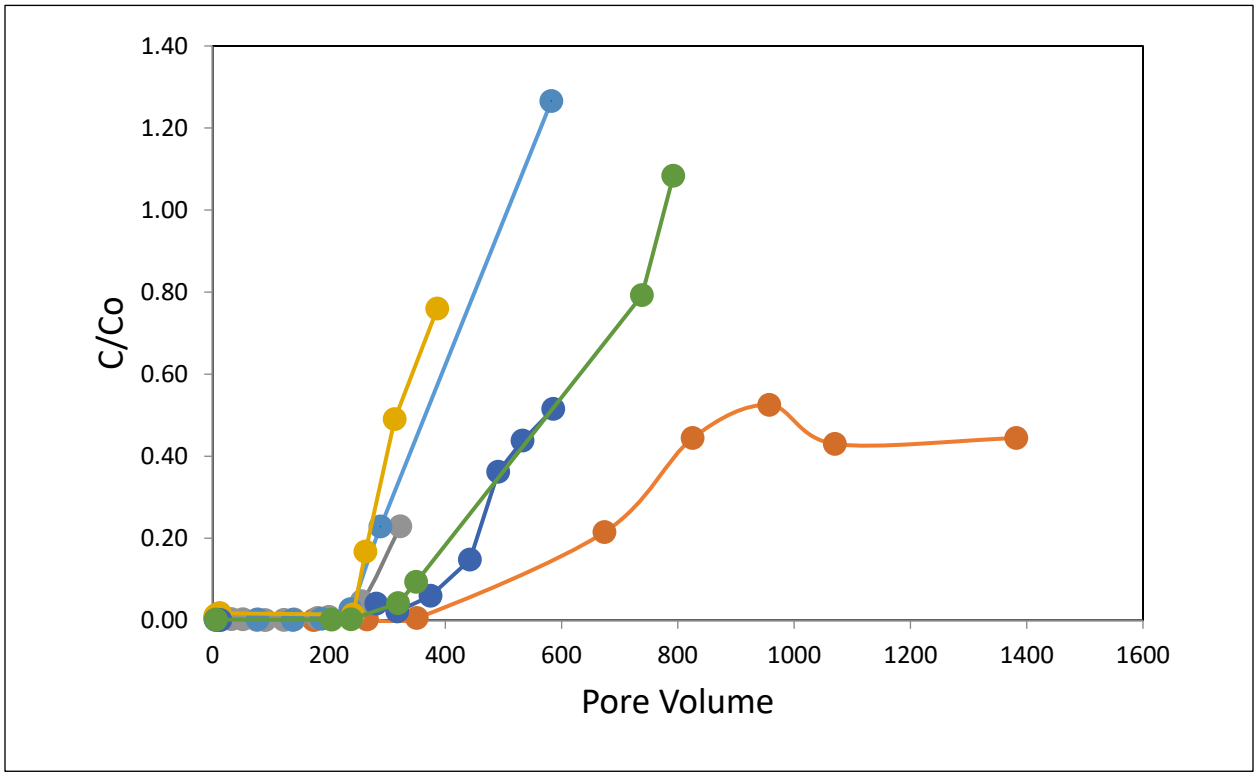

Figure 22: Breakthrough curves of 3-day column filtration experiments, which was used to calculate the fractional coverage (f) at breakthrough).

The outcome of this study provides a chemical-free solution employing Moringa seed extract to enable sustainable drinking water supply in the developing world. Using the α and f experimentally determined here, preliminary scale-up analyses were conducted considering a point-of-use household scale (5 people) filter. Desired performance dictates that the filter provide 10 L/day and >4 log removal of 1 μm particles given a heavily contaminated source water (10^4/100ml) with an appropriate head loss.

Two different scales were considered for scale up: 5-person household scale for point-of-use, and 1000-person community scale. Filter flow rate was first determined based on the amount of people served for each scale assuming 2 liters per day of drinking water. In addition, a sand diameter of 0.5 mm was specified. The specific diameter and length of the column was then determined in order to meet two requirements: 1) reasonable head required and 2) 4 log removal

of 1 μm particles at a concentration of 10^4 /ml concentration. The amount of sand was then calculated based on the porosity (0.37) while the mass of seeds was calculated using 5.6 g seed/m^2 sand area. The lifetime of the filter based on saturation was then calculated using 4% maximum fractional coverage (f). The head loss was calculated using an non-linear Forchheimer flow equation when Re>1:

$$\frac{H_L}{L} = \frac{K_v(1-\varepsilon)^2 v\mu L}{\rho_W g d\varepsilon^3} + \frac{K_I(1-\varepsilon)v^2 L}{\rho_W d\varepsilon^3}$$

In this equation, K_v is the head loss coefficient due to viscous forces and K_I is the head loss coefficient due to inertial forces. Typical values for sand, K_v=110 and K_I =2^6, were used for the calculations.

Log removal increases with column diameter due to decreased superficial velocity and increased collector efficiency. Log removal also increases with column length given the CBF model. However, column diameter and length are disproportional to each other due to given a fixed column volume. Analysis shows that, in order to meet both requirements, column volume has to be scaled large enough to generate the dimensions shown in Table 3.

The minimal volume was found to reach breakthrough in 140 years given the fraction of coverage of 4%. Therefore, in reality, the longevity of the column will not depend on the column capacity but rather depend on the duration and stability of adsorbed protein over extended period of time.

Analyses also suggest that the column filtration rate will be operated at low Re number <1, and with very small head required (<0.1 m). It was also found that the column with a larger media size such as 0.8 mm would result in a column (community scale) dimension providing too small of required head (0.008 m with a dimension of 1 m diameter and 1.6 m long) or too large of a scale (1.6 m head loss but a dimension of 0.2 m diameter and 12.5 m long) that is unrealistic for

implementation in the field.

Assuming 2 L water/day/person, 1 µm particles at 10^4/ml concentration, 0.5 mm sand size, and 4% coverage at breakthrough, a filter will not reach breakthrough in 140 years, although filters are assumed to be replaced every three months based on protein stability and effectiveness with sand being reused. These analyses, shown in Table 3, also indicate that an moringa coated sand filter, with dimensions of 5 cm diameter and 1 m length (and 3 cm head loss) would require 0.21 kg seed/year, as a two-year old *Moringa* tree produces 480 kg seed/year (Ayerza, 2012). In addition, these calculations show that the filter longevity was not limited by the sand surface area capacity. As moringa coated sand flocculating properties remained unchanged after four-month storage in both wet and dry form (Jerri, Adolfsen, McCullough, Velegol, & Velegol, 2011), it is suggested that filters can be replaced every three months. In addition, a hybrid column with a regular sand column on top of an moringa coated sand column may be used for treating highly turbid source waters.

Table 3: Scale up specifics of f-sand filter.

Design parameters		**Point of use (5 people)**	**Community based (1000 people)**	**Typical slow sand filter**	**Rapid sand filter**
Treatment capacity	Daily output (L)	10	2000	NA	NA
	Yearly output (L)	3650	730,000	NA	NA
	Flow rate (L/d)	10	2000	NA	NA
	Filtration rate (m/h)	0.21	0.22	0.05-0.2	5-15
Column specifics	Filter volume (m3, porosity 0.39)	0.002	0.41	NA	NA
	Media diameter (mm)	0.50		0.3-0.45	0.5-1.2
	Filter diameter (m)	0.05	0.70	NA	NA
	Filter length (m)	1.04	1.06	0.9-1.5	0.6-1.8
	Log removal	4.1	4.2		
	Minimal head (m)	0.03	0.03	0.9-1.5	1.8-3
	Total sand (kg)	2.1	423	NA	NA

Sand and seed consumption	Cost of sand/ person/ year ($) Unit price: $0.018/kg (Ladlow, 2015)	0.03		NA	NA
	Total seed (kg)	0.054	42.91	NA	NA
	Seed kg /person/ year	0.043		NA	NA

A table showing all column experiments that were run to obtain the results in this section can be found in Appendix D.

Wastewater Column Tests

In these experiments, moringa-coated-sand columns were created and used to filter wastewater effluent. This was compared to typical wastewater effluent values of total coliform and E. coli. It is predicted that wastewater effluent with “real” bacteria will behave similarly to the lab-grown E. coli solutions when ran through an f-sand column filter, and a similar log removal of E. coli and total coliforms is expected.

Materials and Methods

Moringa-coated sand filter preparation: Whole, unshelled, Nicaraguan moringa seeds were ground with a coffee grinder and mixed with DI water for 5 minutes followed by filtration through a 1.5 μm Whatman glass fiber filter and a 0.22 μm Millipore cellulose acetate filter. Seed dosage was 5.6 grams of seed per square meter of sand surface area. The seed extract was mixed with unwashed Sigma Aldrich glass beads for 5 minutes; the supernatant was discarded, and the glass beads were rinsed with DI water three times to remove organic residues. The glass bead slurry (moringa-coated-sand) was then used to pack glass columns.

Wastewater: Wastewater was obtained from the Penn State University Park Wastewater Treatment Plant. Each sample taken was about 900 mL of secondary effluent, taken off of the trickling filter weir. Wastewater was filtered through 30 um filter paper before use as influent to remove large chunks of organic matter.

Column experiments: Wastewater filtration tests were performed with Bio-Rad glass columns, 10 cm in length and 1.6 cm in inner diameter. The porosity of the packed sand column was

gravimetrically determined to be 0.37. 25 grams of 106 μm collector particles were used as the 'sand'. The moringa-coated-sand slurry was quickly poured into the glass column and gently mixed by rotation along the length of the column to remove any trapped bubbles. The columns were then packed with sterile DI water overnight and equilibrated with sterile, filtered 1mM as background electrolyte for 20 pore volumes before switching to appropriate influent solutions. Sterilized vials were used to collect effluent samples. A constant flow rate of 1.6 mL/min was achieved using a Cole-Parmer peristaltic pump with the feed solution entering from the top of the column. Experimental log removal for was calculated using this equation:

$$\log_{10} removal_{experimental} = -\log_{10}\left(\frac{N}{N_0}\right)$$

where N and N_0 are the effluent and influent coliform concentrations, respectively.

Total coliform and E. coli testing: Total coliform and E. coli testing was performed at the Penn State Agricultural Analytical Services Laboratory. 10 mL samples were taken at various pore volumes and diluted with 90 mL of sterile DI water to provide the needed 100 mL for each test.

Results

Figure 23 shows log removal of E. coli and total coliforms from wastewater by filtration through sand columns and moringa-coated sand columns (all other conditions held constant). Preliminary data (i.e. no repeated trials) shows that removal of total coliform and E. coli bacteria was lower in moringa-coated sand filters than in sand filters, perhaps due to differing interactions between the moringa-coated filter at low concentrations or complication due to the extremely

variable and complex wastewater composition. Note: trials were not repeated, so error bars are not shown. Raw data can be found in Appendix E.

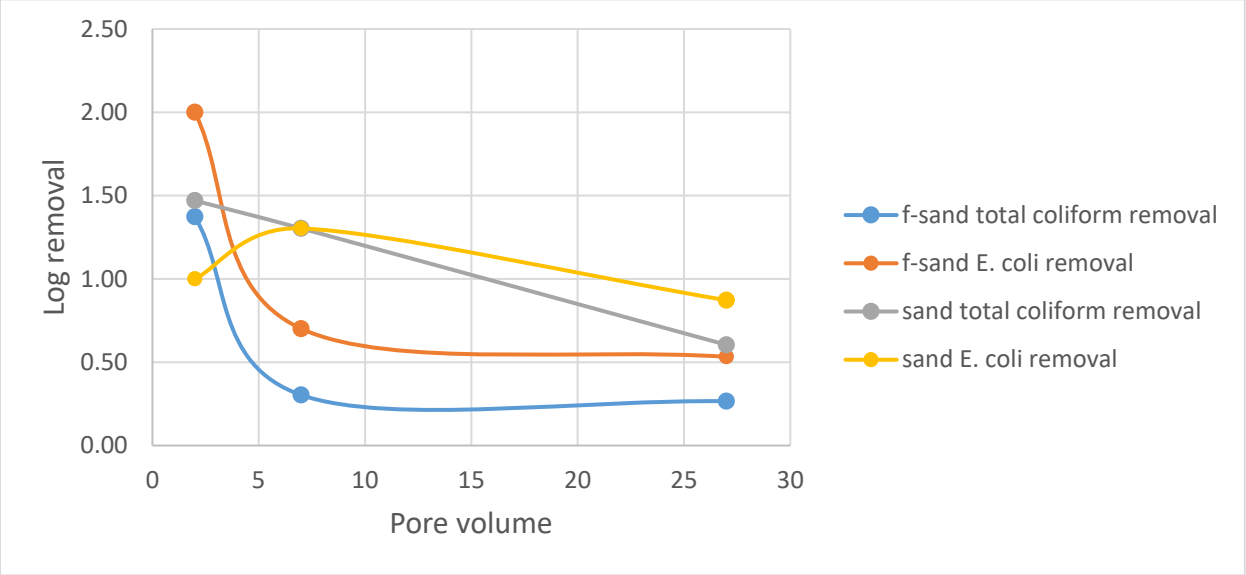

Figure 23: Column removal of coliforms from wastewater effluent.

As preliminary data does not show that moringa-coated sand columns are more effective than sand columns, literature review and testing of controlled moringa-coated sand columns at low concentrations of lab-grown E. coli is needed to determine why the columns are reacting this way.

Low Concentration E. Coli Column Tests

Preliminary data from moringa-coated sand columns indicated that both moringa-coated sand and bare sand columns acted similarly when filtering wastewater. This resulted in a hypothesis that controlled moringa-coated sand columns may act differently at low concentrations of lab-grown E. coli influent. As a result, column experiments, similarly to those performed at higher concentrations in earlier experiments, were performed at concentrations of 10^1, 10^2, and 10^3 CFU/mL.

Moringa-coated sand filter preparation: Whole, unshelled, Nicaraguan moringa seeds were ground with a coffee grinder and mixed with DI water for 5 minutes followed by filtration through a 1.5 μm Whatman glass fiber filter and a 0.22 μm Millipore cellulose acetate filter. Seed dosage was 5.6 grams of seed per square meter of sand surface area. The seed extract was mixed with unwashed Sigma Aldrich glass beads for 5 minutes; the supernatant was discarded, and the glass beads were rinsed with DI water three times to remove organic residues. The glass bead slurry (moringa-coated-sand) was then used to pack glass columns.

Column experiments: The moringa-coated sand was poured into a Bio-rad glass column of 10 cm in length and 1.5 cm inner diameter. The porosity of the packed sand column was gravimetrically determined to be 0.37. The columns were packed with DI water overnight and then equilibrated with PBS solution for 20 pore volumes before switching to appropriate influent solutions prepared in the same PBS solution. A constant flow rate was achieved using a Cole-Parmer peristaltic pump.

Influent preparation: Influent was prepared using Escherichia coli strain TGI (E. coli) containing plasmids that express red fluorescent protein (pCA24N-rfp-lasR) to be used as model

pathogens. Sterilized water and 10 times diluted Phosphate buffered saline (PBS) were used as buffer while sterilized vials were used to collect effluent samples at specified pore volumes. Effluent samples were then analyzed by counting the number of colonies grown with a conventional plate counting method to quantify colony-forming-units.

Results

Contrary to what was hypothesized, E. coli removal in influent with a lower concentration was not similar between moringa-coated sand and bare sand filters. As shown in Figure 24, bare sand filters, which were predicted to remove approximately 0.055 log of E. coli, consistently removed less than 0.5 log removal of E. coli at all measured pore volumes. This result was not surprising. However, on every occasion aside from one, moringa-coated sand columns completely removed all of the E. coli in solution at different pore volumes and low concentrations. One sample of effluent from an E. coli column, taken at 8 pore volumes and 10^3 CFU/mL of E. coli influent, one colony grew from the plated effluent. This could suggest that the column did not remove all of the E. coli, but it is more likely that contamination occurred, especially when one considers the high consistency between other samples taken from moringa-coated sand columns (there are no error bars at any of the other data points because 100% of the time, 100% of E. coli was removed by the moringa-coated sand columns).

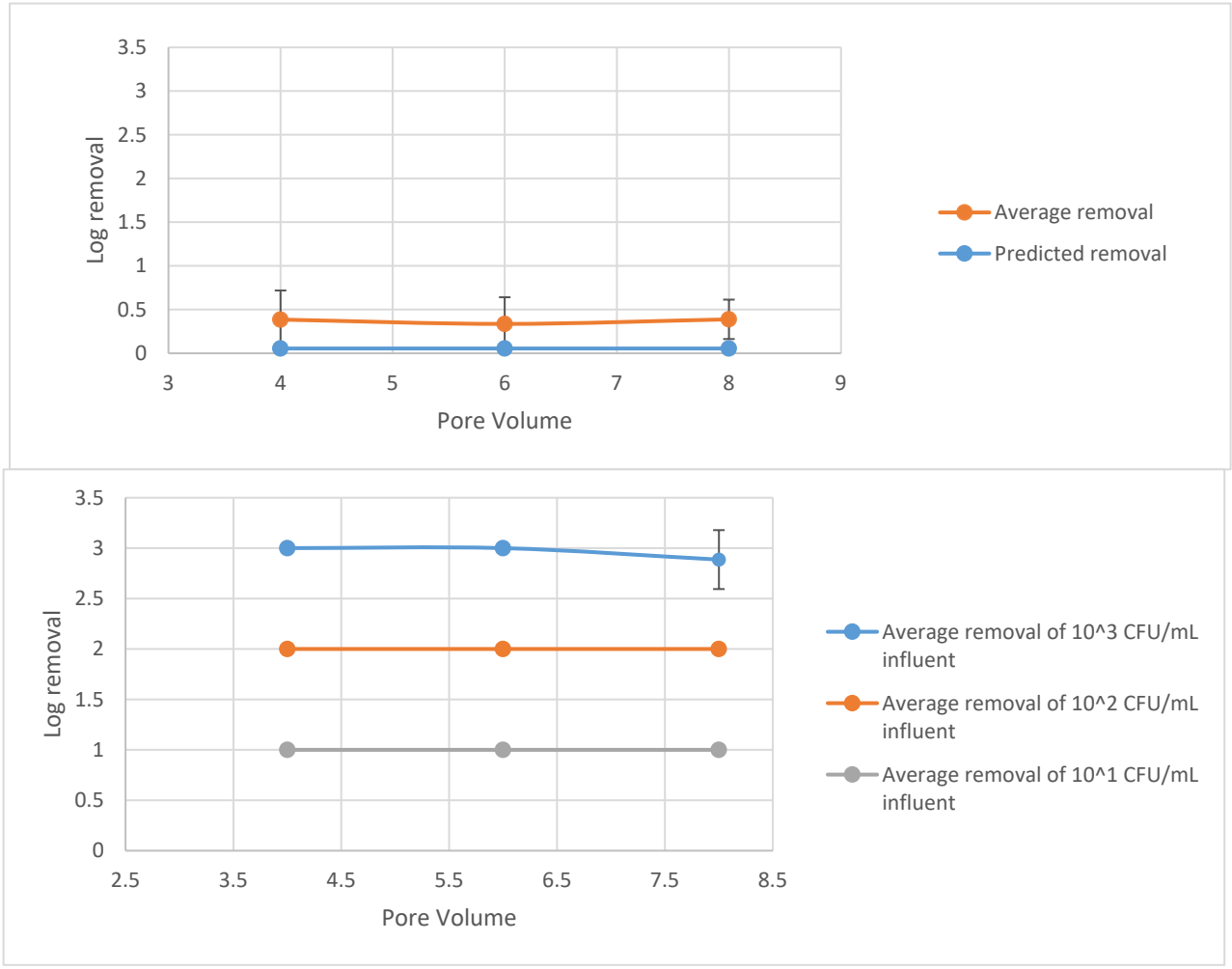

Figure 24: Top: Log removal of E. coli at varying pore volumes at low concentrations of E. coli. Bottom: Log removal of E. coli at varying pore volumes at low concentrations of E. coli.

As this data suggest that moringa-coated sand columns act similarly at both low and high concentrations of E. coli influent, it is likely that there is something else occurring in the wastewater columns discussed in the last section. More research must be done in order to determine what makes the model not accurate for predicting the removal coliforms in wastewater when run through moringa-coated sand columns. It is likely that another component found in wastewater that is not found in our model E. coli-contaminated influent is affecting the filter performance.

Successful Implementation of Moringa-Coated-Sand

One of the biggest challenges is determining if this method of cleaning water will be culturally and socially acceptable in places around the world. In many places in Africa, Moringa trees have been known as “miracle trees” due to many amazing properties. The oil has anti-aging effects, and the leaves are extremely nutritious (Anwar, Latif, Ashraf, & Gilani, 2007). In addition, Moringa seeds have been used for years in an indigenous tradition to clean water. People would crush the seeds and simply add them to water, which could remove 90% of contaminants from water (Santos, Argolo, Coelho, & Paiva, 2005). If it is tradition for people in some African cultures to use the Moringa seed to clean water, our water purification process is more likely to be culturally and socially accepted.

However, some people might have a problem with using Moringa seeds to clean their water. In Rwanda in the early 2000s, there was a big push by the World Food Program, who were trying to help the Rwandan economy (Mbonyinshuti, 2013). They convinced many farmers to risk their livelihood and switch from growing other crops to buying and growing Moringa, but the market was not big enough and most farmers were forced into debt (Mbonyinshuti, 2013). Will Rwandans trust this method and put faith in moringa seeds to clean their water? Since 2012, a company called Asili Oils, a company which extracts oil from Moringa seeds for cosmetics, has been helping to change the attitude towards Moringa seeds by employing locals (Asili Natural Oils: A Social Impact Company, 2018). To some, Moringa is still a bad word – but to others, it has changed to embody a much more positive connotation. To implement our Moringa-coated-sand filters in Rwanda, we will have to be extremely wary and mindful of this mixed history.

An ideal way to approach sustainable implementation of moringa-coated-sand filters involves oil extraction. In Rwanda, Asili Oils removes oil from the seeds and leaves behind seed cake (Asili Natural Oils: A Social Impact Company, 2018). Previous work in our lab has shown that moringa-coated-sand can be created from moringa seed cake and is just as effective as that created from dried moringa seeds. We propose a partnership with oil extraction companies, where the seed cake waste can be used as a resource to create water filters. As many Rwandans have accepted the use of moringa seeds for use in cosmetics, they will be more likely to accept the use in water filters if it partners with a company they already trust.

Another novel way to sustainably implement this process is to potentially take the dirty moringa-coated-sand and sell it to concrete cement companies. The cement creation process would kill any remaining bacteria in the sand, and the MOCP protein is not likely to affect the final cement. This would allow for safe disposal of the clogged filter media in a way that does not create waste. Creative implementation methods such as these will make this sustainable process even more sustainable and will make it both technologically effective and culturally accepted.

Moringa-coated sand can remove E. coli and model bacteria. It can also be modeled mathematically for scale-up and performance prediction. However, this is only half of the work. Any engineering solution must not only be technically effective, but it must also be economically and culturally effective. A solution that passes both the technical and social needs into account is one that will actually make the changes we need in our society.

Conclusion

The moringa-coated-sand filter is one way that water can be treated in a low-technology fashion. An antimicrobial cationic protein from the seeds of the Moringa tree are adsorbed to sand and packed into a column. This filter can remove E. coli and model bacteria and can analyzed in either low or high technology situations to determine its effectiveness before it is used to clean water. It can also be modeled mathematically for scale-up and performance predictions. However, this solution must not only be technically effective, but it must also be economically and culturally effective. When implemented, a collaboration between moringa oil companies, concrete cement companies, and the moringa-coated sand filter team is proposed to sustainable and effectively apply this process outside of the lab and in the real world.

APPENDIX A

Raw data: total nitrogen and total organic carbon moringa-coated-sand tests

MO Serum Sample	Mixing Time (min)	TOC (mg C/L)	TN (mg N/L)	Total protein (mg/L) (Nitrogen*6.25)	% Dissolved protein by Nitrogen
A	5	1003	168	1050	26.25
B	10	784	99	618.75	15.46875
C	20	786	87	543.75	13.59375
D	30	710	63	393.75	9.84375
E	40	711	57	356.25	8.90625
F	60	703	56	350	8.75
A	5	886	167	1043.75	26.09375
B	20	709	90	562.5	14.0625
C	60	616	56	350	8.75
D	5	968	181	1131.25	28.28125
E	20	727	92	575	14.375
F	60	618	58	362.5	9.0625

	A (5)	B (20)	C (60)	D (5)	E (20)	F (60)	A (5)	B (20)	C (60)
Initial mass (g)	0.69	0.64	0.7	0.67	0.69	0.66	0.9361	0.943	0.9518
Dry filter mass (g)	0.9792	1.0084	1.0939	0.9633	1.0494	1.0199	1.025	1.0259	1.0478
Calculated solid mass (g)	0.2892	0.3684	0.3939	0.2933	0.3594	0.3599	0.0889	0.0829	0.096

Initial seed mass added (g)	0.5	0.51	0.52	0.5	0.5	0.51	0.1598	0.1476	0.1565
% MO seed removed by filter	57.8	72.2	75.8	58.7	71.9	70.6	55.6	56.2	61.3

APPENDIX B

Raw data: sPSL counts for effectiveness tests

60 mins unrinsed glass sand		60 mins unrinsed real sand		120 mins unrinsed Chang Mai glass f-sand		120 mins unrinsed Nigerian glass f-sand		120 mins unrinsed Nigerian real f-sand		60 mins unrinsed glass sand	
#	sPSL stuck	#	sPSL stuck	#	sPSL stuck	#	sPSL stuck	#	sPSL stuck	#	sPSL stuck
1	0	2	1	3	9	4	12	7	14	12	2
1	1	2	0	3	14	4	15	7	8	12	2
1	1	2	1	3	8	4	17	7	8	12	1
1	0	2	1	3	8	4	8	7	8	12	0
1	1	2	2	3	9	4	18	7	17	12	1
1	0	2	2	3	8	4	18	7	12	12	0
1	2	2	1	3	9	4	17	7	20	12	0
1	0	2	3	3	10	4	19	7	12	12	0
1	2	2	2	3	16	4	19	7	6	12	1
1	0	2	1	3	16	4	21	7	7	12	1
1	2	2	0	3	15	4	10	7	9	12	1
1	1	2	1	3	15	4	15	7	9	12	2
1	2	2	2	3	22	4	15	7	10	12	0
1	2	2	0	3	8	4	8	7	10	12	1
1	0	2	1	3	8	4	14	7	10	12	1
1	1	2	1	3	10	4	11	7	15	12	0
1	1	2	2	3	15	4	12	7	11	12	0
1	2	2	1	3	10	4	14	7	14	12	0
1	2	2	0	3	9	4	12	7	15	12	1
1	1	2	1	3	12	4	16	7	16	12	2
1	1	2	1	3	13	4	13	7	11	12	0
1	1	2	2	3	9	4	14	7	16	12	0
1	0	2	0	3	13	4	13	7	20	12	0
1	1	2	0	3	10	4	18	7	14	12	1
1	2	2	0	3	14	4	16	7	16	12	1
1	0	2	3	3	10	4	16	7	14	12	0
1	0	2	2	3	16	4	12	7	17	12	0
1	1	2	1	3	9	4	13	7	13	12	0
1	0	2	1	3	12	4	16	7	13	12	1
1	1	2	0	3	11	4	13	7	16	12	1
		2	0	3	12	4	7				

		2	1	3	25	4	7				
		2	1	3	9	4	9				
		2	1	3	12	4	8				
		2	0	3	12	4	9				
		2	1	3	9	4	10				
		2	0	3	12	4	10				
		2	1	3	9	4	9				
		2	0	3	9	4	8				
		2	0	3	8	4	8				
		2	0	3	7	4	10				
		2	1	3	10	4	9				
				3	12						
				3	8						
				3	17						
				3	8						
				3	11						
				3	13						
				3	16						
				3	12						
				3	10						
				3	8						
				3	11						
				3	8						
				3	8						
				3	16						
				3	11						
				3	12						
				3	8						
				3	9						
				3	13						
				3	15						
				3	13						
				3	8						
				3	9						
				3	9						
				3	11						
				3	12						
				3	10						
				3	13						
				3	7						
av	0.933333	av	0.928571	av	11.26761	av	12.83333	av	12.7	av	0.666667

10 mins unrinsed Nigerian glass f-sand		10 mins unrinsed Chang Mai glass f-sand		10 mins unrinsed Haitian glass f-sand (poor germination)		10 mins unrinsed Indian Seed Cake glass f-sand	
#	sPSL stuck	#	sPSL stuck	#	sPSL stuck	#	sPSL stuck
13	14	14	14	16	10	17	10
13	10	14	14	16	11	17	11
13	12	14	13	16	12	17	12
13	13	14	12	16	18	17	11
13	14	14	13	16	15	17	13
13	10	14	13	16	15	17	10
13	12	14	12	16	15	17	16
13	14	14	12	16	16	17	12
13	11	14	11	16	18	17	13
13	11	14	14	16	17	17	13
13	10	14	13	16	11	17	16
13	11	14	13	16	13	17	16
13	11	14	14	16	17	17	12
13	13	14	13	16	16	17	12
13	12	14	12	16	14	17	11
13	12	14	15	16	18	17	12
13	10	14	10	16	12	17	13
13	11	14	14	16	13	17	12
13	12	14	12	16	17	17	14
13	10	14	12	16	13	17	15
av	11.65	av	12.8	av	14.55	av	12.7

10 mins unrinsed Nicaraguan glass f-sand		10X dilution of moringa glass f-sand		100X dilution of moringa glass f-sand		1000X dilution of moringa glass f-sand	
#	sPSL stuck	#	sPSL stuck	#	sPSL stuck	#	sPSL stuck
15	8	18	6	19	3	20	1
15	12	18	7	19	3	20	1
15	10	18	5	19	4	20	2
15	12	18	4	19	3	20	3
15	8	18	5	19	5	20	2
15	8	18	5	19	3	20	0
15	17	18	5	19	4	20	2
15	14	18	9	19	3	20	2
15	15	18	7	19	4	20	3

15	10	18	4	19	3	20	1
15	11	18	6	19	3	20	1
15	12	18	6	19	3	20	0
15	11	18	6	19	4	20	1
15	10	18	4	19	4	20	0
15	18	18	5	19	3	20	2
15	17	18	5	19	4	20	1
15	13	18	4	19	3	20	1
15	11	18	6	19	3	20	2
15	16	18	5	19	2	20	0
15	12	18	6	19	4	20	2
average	12.25	average	5.5	average	3.4	average	1.35
1X dilution of moringa glass f-sand, 60-5 mix		10X dilution of moringa glass f-sand, 60-5 mix		100X dilution of moringa glass f-sand, 60-5 mix		1000X dilution of moringa glass f-sand, 60-5 mix	
#	sPSL stuck	#	sPSL stuck	#	sPSL stuck	#	sPSL stuck
21	10	22	7	23	5	24	1
21	9	22	6	23	3	24	3
21	12	22	8	23	3	24	1
21	15	22	6	23	3	24	1
21	13	22	7	23	4	24	0
21	10	22	4	23	2	24	1
21	13	22	6	23	3	24	0
21	12	22	6	23	5	24	0
21	14	22	7	23	2	24	2
21	9	22	5	23	4	24	1
21	13	22	5	23	5	24	0
21	11	22	7	23	3	24	1
21	11	22	5	23	4	24	1
21	9	22	6	23	2	24	0
21	11	22	6	23	3	24	1
21	13	22	8	23	2	24	0
21	12	22	6	23	1	24	1
21	13	22	6	23	3	24	0
21	12	22	7	23	2	24	0
21	14	22	5	23	3	24	1
average	11.8	average	6.15	average	3.1	average	0.75
10 mins unrinsed Nigerian glass f-sand Bashir A		10 mins unrinsed Nigerian glass f-sand Bashir B		10 mins unrinsed Nigerian glass f-sand Bashir C		10 mins unrinsed Nigerian glass f-sand Bashir D	
	sPSL stuck						sPSL stuck

sample number		sample number	sPSL stuck	sample number	sPSL stuck	sample number	
25	12	26	10	27	12	28	13
25	13	26	12	27	13	28	10
25	10	26	11	27	11	28	11
25	12	26	12	27	12	28	13
25	12	26	13	27	14	28	12
25	13	26	11	27	11	28	9
25	11	26	11	27	12	28	11
25	12	26	10	27	12	28	13
25	10	26	9	27	10	28	10
25	11	26	11	27	10	28	9
25	14	26	9	27	11	28	11
25	10	26	12	27	12	28	11
25	11	26	13	27	10	28	12
25	12	26	9	27	11	28	10
25	9	26	10	27	10	28	14
25	11	26	11	27	11	28	13
25	12	26	12	27	12	28	13
25	11	26	11	27	10	28	9
25	12	26	13	27	12	28	12
25	11	26	12	27	14	28	11
average	11.45	average	11.1	average	11.5	average	11.35

10 mins unrinsed Haitian glass f-sand Kluny Tree 1		10 mins unrinsed Haitian glass f-sand Kluny Tree 2		10 mins unrinsed Haitian glass f-sand Josline		10 mins unrinsed Haitian glass f-sand Nana		10 mins unrinsed Haitian glass f-sand Melija	
sample number	sPSL stuck	sample number	sPSL stuck	sample number	sPSL stuck	sample number	sPSL stuck	sample number	sPSL stuck
31	12	32	13	33	13	34	10	35	12
31	13	32	12	33	11	34	12	35	11
31	12	32	10	33	12	34	10	35	13
31	13	32	16	33	14	34	12	35	13
31	10	32	15	33	11	34	13	35	11
31	11	32	12	33	9	34	12	35	13
31	13	32	11	33	11	34	11	35	10
31	8	32	14	33	13	34	13	35	13
31	13	32	11	33	10	34	12	35	8
31	12	32	9	33	9	34	15	35	11
31	14	32	10	33	12	34	12	35	10
31	13	32	11	33	9	34	10	35	11
31	11	32	16	33	11	34	10	35	14

31	8	32	12	33	13	34	11	35	11
31	11	32	11	33	13	34	13	35	12
31	11	32	11	33	12	34	9	35	10
31	10	32	7	33	13	34	10	35	12
31	9	32	11	33	10	34	13	35	10
31	15	32	11	33	12	34	11	35	11
31	10	32	11	33	13	34	11	35	13
average	11.45	average	11.7	average	11.55	average	11.5	average	11.45

10 mins unrinsed Kenya glass f-sand White seeds		10 mins unrinsed Kenya glass f-sand Brown seeds	
sample number	sPSL stuck	sample number	sPSL stuck
29	14	30	11
29	12	30	11
29	11	30	14
29	9	30	17
29	11	30	13
29	8	30	11
29	12	30	11
29	12	30	12
29	13	30	8
29	9	30	14
29	14	30	11
29	9	30	12
29	12	30	10
29	10	30	11
29	12	30	9
29	13	30	16
29	11	30	9
29	13	30	11
29	11	30	10
29	12	30	13
average	11.4	average	11.7

Shaking mixing 10 minutes		Stir plate mixing 10 minutes	

sample number	sPSL stuck	sample number	sPSL stuck
29	14	13	14
29	12	13	10
29	11	13	12
29	9	13	13
29	11	13	14
29	8	13	10
29	12	13	12
29	12	13	14
29	13	13	11
29	9	13	11
29	14	13	10
29	9	13	11
29	12	13	11
29	10	13	13
29	12	13	12
29	13	13	12
29	11	13	10
29	13	13	11
29	11	13	12
29	12	13	10
30	11	14	14
30	11	14	14
30	14	14	13
30	17	14	12
30	13	14	13
30	11	14	13
30	11	14	12
30	12	14	12
30	8	14	11
30	14	14	14
30	11	14	13
30	12	14	13
30	10	14	14
30	11	14	13
30	9	14	12
30	16	14	15
30	9	14	10
30	11	14	14
30	10	14	12
30	13	14	12

32	13	17	10
32	12	17	11
32	10	17	12
32	16	17	11
32	15	17	13
32	12	17	10
32	11	17	16
32	14	17	12
32	11	17	13
32	9	17	13
32	10	17	16
32	11	17	16
32	16	17	12
32	12	17	12
32	11	17	11
32	11	17	12
32	7	17	13
32	11	17	12
32	11	17	14
32	11	17	15
33	13	average	12.38333
33	11		
33	12		
33	14		
33	11		
33	9		
33	11		
33	13		
33	10		
33	9		
33	12		
33	9		
33	11		
33	13		
33	13		
33	12		
33	13		
33	10		
33	12		
33	13		
34	10		
34	12		

34	10		
34	12		
34	13		
34	12		
34	11		
34	13		
34	12		
34	15		
34	12		
34	10		
34	10		
34	11		
34	13		
34	9		
34	10		
34	13		
34	11		
34	11		
35	12		
35	11		
35	13		
35	13		
35	11		
35	13		
35	10		
35	13		
35	8		
35	11		
35	10		
35	11		
35	14		
35	11		
35	12		
35	10		
35	12		
35	10		
35	11		
35	13		
average	11.55		

APPENDIX C

Samples passing both the "stick" test and microscope analysis

Serum mixing time	Sand mixing time	Collector type and size	Original seed location and notes
60 minutes	60 minutes	250-300 μm glass beads	Chang Mai
60 minutes	60 minutes	250-300 μm glass beads	Nigeria
5 minutes	60 minutes	250-300 μm sand	Nigeria
20 minutes	60 minutes	250-300 μm sand	Nigeria
60 minutes	60 minutes	250-300 μm sand	Nigeria
60 minutes	60 minutes	106 μm glass beads	Chang Mai
20 minutes	60 minutes	250-300 μm glass beads	Chang Mai
60 minutes	60 minutes	250-300 μm glass beads	Chang Mai
5 minutes	5 minutes	250-300 μm glass beads	Nigeria
5 minutes	5 minutes	250-300 μm glass beads	Chang Mai
5 minutes	5 minutes	250-300 μm glass beads	Nicaragua
5 minutes	5 minutes	250-300 μm glass beads	Haiti *Note: poor seed germination
5 minutes	5 minutes	250-300 μm glass beads	India *Note: seed cake was used instead of dried seeds
5 minutes	5 minutes	250-300 μm glass beads	Nigeria *Note: green, not dried on tree, picked during dry season
5 minutes	5 minutes	250-300 μm glass beads	Nigeria B *Note: brown, not dried on tree, picked during dry season
5 minutes	5 minutes	250-300 μm glass beads	Nigeria C *Note: brown, dried on tree, picked during dry season

5 minutes	5 minutes	250-300 μm glass beads	Nigeria D *Note: brown, dried on tree, picked during rainy season
5 minutes	5 minutes	250-300 μm glass beads	Tharaka, Kenya *Note: white
5 minutes	5 minutes	250-300 μm glass beads	Tharaka, Kenya *Note: brown
5 minutes	5 minutes	250-300 μm glass beads	Haiti *Note: Kluny tree 1
5 minutes	5 minutes	250-300 μm glass beads	Haiti *Note: Kluny tree 2
5 minutes	5 minutes	250-300 μm glass beads	Haiti *Note: Josline
5 minutes	5 minutes	250-300 μm glass beads	Haiti *Note: Nana
5 minutes	5 minutes	250-300 μm glass beads	Haiti *Note: Melija

APPENDIX D

Column Experiment Test Log

Factor	Date	Column Size	Seed Load (ug/ mL)	Seed (g)	Serum (mL)	Sand (g)	Sand Size (um)	Salt (mM)	Influent (#/mL)	Flow (mL/ min)	#
Seed Loading	07/22/16	10cm L, 1.6cm ID	10000	2.4996	250	25.3208	106	1 mM	10^6/mL	1.6	1
Seed Loading	07/24/16	10cm L, 1.6cm ID	10000	N/A	610	N/A	106	1 mM	10^6/mL	1.6	2
Seed Loading	07/24/16	10cm L, 1.6cm ID	10000	N/A	610	N/A	106	1 mM	10^6/mL	1.6	3
Seed Loading	07/25/16	10cm L, 1.6cm ID	1000	0.5998	610	25.2429	106	10 mM	10^6/mL	1.6	1
Seed Loading	07/25/16	10cm L, 1.6cm ID	1000	0.6012	610	25.1575	106	10 mM	10^6/mL	1.6	2
Seed Loading	07/25/16	10cm L, 1.6cm ID	1000	N/A	610	N/A	106	1 mM	10^6/mL	1.6	2
Seed Loading	07/26/16	10cm L, 1.6cm ID	1000	0.5998	610	25.2429	106	30 mM	10^6/mL	1.6	1
Seed Loading	07/26/16	10cm L, 1.6cm ID	5000	3.012	610	25.3358	106	1 mM	10^6/mL	1.6	1
Seed Loading	07/26/16	10cm L, 1.6cm ID	5000	3.0146	610	25.2781	106	1 mM	10^6/mL	1.6	2
Seed Loading	7/26/16	10cm L, 1.6cm ID	1000	0.5998	610	25.2429	106	5 mM	10^6/mL	1.6	1
Seed Loading	07/31/16	10cm L, 1.6cm ID	1000	2.0046	2000	25.3898	106	5 mM	10^6/mL	1.6	2
Seed Loading	07/31/16	10cm L, 1.6cm ID	1000			25.2727	106	5 mM	10^6/mL	1.6	3
Seed Loading	07/31/16	10cm L, 1.6cm ID	1000			25.1896	106	5 mM	10^6/mL	1.6	4
Seed Loading	08/09/16	10cm L, 1.6cm ID	10000	6.1063	610	25.0066	106	1 mM	10^6/mL	1.6	4
Seed Loading	08/09/16	10cm L, 1.6cm ID	5000	3.057	610	25.0408	106	1 mM	10^6/mL	1.6	3
Seed Loading	08/09/16	10cm L, 1.6cm ID	1000	0.6103	610	25.0394	106	1 mM	10^6/mL	1.6	3
Seed Loading	08/10/16	10cm L, 1.6cm ID	1000	0.6182	610	25.0462	106	1 mM	10^6/mL	1.6	4
Seed Loading	08/30/16	10cm L, 1.6cm ID	1000	0.6108	610	24.9912	106	1 mM	10^6/mL	1.6	5
Seed Loading	8/31/16	10cm L, 1.6cm ID	1000	0.6062	610	25.1348	106	1 mM	10^6/mL	1.6	6
Seed Loading	09/21/16	10cm L, 1.6cm ID	10000	12.2187	1220	25.0766	106	1 mM	10^6/mL	1.6	5
Seed Loading	09/21/16	10cm L, 1.6cm ID	10000			25.0293	106	1 mM	10^6/mL	1.6	6

Seed Loading	7/21/16	10cm L, 1.6cm ID	Bare sand	0	0	N/A	106	1 mM	10^6/mL	1.6	C1
Seed Loading	7/21/16	10cm L, 1.6cm ID	Bare sand	0	0	N/A	106	1 mM	10^6/mL	1.6	C2
Seed Loading	7/22/16	10cm L, 1.6cm ID	Bare sand	0	0	24.9511	106	1 mM	10^6/mL	1.6	C3
Seed Loading	7/24/16	10cm L, 1.6cm ID	Bare sand	0	0	N/A	106	1 mM	10^6/mL	1.6	C4
Seed Loading	7/26/16	10cm L, 1.6cm ID	Bare sand	0	0	25.6287	106	5 mM	10^6/mL	1.6	C1
Seed Loading	7/18/16	10cm L, 1.6cm ID	Bare sand	0	0	24.9812	106	10 mM	10^6/mL	1.6	C1
Seed Loading	7/18/16	10cm L, 1.6cm ID	Bare sand	0	0	25.3248	106	10 mM	10^6/mL	1.6	C2
Seed Loading	7/18/16	10cm L, 1.6cm ID	Bare sand	0	0	25.0116	106	10 mM	10^6/mL	1.6	C3
Seed Loading	7/18/16	10cm L, 1.6cm ID	Bare sand	0	0	25.2109	106	10 mM	10^6/mL	1.6	C4
Seed Loading	7/19/16	10cm L, 1.6cm ID	Bare sand	0	0	N/A	106	10 mM	10^6/mL	1.6	C5
Seed Loading	7/19/16	10cm L, 1.6cm ID	Bare sand	0	0	N/A	106	10 mM	10^6/mL	1.6	C6
Seed Loading	7/26/16	10cm L, 1.6cm ID	Bare sand	0	0	25.6287	106	30 mM	10^6/mL	1.6	C1
Seed Loading	7/31/16	10cm L, 1.6cm ID	Bare sand	0	0	25.4514	106	30 mM	10^6/mL	1.6	C2
Seed Loading	8/1/16	10cm L, 1.6cm ID	Bare sand	0	0	25.3497	106	30 mM	10^6/mL	1.6	C3
Seed Loading	8/1/16	10cm L, 1.6cm ID	Bare sand	0	0	24.9811	106	30 mM	10^6/mL	1.6	C4
Salt Concentration	7/26/16	10cm L, 1.6cm ID	5000	3.012	610	25.3358	106	1 mM	10^6/mL	1.6	1
Salt Concentration	7/26/16	10cm L, 1.6cm ID	5000	3.0146	610	25.2781	106	1 mM	10^6/mL	1.6	2
Salt Concentration	8/9/16	10cm L, 1.6cm ID	5000	3.057	610	25.0408	106	1 mM	10^6/mL	1.6	3
Salt Concentration	7/26/16	10cm L, 1.6cm ID	5000	N/A	610	N/A	106	5 mM	10^6/mL	1.6	1
Salt Concentration	10/11/16	10cm L, 1.6cm ID	5000	3.0518	610	25.0769	106	5 mM	10^6/mL	1.6	2
Salt Concentration	10/11/16	10cm L, 1.6cm ID	5000	3.0571	610	24.9922	106	5 mM	10^6/mL	1.6	3
Salt Concentration	10/18/16	10cm L, 1.6cm ID	5000	3.0524	610	25.0863	106	5 mM	10^6/mL	1.6	4

Salt Concentration	10/18/16	10cm L, 1.6cm ID	5000	3.0531	610	24.9881	106	10 mM	10^6/mL	1.6	1
Salt Concentration	10/25/16	10cm L, 1.6cm ID	5000	3.0533	610	24.9037	106	10 mM	10^6/mL	1.6	2
Salt Concentration	10/25/16	10cm L, 1.6cm ID	5000	3.0533	610	24.9902	106	10 mM	10^6/mL	1.6	3
Longevity	1/25/17	5 cm L, 1 cm ID	5000	0.286	55	5.5245	106	1 mM	10^6/mL	1.6	1
Longevity	1/30/17	5 cm L, 1 cm ID	5000	0.2822	56	5.5046	106	1 mM	10^6/mL	1.6	2
Longevity	2/5/17	5 cm L, 1 cm ID	5000	0.2809	55	5.5044	106	1 mM	10^6/mL	1.6	3
Longevity	2/6/17	5 cm L, 1 cm ID	5000	0.2802	55	5.4998	106	1 mM	10^6/mL	1.6	4
Flow rates	2/13/17	10cm L, 1.6cm ID	5000	3.01	610	25.001	106	1 mM	10^6/mL	15	1
Flow rates	2/13/17	10cm L, 1.6cm ID	5000	3.04	610	24.94	106	1 mM	10^6/mL	15	2
Flow rates	2/16/17	10cm L, 1.6cm ID	5000	3.045	610	25.0253	106	1 mM	10^6/mL	15	3
Flow rates	2/16/17	10cm L, 1.6cm ID	5000	3.0014	610	25.0009	106	1 mM	10^6/mL	15	4
Flow rates	2/19/17	10cm L, 1.6cm ID	5000	3.0543	610	25.074	106	1 mM	10^6/mL	15	5
Flow rates	2/19/17	10cm L, 1.6cm ID	5000	3.0049	610	25.0101	106	1 mM	10^6/mL	15	6
Flow rates	2/23/17	10cm L, 1.6cm ID	5000	3.051	610	24.949	106	1 mM	10^6/mL	15	7
Flow rates	2/23/17	10cm L, 1.6cm ID	5000	3.0515	610	25.004	106	1 mM	10^6/mL	15	8
Flow rates	2/26/17	10cm L, 1.6cm ID	5000	3.0498	610	25.0063	106	1 mM	10^6/mL	0.7	1
Flow rates	2/26/17	10cm L, 1.6cm ID	5000	3.0515	610	24.9996	106	1 mM	10^6/mL	0.7	2
Flow rates	3/2/17	10cm L, 1.6cm ID	5000	3.0503	610	25.0014	106	1 mM	10^8/mL	0.7	3
Flow rates	3/2/17	10cm L, 1.6cm ID	5000	3.0501	610	24.9978	106	1 mM	10^8/mL	0.7	4
Flow rates	3/12/17	10cm L, 1.6cm ID	5000	3.0501	610	24.9567	106	1 mM	10^8/mL	0.7	5
Flow rates	3/12/17	10cm L, 1.6cm ID	5000	3.0511	610	25.002	106	1 mM	10^8/mL	0.7	6
Flow rates	3/15/17	10cm L, 1.6cm ID	5000	3.0498	610	25.0031	106	1 mM	10^8/mL	0.7	7
Flow rates	3/15/17	10cm L, 1.6cm ID	5000	3.0508	610	24.9936	106	1 mM	10^8/mL	0.7	8
Flow rates	3/19/17	10cm L, 1.6cm ID	5000	3.0509	610	25.0993	106	1 mM	10^8/mL	0.7	9
Flow rates	3/19/17	10cm L, 1.6cm ID	5000	3.0507	610	24.9979	106	1 mM	10^8/mL	0.7	10

Collector size	3/23/17	10cm L, 1.6cm ID	5000	1.2996	266	25.9858	256	1 mM	10^6/mL	1.6	1
Collector size	3/23/17	10cm L, 1.6cm ID	5000	1.3033	266	25.9748	256	1 mM	10^6/mL	1.6	2
Collector size	3/26/17	10cm L, 1.6cm ID	5000	0.7029	133	26.0295	512	1 mM	10^6/mL	1.6	1
Collector size	3/26/17	10cm L, 1.6cm ID	5000	0.702	133	25.9989	512	1 mM	10^6/mL	1.6	2
Collector size	3/29/17	10cm L, 1.6cm ID	5000	1.2996	266	25.9567	256	1 mM	10^6/mL	1.6	3
Collector size	3/29/17	10cm L, 1.6cm ID	5000	1.3014	266	26.0046	256	1 mM	10^6/mL	1.6	4
Collector size	4/2/17	10cm L, 1.6cm ID	5000	0.7037	133	26.0777	512	1 mM	10^6/mL	1.6	3
Collector size	4/2/17	10cm L, 1.6cm ID	5000	0.7022	133	25.9973	512	1 mM	10^6/mL	1.6	4
Collector size	4/5/17	10cm L, 1.6cm ID	5000	3.0501	610	25.0009	106	1 mM	10^6/mL	1.6	1
Collector size	4/5/17	10cm L, 1.6cm ID	5000	3.0503	610	24.9998	106	1 mM	10^6/mL	1.6	2
Collector size	4/9/17	10cm L, 1.6cm ID	5000	3.0444	610	25.109	106	1 mM	10^6/mL	1.6	3
Collector size	4/9/17	10cm L, 1.6cm ID	5000	3.0552	610	25.1489	106	1 mM	10^6/mL	1.6	4
Collector size	4/12/17	10cm L, 1.6cm ID	5000	0.6977	133	25.9878	512	1 mM	10^6/mL	1.6	5
Collector size	4/12/17	10cm L, 1.6cm ID	5000	0.7004	133	26.0112	512	1 mM	10^6/mL	1.6	6
Longevity	4/16/17	5 cm L, 1 cm ID	5000	0.2799	56	5.4922	106	1mM	10^7/mL	0.7	1
Longevity	4/16/17	5 cm L, 1 cm ID	5000	0.2805	56	5.4988	106	1mM	10^7/mL	0.7	2
Longevity	4/19/17	5 cm L, 1 cm ID	5000	0.2787	55	5.5032	106	1 mM	10^7/mL	0.7	3
E. Coli	6/6/17	10cm L, 1.6cm ID	5000	3.049	610	24.992	106	N/A	10^6/mL	1.6	1
E. Coli	6/6/17	10cm L, 1.6cm ID	5000	3.052	610	25.016	106	1 mM	10^6/mL	1.6	2
Flow rates	6/11/17	10cm L, 1.6cm ID	5000	3.05	610	24.8	106	1 mM	10^6/mL	3.2	1
Flow rates	6/11/17	10cm L, 1.6cm ID	5000	3.05	610	24.99	106	1 mM	10^6/mL	3.2	2
Flow rates	6/11/17	10cm L, 1.6cm ID	5000	3.052	610	25	106	1 mM	10^6/mL	3.2	3
Flow rates	6/12/17	10cm L, 1.6cm ID	5000	3.05	610	24.9929	106	1 mM	10^6/mL	7	1
Flow rates	6/12/17	10cm L, 1.6cm ID	5000	3.0505	610	24.9905	106	1 mM	10^6/mL	7	2
Flow rates	6/12/17	10cm L, 1.6cm ID	5000	3.0501	610	25.0026	106	1 mM	10^6/mL	7	3
Flow rates	6/14/17	10cm L, 1.6cm ID	5000	3.051	610	24.9982	106	1 mM	10^6/mL	3.2	1
Flow rates	6/14/17	10cm L, 1.6cm ID	5000	3.049	610	25.0056	106	1 mM	10^6/mL	3.2	2

Flow rates	06/15/17	10cm L, 1.6cm ID	5000	3.0498	610	24.9999	106	1 mM	10^6/mL	3.2	3
Flow rates	06/15/17	10cm L, 1.6cm ID	5000	3.051	610	25.0021	106	1 mM	10^6/mL	3.2	4
Flow rates	6/18/17	10cm L, 1.6cm ID	5000	3.0501	610	25.0225	106	1 mM	10^6/mL	7	1
Flow rates	6/18/17	10cm L, 1.6cm ID	5000	3.0501	610	25.0052	106	1 mM	10^6/mL	7	2
Flow rates	06/19/17	10cm L, 1.6cm ID	5000	3.0498	610	24.9997	106	1 mM	10^6/mL	7	3
Flow rates	06/19/17	10cm L, 1.6cm ID	5000	3.0499	610	25	106	1 mM	10^6/mL	7	4
E. Coli	06/19/17	10cm L, 1.6cm ID	5000	3.0499	610	24.9998	106	N/A	10^6/mL	1.6	1
E. Coli	06/19/17	10cm L, 1.6cm ID	5000	3.0498	610	24.9995	106	N/A	10^6/mL	1.6	2
E. Coli	6/26/17	10cm L, 1.6cm ID	5000	3.0513	610	24.999	106	N/A	10^6/mL	1.6	3
E. Coli	6/26/17	10cm L, 1.6cm ID	5000	3.0492	610	24.9959	106	N/A	10^6/mL	1.6	4
E. Coli	7/5/17	10cm L, 1.6cm ID	5000	3.0477	610	24.9649	106	N/A	10^6/mL	1.6	5
E. Coli	7/5/17	10cm L, 1.6cm ID	5000	3.0501	610	25.0321	106	N/A	10^6/mL	1.6	6
E. Coli	7/5/17	10cm L, 1.6cm ID	5000	3.0491	610	25.0201	106	N/A	10^6/mL	1.6	7
E. Coli	7/5/17	10cm L, 1.6cm ID	Bare sand	0	610	25.0002	106	N/A	10^6/mL	1.6	C1
E. Coli Longevity	7/17/17	5 cm L, 1 cm ID	5000	0.2804	55	5.50008	106	N/A	10^6/mL	1.6	1
E. Coli Longevity	7/17/17	5 cm L, 1 cm ID	5000	0.2803	55	5.50009	106	N/A	10^6/mL	1.6	2
E. Coli	7/19/17	10cm L, 1.6cm ID	5000	3.0507	610	25.0006	106	N/A	10^6/mL	1.6	8
E. Coli	7/19/17	10cm L, 1.6cm ID	5000	3.0505	610	25.0002	106	N/A	10^6/mL	1.6	9
E. Coli	7/19/17	10cm L, 1.6cm ID	5000	3.0497	610	24.9993	106	N/A	10^6/mL	1.6	10
E. Coli	8/24/17	10cm L, 1.6cm ID	5000	3.0503	610	25.0001	106	N/A	10^6/mL	1.6	11
E. Coli	8/24/17	10cm L, 1.6cm ID	5000	3.0503	610	25.0002	106	N/A	10^6/mL	1.6	12
E. Coli	8/24/17	10cm L, 1.6cm ID	5000	3.0504	610	25.0004	106	N/A	10^6/mL	1.6	13
Wastewater influent	9/12/17	10cm L, 1.6cm ID	Bare sand	0	610	25.0233	106	1 mM	Unknown	1.6	C1
Wastewater influent	9/12/17	10cm L, 1.6cm ID	5000	3.0474	610	25.028	106	1 mM	Unknown	1.6	1

Wastewater influent	9/26/17	10cm L, 1.6cm ID	Bare sand	0	610	24.9611	106	1 mM	Unknown	1.6	C2
Wastewater influent	9/26/17	10cm L, 1.6cm ID	5000	3.0501	610	25.109	106	1 mM	Unknown	1.6	2
Shelf tests	9/17/17	10cm L, 1.6cm ID	5000	3.0489	610	24.886	106	1 mM	10^6/mL	1.6	1
Shelf tests	9/17/17	10cm L, 1.6cm ID	5000	3.0518	610	24.99	106	1 mM	10^6/mL	1.6	2
Shelf tests	9/17/17	10cm L, 1.6cm ID	5000	3.0502	610	25.046	106	1 mM	10^6/mL	1.6	3
Shelf tests	9/17/17	10cm L, 1.6cm ID	5000	3.0518	610	25.0055	106	1 mM	10^6/mL	1.6	4
Shelf tests	9/17/17	10cm L, 1.6cm ID	5000	3.0505	610	24.9812	106	1 mM	10^6/mL	1.6	5
Shelf tests	9/17/17	10cm L, 1.6cm ID	5000	3.0505	610	25.001	106	1 mM	10^6/mL	1.6	6
Shelf tests	9/29/17	10cm L, 1.6cm ID	5000	3.0522	610	24.929	106	1 mM	10^6/mL	1.6	7
Shelf tests	9/29/17	10cm L, 1.6cm ID	5000	3.0473	610	25.104	106	1 mM	10^6/mL	1.6	8
Shelf tests	9/29/17	10cm L, 1.6cm ID	5000	3.0477	610	24.9862	106	1 mM	10^6/mL	1.6	9
Shelf tests	9/30/17	10cm L, 1.6cm ID	5000	3.0486	610	25.0088	106	1 mM	10^6/mL	1.6	10
Shelf tests	9/30/17	10cm L, 1.6cm ID	5000	3.0497	610	25.0018	106	1 mM	10^6/mL	1.6	11
Shelf tests	9/30/17	10cm L, 1.6cm ID	5000	3.0483	610	24.981	106	1 mM	10^6/mL	1.6	12
Shelf tests	10/23/17	10cm L, 1.6cm ID	5000	3.0507	610	25.1875	106	1 mM	10^6/mL	1.6	13
Shelf tests	10/23/17	10cm L, 1.6cm ID	5000	3.0522	610	25.2231	106	1 mM	10^6/mL	1.6	14
Shelf tests	10/23/17	10cm L, 1.6cm ID	5000	3.0506	610	25.9052	106	1 mM	10^6/mL	1.6	15
Low concentration E. coli	12/4/17	10cm L, 1.6cm ID	5000	3.0458	610	25.0056	106	N/A	10^3/mL	1.6	1
Low concentration E. coli	12/4/17	10cm L, 1.6cm ID	5000	3.0505	610	24.9998	106	N/A	10^3/mL	1.6	2
Low concentration E. coli	12/4/17	10cm L, 1.6cm ID	5000	3.0476	610	24.9828	106	N/A	10^3/mL	1.6	3
Low concentration E. coli	12/4/17	10cm L, 1.6cm ID	5000	3.0533	610	25.0321	106	N/A	10^3/mL	1.6	4
Low concent	1/19/18	10cm L, 1.6cm ID	Bare sand	0	610	24.9705	106	N/A	10^3/mL	1.6	C1

ration E. coli											
Low concentration E. coli	1/19/18	10cm L, 1.6cm ID	5000	3.0563	610	24.9901	106	N/A	10^3/mL	1.6	5
Low concentration E. coli	1/19/18	10cm L, 1.6cm ID	5000	3.0469	610	24.9705	106	N/A	10^3/mL	1.6	6
Low concentration E. coli	1/31/18	10cm L, 1.6cm ID	Bare sand	0	610	25.0209	106	N/A	10^3/mL	1.6	C2
Low concentration E. coli	1/31/18	10cm L, 1.6cm ID	5000	3.0502	610	24.9829	106	N/A	10^3/mL	1.6	7
Low concentration E. coli	1/31/18	10cm L, 1.6cm ID	5000	3.0503	610	25.0638	106	N/A	10^3/mL	1.6	8
Low concentration E. coli	2/5/18	10cm L, 1.6cm ID	Bare sand	0	610	25.0252	106	N/A	10^1/mL	1.6	C3
Low concentration E. coli	2/5/18	10cm L, 1.6cm ID	5000	3.0534	610	25.044	106	N/A	10^1/mL	1.6	1
Low concentration E. coli	2/5/18	10cm L, 1.6cm ID	5000	3.0497	610	25.0109	106	N/A	10^1/mL	1.6	2
Low concentration E. coli	2/14/18	10cm L, 1.6cm ID	5000	3.05	610	24.9984	106	N/A	10^2/mL	1.6	1
Low concentration E. coli	2/14/18	10cm L, 1.6cm ID	5000	3.05	610	25.0199	106	N/A	10^2/mL	1.6	2
Low concentration E. coli	2/14/18	10cm L, 1.6cm ID	Bare sand	0	610	25.0131	106	N/A	10^2/mL	1.6	C4
Low concentration E. coli	2/20/18	10cm L, 1.6cm ID	5000	3.0565	610	25.0154	106	N/A	10^3/mL	1.6	9
Low concentration E. coli	2/20/18	10cm L, 1.6cm ID	5000	3.0556	610	25.097	106	N/A	10^3/mL	1.6	10

Low concentration E. coli	2/20/18	10cm L, 1.6cm ID	5000	0	610	25.008	106	N/A	10^3/mL	1.6	C5
Low concentration E. coli	2/27/18	10cm L, 1.6cm ID	5000	3.0571	610	25.0258	106	N/A	10^3/mL	1.6	11
Low concentration E. coli	2/27/18	10cm L, 1.6cm ID	5000	3.0506	610	25.0929	106	N/A	10^3/mL	1.6	12
Low concentration E. coli	2/27/18	10cm L, 1.6cm ID	bare sand	0	610	25.0333	106	N/A	10^3/mL	1.6	C6

APPENDIX E

Raw data: Wastewater Column Tests

	9/13/17	9/13/17	9/13/17	9/27/17	9/27/17	9/27/17	9/27/17	9/27/17
Sample	Influent	Moringa-coated-sand	Bare sand	Influent	Moringa-coated-sand	Moringa-coated-sand	Bare sand	Bare sand
Approx. pore volume filtered (pore volume)	N/A	0 to 2	0 to 2	N/A	5 to 7	7 to 27	5 to 7	7 to 27
	N/A	2	2	N/A	7	27	7	27
Dilution	1/10 dilution	1/10 dilution	1/10 dilution	N/A	1/10 dilution	N/A	1/10 dilution	N/A
Total Coliform (measured) (MPN2/100 mL)	118	5	4	201	10	109	1	50
Total Coliform (actual) (MPN2/100 mL)	1180	50	40	201	100	109	10	50
Total Coliform log removal	N/A	1.37	1.47	N/A	0.30	0.27	1.30	0.60
E. coli (measured) (MPN2/100 mL)	10	0	1	201	4	59	1	27
E. coli (actual) (MPN2/100 mL)	100	0	10	201	40	59	10	27
E. coli log removal	N/A	2.00	1.00	N/A	0.70	0.53	1.30	0.87

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- Presented research in 7 different research symposiums, exhibitions, and competitions
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