THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

REMOVAL OF MODEL MICROBES USING MORINGA OLEIFERA PROTEIN COVERED SAND

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ABSTRACT

Standard water treatment methods include coagulation, flocculation, sedimentation, disinfection, membrane filtration, reverse osmosis and ultraviolet light (UV) (Yongabi *et*. *al* 2011). However, not all of these methods are available in the rural and impoverished areas that need clean water the most. Previous work has shown the feasibility of a water filter created from sand coated with cationic proteins from seeds from the *Moringa oleifera* tree (known as *f*-sand). F-sand has been shown to remove and even inactivate microbes. In order to scale up and *f*-sand filter, many parameters must be known. One of those parameters is the fractional coverage of microbes that will coat the surface of the *f*-sand. Here we developed a method to test the fractional coverage of microbes on the surface of *f*-sand. The fractional coverage of *f*-sand ranges from 9.1% to 16.7% in DI and low salt solutions, and coverage remains constant over time. On average, model microbes cover 13% of the surface of *f*-sand particles. The fractional coverage of *f*-sand is greater in tap water solutions, with a range of values from 11.9% to 26.4%. Coverage in tap water solutions is variable and unpredictable over time. After creating *f*-sand and testing its fractional coverage using seeds from Thailand, Nicaragua, and Tanzania, we determined that the fractional coverage is constant with location and time.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Stephanie Velegol for serving as my thesis advisor. Your support and guidance, both in academic research and beyond, has helped me grow as a student and professional. I am incredibly grateful for the opportunity to work on such a rewarding project that aims to provide a simple, yet sustainable, method for producing clean, safe drinking water. I would also like to thank Dr. Darrell Velegol and his research group for allowing me to use their lab and materials. Thanks to my fellow undergraduate researchers and friends, Krista Liguori, Kathleen Lauser, and Adam Uliana, for helping me with data collection, experimentation techniques, and modeling. Special thank you to Dr. Christopher Gorski for exciting my passion for water treatment technologies and directing me to the Moringa Research Group.

Chapter 1

Preface

Since a young age I have been fascinated with water. Growing up, my family traveled to various countries throughout the tropics. Every year brought a new adventure. And each new adventure brought to light new water problems. These beautiful islands, surrounded by water, barely had enough fresh water to drink. We were advised not to drink the water in nearly every country we visited. If you drank the tap water you risked contracting a water-borne illness. It was then that I knew I had to do something. Clean water shouldn't be a luxury; it should be something that everyone has. My passion for water didn't stop when the family vacations did. In the summer of 2012 I travelled to Costa Rica for two weeks to study renewable energy and technology. While there we helped install rainwater collection systems on homes in a local village. Seeing how grateful everyone was for their new collection systems made me realize the true importance of water and how we often take for granted the constant, clean supply of water that flows out of our taps. The following summer my passion for water took me to Fiji through a Social and Environmental Sustainability program. While there, I tested the water quality in several locations, looking specifically for fecal bacteria. My results showed heavily polluted water near areas of development—the areas where people were living, where water was needed most. When I returned to Penn State for my Fall 2013 semester, I searched for an opportunity to continue my passion for clean water solutions. I found the perfect fit in the Moringa Research Group led by Dr. Stephanie Velegol. It is here that I get to work towards a solution that will make safe, clean water accessible to everyone.

Introduction

Clean, safe drinking water is scarce. Approximately 1.6 million people are forced to use contaminated water globally (Yongabi *et. al* 2011). Contaminated water carries disease-causing microorganisms that affect both adults and children. The World Health Organization estimates that 80% of all diseases are caused by inadequate sanitation, contaminated water, and the inaccessibility of water (Yongabi *et. al* 2011).

Standard water treatment methods include coagulation, flocculation, sedimentation, disinfection, membrane filtration, reverse osmosis and ultraviolet light (UV) (Yongabi *et. al* 2011). However, not all of these methods are available in the rural and impoverished areas that need clean water the most. The seeds of the *Moringa oleifera* tree may hold the solution to this problem. There are 14 different species of Moringa trees, but *Moringa oleifera* is the most widespread (Ndabigengesere *et. al* 1995). The map below highlights areas where Moringa trees are found, and the dots on the map indicate the locations of seeds that we have used for testing.

Figure 1: Map of locations where Moringa trees grow. Red dots highlight locations of seeds used for testing.

The seeds of the Moringa tree contain cationic proteins (MOCP). What is so unique about these cationic proteins is that they act as both a coagulant and an antimicrobial agent. The positive charge of the protein allows it to capture negatively charged particles and remove them from solution. One of the

dominant mechanisms of MOCP antimicrobial activity is membrane fusion (Shebek *et. al* 2015). *F*-sand is created by adsorbing the MOCP from *Moringa oleifera* seeds onto silica particles. This *f*-sand is then packed into a column to create a filter for water treatment.

Water is considered safe for drinking when it is free of pathogenic organisms, toxic substances, and an excess of mineral and organic debris (Yongabi 2010). Two of the most common microbes found in contaminated water are protozoans such as *Giardia* and *Cryptosporidium*. Although the size of these microbes can vary, they typically have diameters between 9-15 micrometers and 3-6 micrometers, respectively (Medema *et. al* 1998). Both of these microbes have negative surface charges, allowing *f*-sand to capture and remove them via adsorption.

The purpose of my thesis was to find the fractional coverage of model microbes on *f*-sand to ultimately determine how much water can be treated by an *f*-sand filter. The diameter of the model microbes used was 3 micrometers, smaller than that of Giardia. By using a model microbe on the lower end of the diameter range, we can test the maximum capturing abilities of *f*-sand. I looked for differences in the fractional coverage of *f*-sand in DI water, tap water, low salt, and high salt solutions. I also considered the effects of different Moringa species on the capturing abilities of *f*-sand.

Chapter 2: Experimental Design

2.1 Materials

Sulfate polystyrene latex particles were used to model microbes such as *Giardia* and *Cryptosporidium* that are often found in contaminated water. These particles have a 3.1µm diameter, similar to that of the microbes. In addition to the *Moringa oleifera* seeds, 50-70 mesh silica was used to create the *f*-sand.

Table 1: Materials Used

Material	Specifications	Supplier	Catalog #	Phone #
Deionized Water	--	Millipore	--	--
Moringa Oleifera Seeds	Chiang Mai, large	Dr. Rick Bates	--	--
Sand (Silica)	50-70 mesh, 250µm diameter	Sigma Aldrich	274739	1-800-325-3010
Sulfate Polystyrene Latex Particles	3.1µm diameter, 8% volume fraction	Life technologies	S37223	1-800-955-6288
Filter Paper	185mm diameter, 11 µm	Whatman	1001-185	1-800-325-3010
Syringe	10 mL syringe with luer-lok tip	Fisher Scientific	309604	1-800-766-7000
Syringe Filter	0.2 µm cellulose acetate membrane	VWR International	28145-477	1-800-932-5000
Falcon Tube	15 mL polypropylene conical tube, 17 x 120	Corning Science	352097	1-800-766-7000
Roller Mixer	Mini labroller dual format rotator	Labnet International, Inc.	H5500	1-732-417-0700
UV-Visible Spectrophotometer	Evolution 201	Thermo Scientific	840-210800	1-800-532-4752

2.2 Calibration

To determine the fractional coverage of model microbes on the *f*-sand, absorbance was measured in a UV-vis spectrophotometer. The absorbance can help us determine the particle concentration in solution. The UV-vis must first be calibrated to ensure accurate measurements. A calibration curve was created from the measurements obtained by the UV-vis. Distilled water was mixed with pre-determined

volumes of sPSL to create a range of six different concentrations from 1.5 µL/mL to 20 µL/mL, or $1.6x10^{-4}$ g/mL to $1.6x10^{-3}$ g/mL. We created 1.5, 4, 8, 12, 16, and 20 µL/mL solutions, but any number of solutions within the range could be used. Each of these solutions was then measured in the UV-vis spectrophotometer at 550 nm wavelength to determine absorbance. See Appendix B to better understand this process. The absorbances were plotted against their known concentrations to create the calibration curve. The slope of this graph is determined by fitting a trend line through the data and origin. This slope is used to calculate the approximate particle concentration of sPSL.

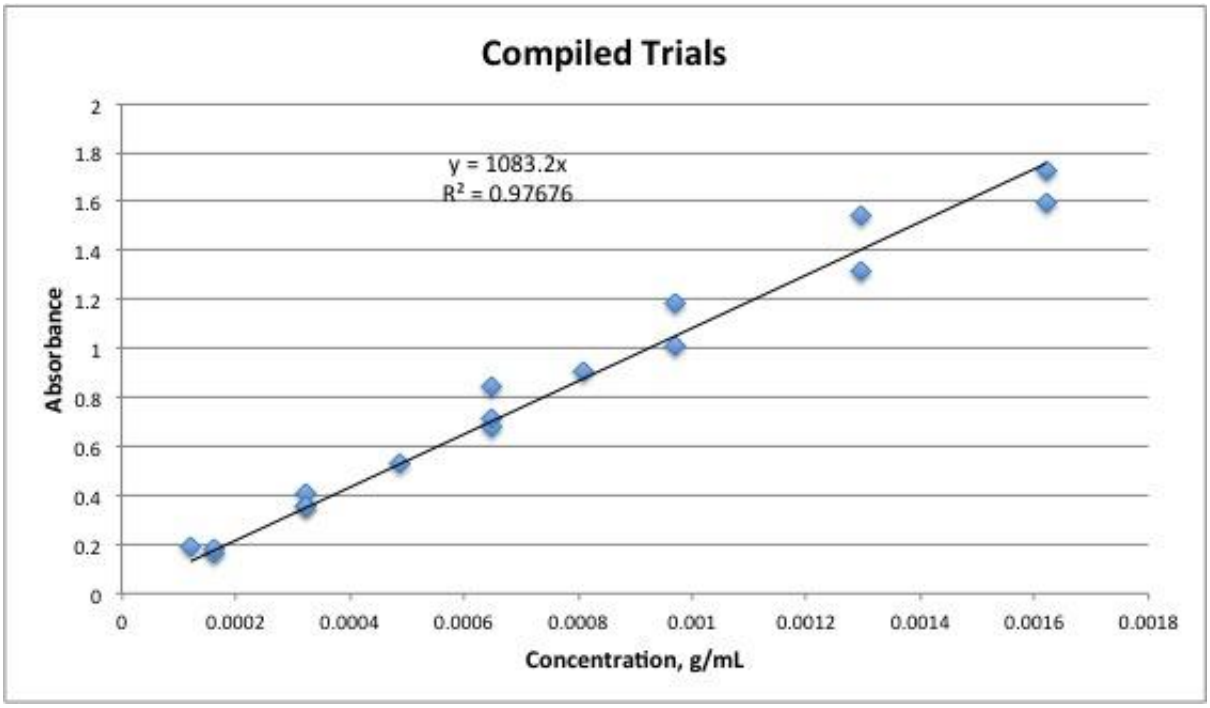

Figure 2: Calibration Curve for sPSL particles in DI water, measured at 550nm

The above calibration curve contains measurements from 3 different trials. This was done to increase confidence in the slope of the curve, which is used to calculate the concentration of sPSL in solution after mixing with *f*-sand. The slope of this curve was used for all experiments conducted to solve for fractional coverage, rather than creating a new calibration curve for every trial.

2.3 Creation of F-sand

The materials listed in Table 1 are needed to create the *f*-sand. To complete the fractional coverage experiments, a sample, control, and control *f*-sand are needed. This section describes the creation of *f*-sand. A 0.1g sample of seed is weighed out and added to a falcon tube. 10mL of DI water is then added to the seed, and the mixture is allowed to roll for one hour. While the mixture is rolling, a 1g samples of silica is weighed out and added to a new falcon tube. After the hour, the mixture is poured through an 11micron filter. A second filtration is completed with a syringe and 0.2micron filter tip. This filtered solution is then added to the falcon tube with the sand and allowed to roll for one hour. Finally, after the hour, the liquid is decanted from the mixture and the sand is rinsed seven times. Further experimentation can now begin with the *f*-sand. For a more detailed procedure, see Appendix C.

2.4 Fractional Coverage Experiments

After the *f*-sand is created, testing for fractional coverage can begin. First, 30 mL of DI are mixed with approximately 270 μL of 8.1% sPSL on a stir plate. After the solution is well mixed for five minutes, approximately 1 mL is added to a cuvette to measure its absorbance in the UV-vis spectrophotometer at 550nm. This serves as a baseline in the experiment. The concentration of sPSL can be varied, but should fall somewhere in the range of 0.300 to 2.000 absorbance. After measuring absorbance, 10 mL of the solution is added to the sample, control, and sPSL control falcon tubes. The sample falcon tube contains 1g f-sand, the control falcon tube contains 1g regular sand, and the sPSL falcon contains just sPSL. Follow protocol #1. After 1 hour, the absorbance of each solution is measured and recorded. Approximately 1 mL of solution should be removed from each falcon tube and placed into a cuvette. Once the absorbance is recorded, the solutions are returned to their respective falcon tubes. The falcon tubes are rolled continuously for 48 hours. Measurements should be taken at least three times over the 48-hour period.

2.4 Controls

To ensure the accuracy of our results, three controls were added to the fractional coverage tests. A total of four samples were tested during each experiment. One control was regular sand with the sPSL mixture. The second control was *f*-sand with DI water. The third control was just the sPSL mixture. These were added to ensure that the sPSL was not flocculating over time and that organic matter was not affecting the absorbance values measured in the UV-vis. To determine the final absorbance, the absorbance value for the control *f*-sand was subtracted from the absorbance value of the *f*-sand sample.

2.5 Modeling

Prediction models are used to determine how much water an *f*-sand filter can treat. The volume of water depends on the size and amount of *f*-sand used, the maximum fractional coverage on an *f*-sand particle, and the concentration of contaminants in the water. The equation below, developed by Dr. Stephanie B. Velegol, is used to calculate the number of milliliters of water a column can treat. This can be scaled up or scaled down depending on the dimensions of the column used.

$$\#\, \boldsymbol{mL\ of\ water} = \frac{\boldsymbol{6f(Dc)^2Hs(1-\varepsilon)}}{\boldsymbol{nDsDm^2}}$$

Table 2: List of variable needed to determine the number of mL of water an f-sand filter can treat.

Variable	Definition
Dc	Diameter of column
Hs	Height of column
ε	Porosity
Ds	Diameter of sand
Dm	Diameter of microbe
f	Fractional coverage of sand surface

The goal of my work was to determine the f value of the sand in various solutions. To determine the f value, the absorbance measurements must be converted into fractional coverage. This can be done via the following equation:

$$f = \frac{\rho_{sand} D_{sand} (Abs_i - Abs_f) V_{solution}}{4 \rho_{sPSL} D_{sPSL} M_{sand} slope}$$

Table 3: List of variables needed to determine the fractional coverage of the surface of a sand particle.

Variable	Definition	Value
ρ_{sand}	Density of sand	2.55 g/mL
D_{sand}	Diameter of sand	250 µm = 250 x 10^{-6} m
$V_{solution}$	Volume of solution	10.1 mL
ρ_{sPSL}	Density of sPSL	1.055 g/mL
D_{sPSL}	Diameter of sPSL	3 µm = 3 x 10^{-6} m
M_{sand}	Mass of sand added	1 g
absorbance	absorbance measured in UV-vis	0.000 - 2.000
slope	slope of calibration curve	1083.2 g/mL

As an example, the following measurements were recorded for fractional coverage tests using seeds from Thailand after 1 hour:

Table 4: Absorbance data measured in the UV-vis at 550nm.

Sample	Absorbance
Sample *f*-sand	0.697
Control *f*-sand	0.002
Control	0.989
sPSL	1.032

Below I derived the equation listed above to convert the absorbance to the fractional coverage. First, we need to know the mass of sPSL stuck to the *f*-sand. Measurements for absorbance are taken in the UV-vis and converted to concentration of sPSL in solution using the slope of the calibration curve:

(1) $$mass\ sPSL\ stuck = \left(\frac{Abs_i}{slope} - \frac{Abs_f}{slope}\right) \times V_{solution}$$

$$mass\ sPSL\ stuck = \left(\frac{0.989}{1083.2\frac{g}{mL}} - \frac{0.697}{1083.2\frac{g}{mL}}\right) \times 10.1mL = 0.0027g$$

The mass of sPSL stuck must then be converted to the volume of sPSL stuck.

(2) $$V_{sPSL\ stuck} = \frac{mass_{sPSL\ stuck}}{\rho_{sPSL}}$$

$$V_{sPSL\ stuck} = \frac{0.0027g}{1.055\frac{g}{mL}} = 0.0026mL$$

Using the volume of sPSL stuck and the volume of one sPSL particle, we can find the number of particle stuck:

(3) $$\#\ particles\ stuck = \frac{V_{sPSL\ stuck}}{V_{one\ sPSL\ particle}} = \frac{mass_{sPSL\ stuck}}{\rho_{sPSL} V_{one\ sPSL\ particle}}$$

$$= \frac{mass_{sPSL\ stuck}}{\rho_{sPSL}\left(\frac{1}{6}\pi D_{sPSL}^3\right)} = \frac{6(Abs_i - Abs_f)V_{solution}}{\pi \rho_{sPSL} D_{sPSL}^3 slope}$$

$$\#\ particles\ stuck = \frac{6(0.0026mL)}{\pi(3x10^{-6}m)^3} = 1.84 \times 10^8$$

From this, we can determine the projected area of all of the sPSL particles stuck to the surface of the *f*-sand:

(4) $$projected\ area = \#\ particles\ stuck\ \times\ area\ of\ one\ particle$$

$$= \frac{6(Abs_i - Abs_f)V_{solution}}{\pi \rho_{sPSL} D_{sPSL}^3 slope} \times \frac{\pi D_{sPSL}^2}{4} = \frac{3(Abs_i - Abs_f)V_{solution}}{2\rho_{sPSL} D_{sPSL} slope}$$

$$projected\ area = 1.84 \times 10^8 \times \frac{\pi(3x10^{-6}m)^2}{4} = 0.0013m^2$$

To calculate the fractional coverage of sPSL particles on the surface of the *f*-sand, we need to know the volume of sand:

(5) $$Volume\ of\ sand = \frac{m_{sand}}{\rho_{sand}}$$

$$Volume\ of\ sand = \frac{1g}{2.55\frac{g}{mL}} = 0.39mL$$

Using the volume of sand, we can find the number of sand particles:

(6) $$\#\ sand\ particles = \frac{m_{sand}}{\rho_{sand}\left(\frac{1}{6}\pi D_{sand}^3\right)} = \frac{6m_{sand}}{\pi\rho_{sand}D_{sand}^3}$$

$$\#\ sand\ particles = \frac{6(0.39mL)}{\pi(250x10^{-6}m)^3} = 47{,}670$$

The number of sand particles must then be converted to total area of the sand particles available for coverage by the sPSL:

(7) $$Total\ area = \#\ sand\ particles\ \times\ area\ of\ sand\ particle$$

$$= \frac{6m_{sand}}{\pi\rho_{sand}D_{sand}^3}(\pi D_{sand}^2) = \frac{6m_{sand}}{\rho_{sand}D_{sand}}$$

$$Total\ area = 47{,}670\pi(250x10^{-6}m)^2 = 0.00936m^2$$

Finally, the fractional coverage, f, can be determined by dividing the total available sand area by the projected sPSL area:

(8) $$f = \frac{projected}{available} = \frac{3(Abs_i - Abs_f)V_{solution}\rho_{sand}D_{sand}}{2\rho_{sPSL}D_{sPSL}slope*6m_{sand}}$$

$$= \frac{(Abs_i - Abs_f)V_{solution}}{4m_{sand}slope}\left(\frac{\rho_{sand}}{\rho_{sPSL}}\right)\left(\frac{D_{sand}}{D_{sPSL}}\right)$$

$$f = \frac{0.0013m^2}{0.00936m^2} = 0.139$$

The fractional coverage of sPSL particles on an *f*-sand particle 13.9% based on the test results obtained using seeds from Thailand. This equation can be used to determine the fractional coverage, f, of sPSL particles on sand.

Chapter 3: Experimental Results

3.1 Fractional Coverage in Varying Solutions

The fractional coverage of sPSL particles on *f*-sand was first measured in triplicate using a DI solution. F-sand was added to a solution of sPSL and DI water and allowed to roll for 48 hours. The absorbance was then measured in the UV-vis spectrophotometer and the value was converted to fractional coverage. Measurements were taken after 1 hour, 24 hours, and 48 hours to note any changes in absorbance.

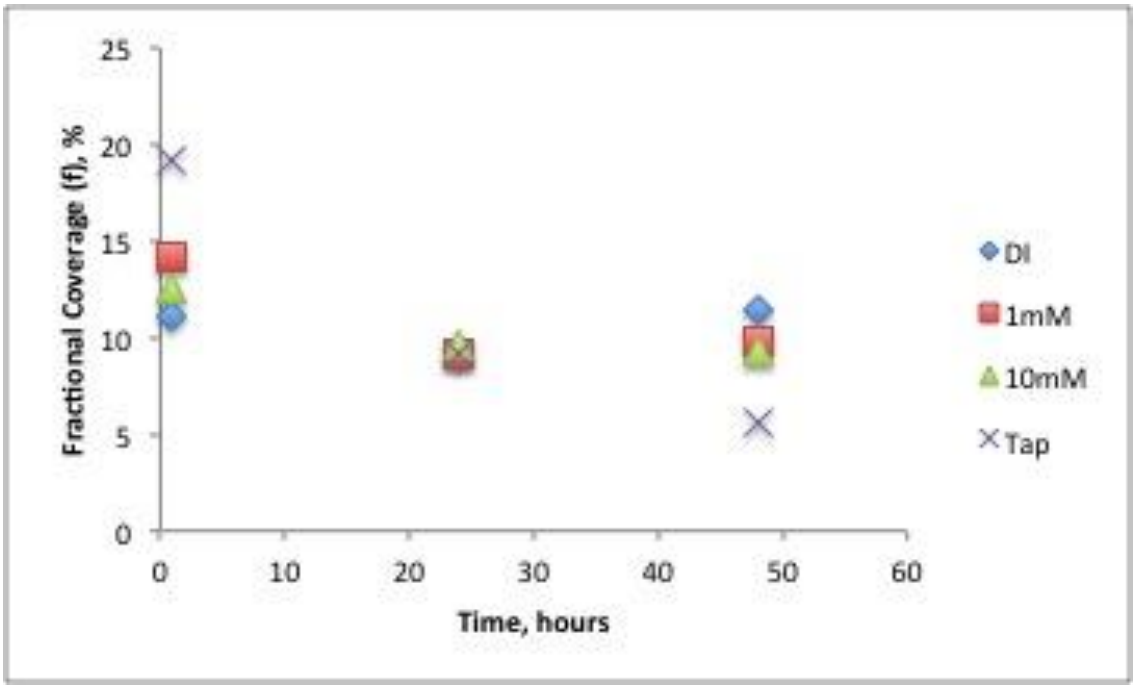

Figure 3: Fractional coverage of sPSL particles on f-sand in various solutions over 48 hours. Measured in UV-vis at 550nm.

The results of those experiments, averaged from 13 different tests, show that the sPSL particles cover 11.2% +/- 1.2% of the surface of *f*-sand after 1 hour. The fractional coverage remains fairly constant over the 48 hours. The same experiment was repeated for f-sand in a solution of sPSL and tap water. Tap water was chosen to determine the effects of hard water. The results of those experiments, averaged from 4 different tests, show that the sPSL particles cover 19.1% +/- 7.1% of the surface of *f*-sand after 1 hour.

The fractional coverage decreased rapidly, and is somewhat unpredictable, over the 48-hour period. The fractional coverage of sPSL particles on *f*-sand was then measured using two low salt solutions. The results of the experiments in 1mM NaCl solution, averaged from 6 different tests, show that the sPSL particles cover 14.2% +/- 2.0% of the surface of *f*-sand after 1 hour. The results of the experiments in 10mM NaCl solution, averaged from 4 different tests, show that the sPSL particles cover 12.6% +/- 4.1% of the surface of *f*-sand after 1 hour. The fractional coverage is constant in low salt solutions over the 48-hour period.

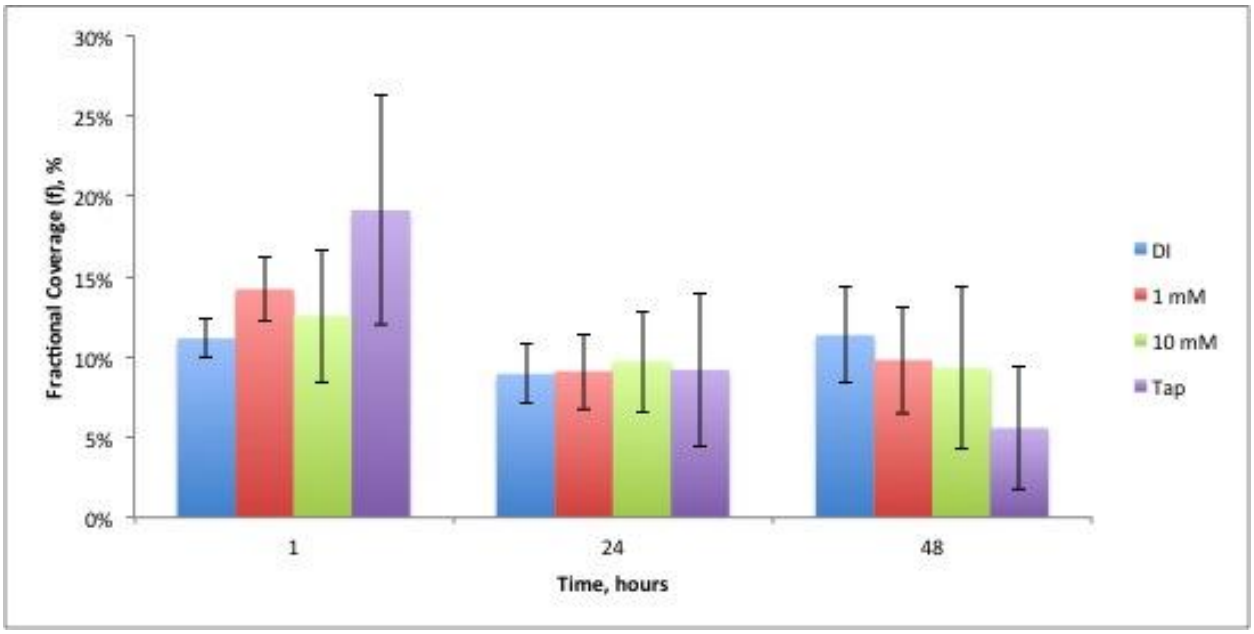

Figure 4: The 95% confidence intervals are shown for the fractional coverage of sPSL particles on f-sand in various solutions over 48 hours.

3.2: Fractional Coverage of *f*-sand created using Moringa seeds from various locations

Preliminary work was completed to determine the fractional coverage of model microbes on *f*-sand created using seeds from Chiang Mai, Thailand. Moringa trees are found around the world in equatorial regions, so we wanted to know if this fractional coverage value could be used for all varieties of the Moringa tree. To assess the capturing abilities of different seeds, we ran tests with the seeds from Thailand, Nicaragua, and Tanzania. Preliminary results showed that the fractional coverage, which represents the capturing abilities of the seeds, is approximately the same for all three varieties. The

fractional coverage of sPSL particles on *f*-sand was measured using the same protocol from Appendix B. The results of those experiments, averaged from 3 different tests, show that the sPSL particles cover 14.2% +/- 1.1% of the surface of *f*-sand created from seeds from Tanzania after 1 hour. The fractional coverage was determined to be 11.9% +/- 0.9% after 1 hour for *f*-sand created from seeds from Nicaragua. As referenced above, the fractional coverage of *f*-sand created from seeds from Thailand show an initial value of 11.2% +/- 1.2%.

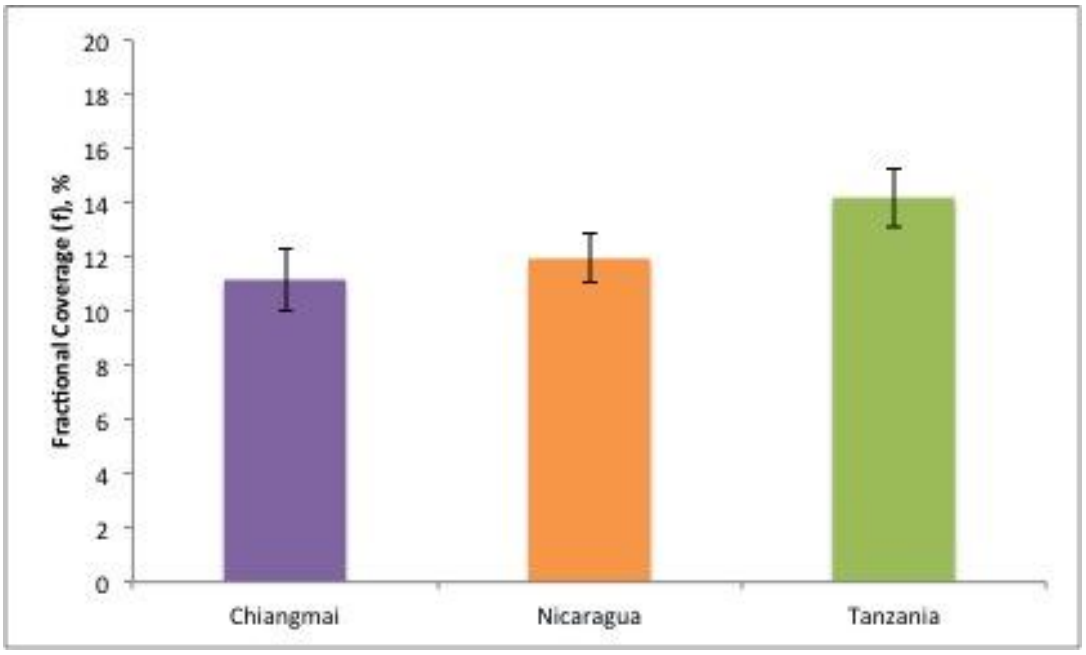

Figure 5: Fractional coverage of f-sand created from Moringa seeds from three different locations. Measurements were taken after 1 hour using a UV-vis at 550nm.

Chapter 4: Conclusions

4.1: Fractional coverage does not change with time for DI and low salt solutions

The fractional coverage of *f*-sand is affected by the composition of the solution it is in. F-sand particles interact not only with the model microbes, but with the other solution constituents as well. The value of f, the fraction of the sand surface covered by microbes, ranges from 9.1% to 16.7% for DI, 1mM and 10mM solutions. It does not change over time in these solutions. Ions such as calcium and magnesium, often found in hard water, affect the value of f both in magnitude and time. The value of f in tap water ranges from 11.9% to 26.4%. These values are larger than those found in DI, 1mM and 10mM, and also fluctuate more over time.

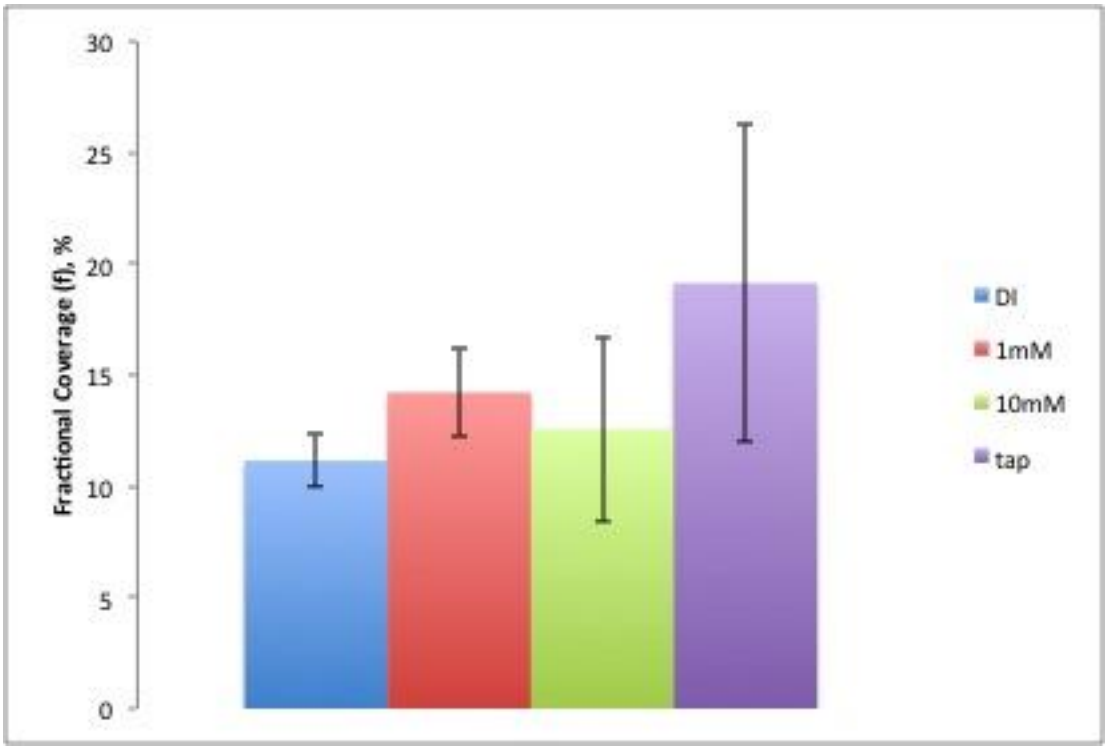

Figure 6: Fractional coverage of f-sand created from seeds from Thailand after 1 hour. Sand rolled in various solutions and coverage measured in a UV-vis at 550nm.

4.2: Fractional coverage is constant with location

The fractional coverage of *f*-sand is independent of the location the seeds are grown in and harvested from. The value of f is the same for three locations of Moringa: Thailand, Tanzania, and

Nicaragua. It ranges from 10.0% to 15.3% after 1 hour. Coverage is constant over the 48 hours. Moringa trees grow throughout the equatorial regions, so the seeds tested represent a fairly comprehensive sampling of the Moringa locations. The similarity in fractional coverage value for all three seed locations suggests that our model for predicting how much water can be treated with an *f*-sand filter should be accurate in all locations where Moringa is found.

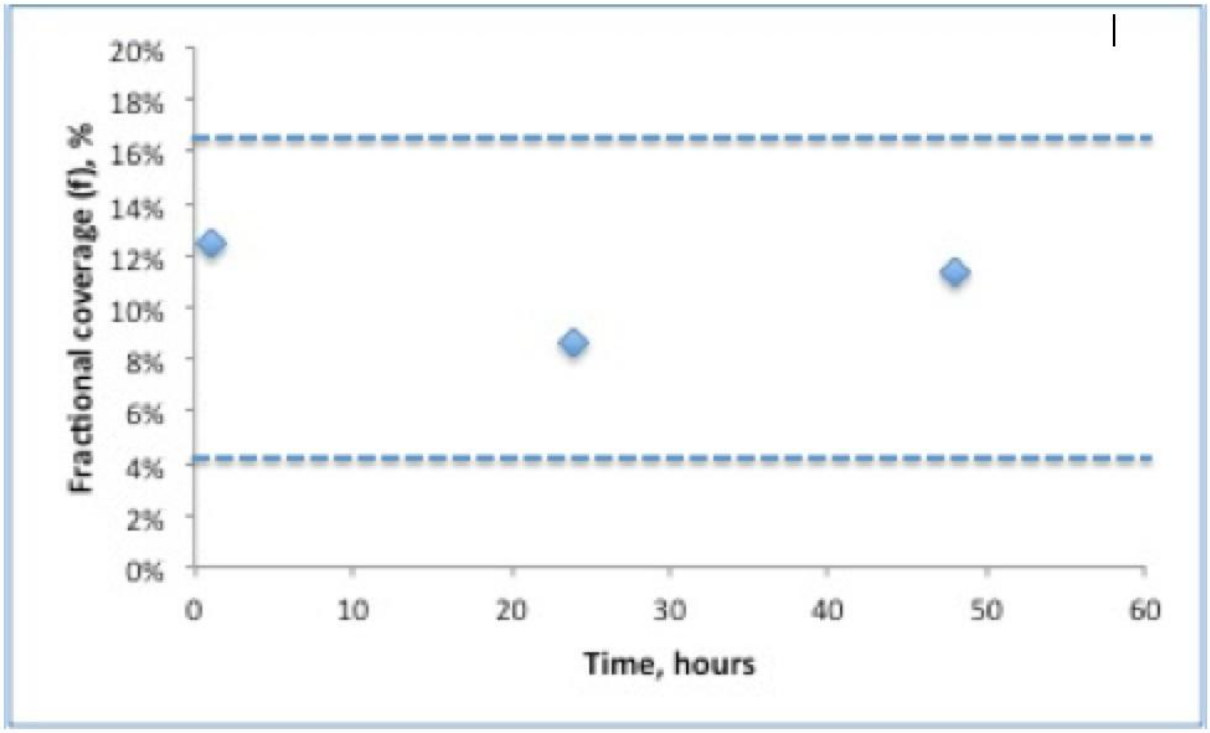

Figure 7: The averaged fractional coverage of f-sand created from Moringa seeds from three different locations over 48 hours. The dashed lines indicate the 95% confidence intervals for coverage. Measurements taken in a UV-vis at 550nm.

4.3: Scale-up of Filter

The results of tests completed indicate that fractional coverage is constant with both location and time. In order for these filters to be implemented in the communities that need them, we need to know how much water they can treat. This is crucial to the size of the filters needed. The amount of water that can be treated, and thereby the size of the filter needed, is dependent on the fractional coverage of microbes and other contaminants on the surface of sand particles. Since fractional coverage is constant

with location and time, we can scale-up our current filter design for use in any location and confidently predict how much water can be treated.

4.4: Future Works

The Moringa Research Group will continue to work towards refining and improving the model. While current fractional coverage testing involves model microbes like sPSL, we are looking to begin testing fractional coverage using microbes such as E. coli. We would also like to test seeds from a few other locations to ensure that fractional coverage is in fact constant with location. Both of these tests would help strengthen our model to be used for filter scale-up. We would also like to test the effect of storage time of seeds on the capturing abilities of *f*-sand.

Additional tests can be completed to determine the fractional coverage of *f*-sand in various solutions such as low salt and tap water. This would further increase the confidence in the fractional coverage values obtained for those solutions.

Appendix A

Protocol #1: How to test fractional coverage of f-sand

Jenna Thomas

Materials Needed:

- Moringa seeds
- Sand
- DI Water
- Appropriately sized containers

1. Weigh out 0.1g large Chiangmai seeds (no shell) and add to "sample" beaker.
    a. Note: Usually 0.1g seed/10mL DI water.
2. Weigh out 0.1g large Chiangmai seeds (no shell) and add to "control with f-sand" beaker.
3. Add 10mL DI to each beaker. This solution is MOCP solution.
4. Roll for 1hr on twirler.
5. Filter 10mL MOCP solution through 11micron filter paper
    a. Note: Wet filter paper with DI water first.
6. Second filtration of 10mL MOCP solution with 10mL syringe through 0.2micron filter paper.
    a. Note: Draw liquid into the syringe first then add the filter tip.
7. Weigh out 1g of 50+70mesh silica and add to "sample" 15mL falcon tube. Rinse 3 times with 10mL DI water and decant.
8. Weigh out 1g of 50+70mesh silica and add to "control with f-sand" 15mL falcon tube. Rinse 3 times with 10mL DI water and decant.
9. Weigh out 1g of 50+70mesh silica and add to "control" 15mL falcon tube. Rinse 3 times with 10mL DI water and decant.
10. Add 10mL MOCP solution to 1g sand for "sample" and "control with f-sand."
    a. Note: Usually 1g sand/0.1g seed.
11. Add 10mL DI water to "control."
12. Roll for 1hr on twirler.
13. Pour off water and rinse sand 7 times with DI water and decant.
14. Mix 30mL DI water and 270microliters of sPSL on stir plate until well mixed.
15. Measure absorbance of sPSL mixture in UV-Visible Spectrophotometer
    a. Note: Add blank cuvette of DI water
16. Add 10mL sPSL mixture to "sample" and "control" falcon tubes
17. Add 10mL DI water to "control with f-sand" falcon tube
18. Measure absorbance of each solution after rolling for 1hr.
19. Continue to roll and measure absorbance of solutions for at least 48hrs.

Appendix B

Protocol #2: Calibration Curve Procedure

Jenna Thomas

1. Label 6 beakers
2. Add designated amounts of sPSL and DI water
    a. See calculations below for each beaker based on serial dilution
3. Stir beakers on stir plate
4. Calculate concentration of each solution
    a. Concentration of sPSL/volume of solution
5. Measure absorbance of each in UV-Vis
6. Graph concentration vs. absorbance

Molarity: $\frac{g\ substance\ x \frac{mol\ substance}{g\ substance}}{volume\ solution}$
sPSL = 8.1g/100mL
180µL sPSL + 9mL DI → 20µL/mL solution
6mL of 20µL/ml solution + 1.5mL DI → 16µL/mL solution
4.5mL of 16µL/ml solution + 1.5mL DI → 12µL/mL solution
4mL of 12µL/ml solution + 2mL DI → 8µL/mL solution
4mL of 8µL/ml solution + 4mL DI → 4µL/mL solution
5 mL of 4µL/ml solution + 5mL DI → 1.5 µL/mL solution

Concentration:
20µL/mL x $\frac{0.001mL}{1\mu L}$ x $\frac{8.1g}{100mL}$ = 0.00162g/mL
16µL/mL x $\frac{0.001mL}{1\mu L}$ x $\frac{8.1g}{100mL}$ = 0.001296g/mL
12µL/mL x $\frac{0.001mL}{1\mu L}$ x $\frac{8.1g}{100mL}$ = 0.000972g/mL
8µL/mL x $\frac{0.001mL}{1\mu L}$ x $\frac{8.1g}{100mL}$ = 0.000648g/mL
4µL/mL x $\frac{0.001mL}{1\mu L}$ x $\frac{8.1g}{100mL}$ = 0.000324g/mL
1.5µL/mL x $\frac{0.001mL}{1\mu L}$ x $\frac{8.1g}{100mL}$ = 0.0001215g/mL

Appendix C

Protocol #3: How to make f-sand

Toni Bechtel
5/10/13

Materials Needed:

- Moringa Protein Serum (M.O. Serum) - from crushed Moringa seeds
- Sand
- DI Water
- Appropriately sized containers

1. Determine how much f-sand you want to make (i.e. how much sand do you need to fill a column or run a jar test)
    a. **Density of "play sand" is around 2.65 g/mL and the estimated void fraction is 0.3**.
2. Rinse the sand with DI water at least 3 times.
    a. Take the amount of sand you will use and add DI water. Shake well and then pour off the water – repeat this at least 3 times.
    b. If you see bubbles that means you have surfactant (soap) in your system. <u>Keep rinsing until you don't see them anymore!!!!</u>
3. Determine the amount of M.O. Serum needed.
    a. **For every 1 g of sand you should have 10 mL of MO Serum**. This is to keep the sand in the water phase and never stuck to the side of the jar
        i. This gets more important as you getting into larger batch sizes!
4. Prepare the appropriate amount of MO Serum based on the total volume needed
    a. Determine the bulk concentration of MO Serum needed by how much surface coverage you want **<u>(monolayer coverage is ~2.2 mg adsorbed/m^2)</u>**
        i. Use the attached graph of surface excess concentration vs. bulk seed dosage
    b. It is probably easiest to prepare a more concentrated MO Serum and then dilute down to the bulk concentration needed to make the f-sand.
5. Combine the sand and the appropriate amount of MO Serum in a jar. Mix for 1 hour (on some type of roller and mixing plate – a horizontal roller works well).
6. Remove the sand from mixing and allow it to settle (about a minute) and then pour of the supernatant (the excess liquid) – this is safe to pour down the drain.
7. Add the same volume of DI water to the sand for rinsing (i.e. if you added 20 mL of MO Serum to the sand initially, add 20 mL of DI to rinse). Shake the mixture well for ~ 30 seconds. Pour off the liquid. Repeat this a total of 3 times.
8. The f-sand can be stored wet at room temperature for ~ 1 month. Store the f-sand in a **WELL-LABELED** (What it is, your initials, and the date) container either in a drawer or in the refrigerator.

Appendix D

Size Analysis for Practical Sand Filter Design

Our goal was to design a filter that could be made from inexpensive, locally-sourced materials and one that required minimal technology to construct it. To test this design and increase the effectiveness of the filter, a size analysis of the sand was performed. Playground sand was obtained from Lowe's, and six 5g samples were tested. A set of 8 sieves, ranging in size from 180-1180 micrometers, was used with a Ro-Tap to complete the size analysis. The initial size analysis revealed that playground sand is composed mainly of particles ranging from 0.2 mm to 0.425 mm.

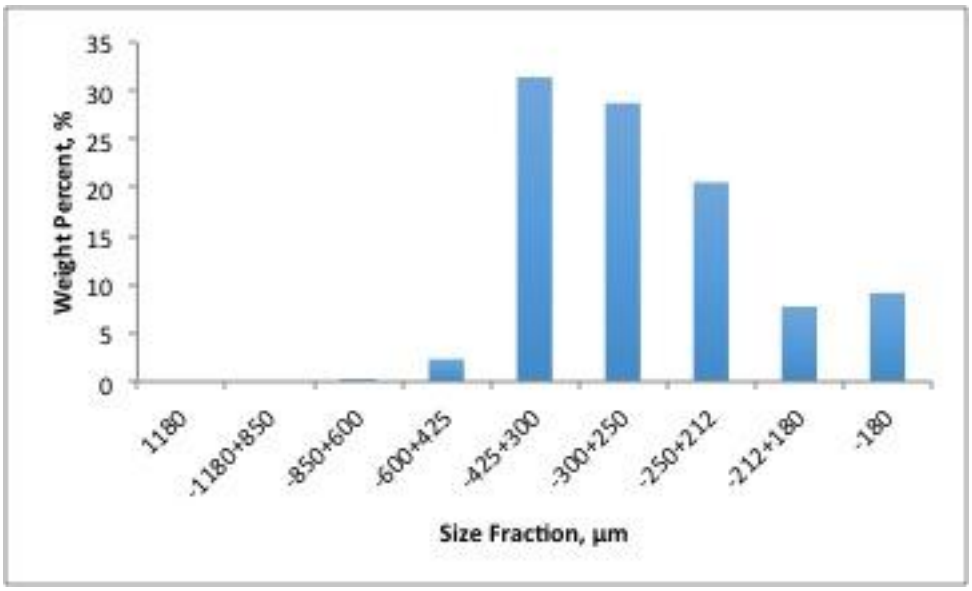

Figure 8: Weight distribution of sand particles based on analysis performed by sieving a 320g sample.

The size of the media affects the efficiency of solids removal. The desired particle size for our filters is 0.5-0.7 mm. To remove the smallest particles from the playground sand, the sand is mixed with water and then decanted based on Stoke's Law analysis. Stoke's Law governs the settling velocity of particles based on their diameter (see equation below).

$$v_t = \frac{gD^2(\rho_p - \rho_l)}{18\mu}$$

Table 5: List of variables used in Stoke's Law equation.

Variable	Definition
v_t	settling velocity
D	diameter of sand particle
ρ_p	density of particle
ρ_l	density of liquid
μ	viscosity of liquid

Using typical silica diameters, the settling time for the various particle sizes was calculated to determine when the water should be decanted. Based on the height of the falcon tube used for settling, particles smaller than 0.5mm could be removed by decanting after 5 seconds. The settling times for various particle sizes are shown in Figure 9 below. Six different mixing and settling combinations were tried to determine the best method for removal: mix for 5 seconds, decant after 2 seconds; mix for 10 seconds, decant after 2 seconds; mix for 5 seconds, decant after 3 seconds; mix for 10 seconds, decant after 3 seconds; mix for 5 seconds, decant after 5 seconds; mix for 10 seconds, decant after 5 seconds.

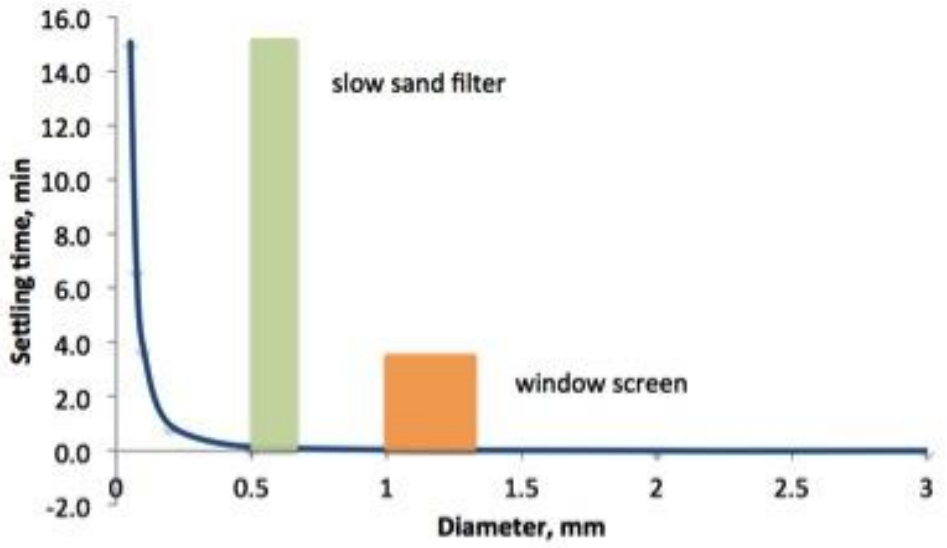

Figure 9: Settling time of various size silica particles, based on Stoke's law. The typical particle size needed for a slow sand filter, as well as the size of a standard window screen, are shown on the graph.

After decanting, the samples were dried and analyzed in the Ro-Tap. The amount of sand present on each sieve screen was weighed to determine the size distribution of the sample. The resulting size distributions varied significantly from what was expected. All of the smaller particles (less than 0.5 mm) that we wanted to remove were present in the samples, as Figure 10 below shows. Due to time constraints, the size analysis was left unfinished. Future testing is needed to determine if the smaller particle sizes can easily be removed in the field.

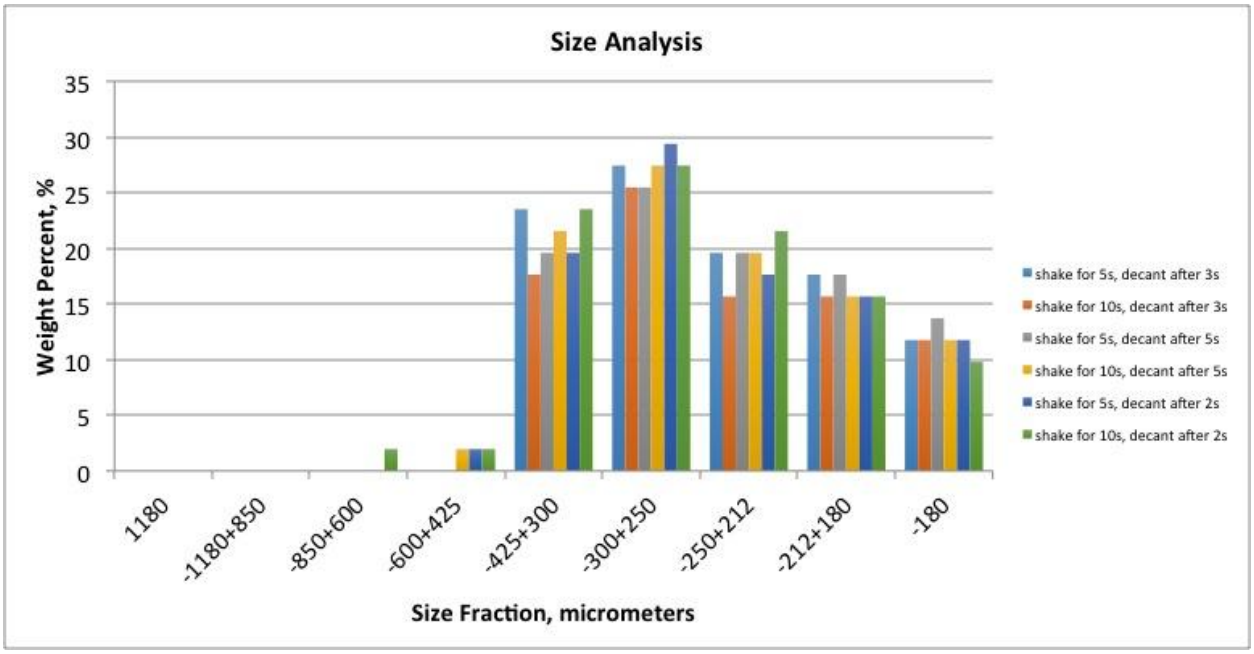

Figure 10: Distribution of particle sizes after mixing and decanting in six different trials.

Appendix E

Field Testing of Moringa Slow Sand Filter

The Moringa research group at Penn State joined forces with the Pure Water Access Project, Inc. to begin the field-testing of Moringa filters. Students at Ohio State University founded the Pure Water Access Project in 2010, and the organization is dedicated to combating the global water crisis by combining water filtration implementation practices with research methodologies.

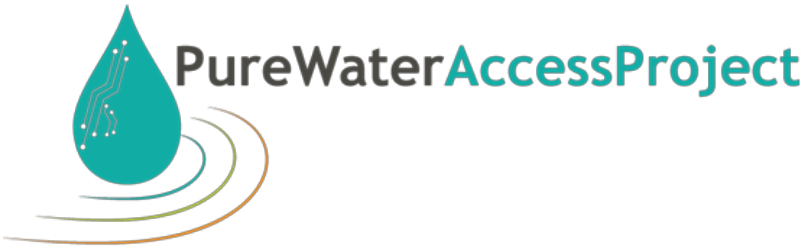

Figure 11: The logo for the Pure Water Access Project, Inc., our new non-profit partner based out of Ohio State.

Through our newfound partnership, they are working with community leaders and a local NGO in San Salvador to address the clean water crisis. Beginning in May 2015, they will begin testing the efficacy of the Moringa seeds by combining *f*-sand with traditional biosand filters. A comparative study will be conducted to determine how effective *f*-sand is at treating contaminated water in several local communities.

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ACADEMIC **VITA**

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EDUCATION

The Pennsylvania State University, **Schreyer Honors College (SHC),** University Park, PA

- Bachelor of Science in Environmental Systems Engineering
- Minors—Geography, Environmental Engineering

EXPERIENCE

Global Safety and Environment Intern, Merck & Co., Inc. **Summer 2014**

- Completed a trend analysis on process safety management and hazardous waste
- Reviewed environmental regulations and collaborated with senior staff to complete air emissions monitoring and sewering permits
- Initiated the redevelopment and standardization of environmental resource manuals to improve site wide environmental compliance

Researcher, Moringa Research Group **2013-Present**

- Extracted proteins from Moringa seeds to model an inexpensive and sustainable water treatment system for disadvantaged areas
- Analyzed the fractional coverage of protein covered sand using model microbes

Study Abroad Student, CAUSE Program, Curacao **May 2014**

- Sampled water, soil, trees, and mud to study the effects of pollution from an oil refinery located on the island
- Completed reef checks to monitor the health of near shore ecosystems

Study Abroad Student, Sustainable Tourism and the Environment, Fiji **May 2013**

- Evaluated water quality and pollution while studying eco-tourism
- Assessed environmental compliance levels by surveying hotel staff

Study Abroad Student, The GREEN Program, Costa Rica **June 2012**

- Studied alternative energy and sustainability at renewable energy plants
- Completed a capstone project on wind and ocean thermal energy technologies

LEADERSHIP

Treasurer, Society of Environmental Systems Engineers **2013-Present**

- Organized meetings, recruited guest speakers, and assisted in professional development

Fundraising Director, College of Earth and Mineral Sciences THON **2014-Present**

- Developed and implemented innovative fundraisers to support the Four Diamonds Fund and the fight against pediatric cancer

Treasurer, UNICEF Penn State **2012-2014**

- Planned fundraisers to raise money for emergency relief and increase awareness about children's rights around the world

Treasurer, Schreyer Honors Student Council **2013-2014**

- Designed, organized, and coordinated the sale of SHC merchandise
- Managed a $10,000 budget to support SHC programming