

Brothers in Christ and team members of Cambodia Village Outreach,

We are pleased to deliver to you the results of our research into the use of moringa for pretreating water to extend the life of ceramic filters installed in homes around Prasat Trew, Cambodia. The first section provides a nutshell summary. Section II details the experiments and results. Section III offers helpful information regarding such things as the cultivation of moringa and sale of other moringa products.

THE BOTTOM LINE: We optimized several steps for cleaning turbid water. Our moringa method consists of adding finely crushed moringa seed directly to highly turbid water (>500 NTU), stirring at approximately 240 rpm, and leaving the mixture to settle for 2-5 hours, after which treated water can be decanted off the top of the container. We recommend crushed moringa seed as a viable tool for rapidly clarifying turbid water for use in household ceramic filters in Cambodia. Further tests should be conducted to upscale the procedure and to determine whether this method can be universally applied around Cambodia.

This report was constructed with diverse information processing styles in mind. Bold text and blue boxes provide snapshots and bottom-line summaries. Some on the team might enjoy reading it top to bottom. CVO leaders will likely be most interested in Section III. Regardless, I hope you all are edified and that our research leads to tangible improvements in drinking water supplies.

Kaley Hallmark, Sweetwater's intern from Oct 2019 – July 2020, drove the laboratory research and should be recognized for her role in any success our moringa method might provide in your battle against suspended sediment. Kaley did a spectacular job digging through the scientific literature to determine what was known and not known about the operation of moringa on suspended sediment, aided in the experimental designs, deftly executed the experiments, and maintained meticulous notes. The whole Sweetwater team prays regularly for the people of Prasat Trew and for the widow *Mary* in particular. Kaley and I were both sorely disappointed when government responses to COVID-19 nixed a personal visit to Cambodia. We remain resolved, Lord willing and if it aligns with your ministry objectives, to aid in follow-up research. We are convinced the moringa method has substantial potential to undergird your efforts to bring clean and Living Water to the people of Cambodia.

In service with you for our King Jesus,

David Pendergrass Executive Director & Lead Scientist Sweetwater Research



Summary

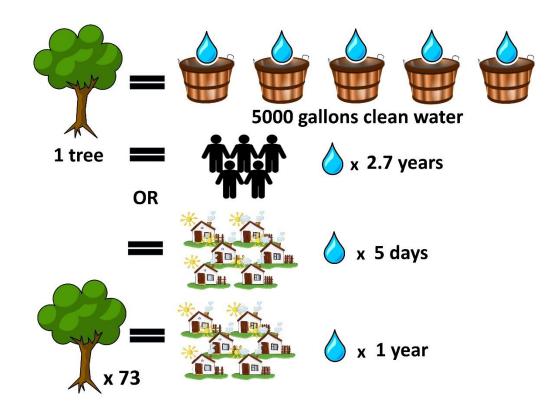
One mature, healthy moringa tree should produce enough seed in one year to clean 5,000 gallons of water. This is sufficient to supply a family of five with pretreated water for nearly three years. A cooperative of six households (30 people) would have pretreated water for five days from the seeds of one tree. The cooperative would need to cultivate 70-80 trees and crush the seeds to pretreat enough water to supply their homes for a year.



These numbers are extrapolated from our research using containers in the 1-liter size class. We recommend

experiments be conducted on larger scales and across different geographies before assurances are given to local entrepreneurs with an interest in moringa water purification systems.

Moringa has the potential to undergird local economic diversification initiatives in addition to its service as a water prefiltering agent. Because there are several steps from tree to prefiltered water, there are several niches for entrepreneurs to fill with their labor. Other moringa products, such as dried leaf powder, can be sold locally or exported to larger markets.



Section II

- 1) Overview of research to optimize the moringa method to pretreat water with dense suspended sediments
- 2) Suggestions for next steps, including experiments for upscaling the moringa method

Introduction

Sweetwater was tasked in early 2019 with extending the life and efficacy of household ceramic filters by developing a method to prefilter exceedingly turbid water using locally available natural materials. Prior to visiting Cambodia, experiments were run on prefiltering techniques that used local muddy water and filter materials that mimicked, as close as could be supposed based on communications with the team, what was found in rural northern Cambodia. A brief video documenting our early experiments and their results can be viewed here. In Cambodia we had three afternoons to recreate our prefilter method, test its efficacy, and make adjustments until the turbidity of the prefiltered water was below 25 NTU, the target turbidity. When it became clear that removing suspended sediment by passing it through a filter was impractical due to the microscopic size of the suspended sediment, we prayed that God would open our eyes to see a locally viable, non-filter method for clarifying water.



Moringa is indigenous to Cambodia and well adapted to its climate, diseases, and pests. The effectiveness of crushed moringa seed as a coagulation agent in water clarification has been common knowledge in tropical communities for many generations. The scientific community picked up this

information from ethnobotanical research and has been publishing studies since the 1970's (e.g., Jahn and Dirar, 1979). Since the turn of the millennium, research has increased dramatically, including experiments to ascertain moringa's effectiveness as a treatment of wastewater (Padhiyar et al., 2020) and disinfection of surface water (Adejumo et al., 2013). A growing body of literature is now focused on identifying the operative coagulant chemicals in moringa seeds, describing their physics, and optimizing the moringa method for both household and industrial uses (Shebek et al., 2015; Adesina et al., 2019).

Moringa Magnetism

The suspended solids that make water cloudy are negatively charged (anionic) particles that repel each other, like same-charged ends of a magnet. The active agent in moringa seed is a water-soluble positively charged (cationic) protein. In more recent literature, this protein has been dubbed MOCP for *Moringa oleifera Cationic Protein* (Keough et al. 2017).

COAGULATION is when anionic suspended solids are neutralized. In the moringa method, MOCP is the neutralizing agent.

FLOCCULATION is when neutralized suspended solids become entangled and "snowball" into larger particles, called flocs.

SEDIMENTATION is when flocs become large enough to settle out.

Putting it together...

- Positive MOCP joins like a magnet to negative suspended solids so they don't repel one another.
- 2) Neutralized solids become entangled into flocs.
- Flocs settle to the bottom of your container and what's left above is water with fewer suspended solids. It is more clear.



Aware of moringa's potential as a non-filter water clarification tool, we performed a hurried search for moringa in the immediate surroundings of the CVO property in Prasat Trew but we did not locate any trees. We did, however, find a bag of seeds at a grocery store in Siem Reap and sought a woman in Prasat Trew to crush the seeds with her mortar and pestle. A teaspoon of powder was added to a 16 oz (1 L) bottle of Rumdoul mineral water, shaken for 20 minutes, and left to settle for one hour. The mixture was then strained through a shirt to obtain moringa seed extract. This extract was added to a bottle of dirty water from the pit at the CVO Prasat Trew property at a ratio of five parts pit water to one part extract. The mix was shaken for 15 seconds and left to settle. Within 30 minutes, there was 2 cm of sludge at the bottom of the bottle and clear water above it.

The success of this initial experiment immediately prompted ideas for further experiments to maximize community resources of time, effort, and moringa seeds. Our research question became, "How can we pretreat the most water with the least expenditure of time, effort, and seeds?"



Sweetwater Research Delivers Clean Water in Cambodia using Moringa

Terminology

For clarity, here are a few notes on terminology. Turbidity refers to the amount of light scattered in a fluid by the particles suspended in it. Turbidimeters shine a light through a vial of test water and read how much light makes it to the other side. If the water is pure, all the light makes it through and the turbidity is o Nephelometric Turbidity Units (NTU). If the water is nearly opaque, such as the muddy water in the Prasat Trew pits, the reading will be in excess of 500 NTU. As mentioned above, the target NTU value we set for water used in ceramic filters was 25 or less. For reference, drinking water facilities in the U.S. aim for 0.1 NTU and many aid organizations working in non-industrialized zones recommend against drinking water with NTU values in excess of five. The figure below from MSR Gear is a helpful visual reference. The Secchi tube used to measure turbidity at the pit in Prasat Trew only read to 500 NTU. Making an educated guess, it was likely above 1000 NTU.





Examples of NTU readings for visual reference. Photo: MSR Water Lag Staff

Ranking different moringa methods required more than simply looking at the final NTU that each method produced. It isn't enough to say, "We got below 25 NTU". It matters how much suspended sediment had to be removed and how quickly it settled. Jars of dirty water that differ in suspended clay type, moringa powder concentration, starting turbidity, stir time, and settling time could all reach the <25 NTU threshold, but some could get there faster than others and use less time, effort, and seeds. A metric is needed that accounts for the difference between starting and ending turbidity and the time it takes to reach the 25 NTU threshold. We read many published experiments that addressed moringa method optimization, yet none of them used a metric that captured these variables. So, we created one.

Scaled Turbidity Reduction Efficiency (TRE₁₀₀) is a metric with a range of values 0-100, where a value of 100 means turbidity is almost completely removed within a few minutes and a value of 0 means there was no reduction in turbidity. The utility of this metric is that it can compare the success of different methods, independent of starting turbidity, clay type, stir time, and so on. For example, the types of soils that dominate northern Cambodia are not the same as in southern Cambodia. If you compared the moringa method in Prasat Trew and Phnom Penh, you might discover suspended solids from Phnom Penh settle more rapidly – but how much more effective was the moringa method relative to when it was used on Prasat Trew pit water? How do you compare the relative efficiency? If you tweak the method how do you know if your adjustments improved the method? TRE₁₀₀ allows one to make relative comparisons. As you read through the results of our tests to optimize the moringa method, TRE₁₀₀ is the number that summarizes all the information into one number that is on a convenient o-100 scale. The higher the TRE₁₀₀, the more you'll like that method because it requires less time to clarify a barrel of turbid water, fewer seeds to grow and harvest, and less effort to crush seeds to powder.

Experiment Parameters

Not all clays are created equal. In order to test whether moringa might have universal application, we used four different clays to make our dirty water: Bentonite, Green Sea, Kaolin, and Rhassoul. These common clays are used in various industrial and cosmetic applications, could be trusted to be uniform in their chemical composition, and were readily available through Amazon.



Some researchers have used moringa seed *extract*, such as we did during our 2019 field experiment. Others merely crushed moringa seed and added the powder directly to the dirty water. **To determine** whether it was necessary to expend more energy and time to produce seed extract, we ran some preliminary experiments and found that direct application of the seed powder was equally effective as the seed extract. In our primary experiment, therefore, we added moringa seed powder directly to the dirty water.

What is the optimal dosage of moringa? That is, what is the least amount of moringa seed powder required to achieve results comparable to higher doses? **Preliminary experiments were run with different dosages on the four clays to ascertain clay-specific optimal moringa dosages.** Table I displays the concentration in grams per liter of clay that had to be added to each flask to attain initial turbidities comparable to those in Cambodia (900 NTU; Green Sea only ever reached 750) and the optimal moringa dosages (milligrams per liter) based on maximum turbidity reduction after 2 hours of settling time.

Clay	Clay Conc. Synthetic Turbid Water (g/L)	Moringa Dosage (mg/L)
Bentonite	4	50
Green Sea	3	50
Kaolin	1.5	200
Rhassoul	2.5	200

Table 1. Clay concentrations for making synthetic turbid water for experiments and optimal moringa dosages for each clay type.

Moringa only works if the positively charged particles of moringa seed, the MOCP (page 2), make contact with the negatively charged particles of suspended clay. Thus, the time spent stirring moringa into dirty water matters. Too little time spent stirring will result in a waste of time because clay particles will not settle out of solution quickly (or at all). Excessive time spent stirring means time and energy wasted and you might even break up the flocs that are forming. To ascertain the optimal stir time, we stirred moringa into flasks of dirty water with a glass rod for 10 sec, 30 sec, 1 min, 5 min, and 10 min at approximately 240 rpm. This steady stir speed was deemed reasonable for most people to maintain in 1-liter containers. After stirring, the moringa & dirty water mixture was left to settle for 12 hours or until turbidity readings were <25 NTU, whichever came first.

In summary, for each of the six reps of our experiment, four flasks of dirty water were prepared (a different clay in each flask) with starting turbidities ranging 600-900. Each flask was dosed directly with moringa seed powder according to the optimal dosage rates determined by preliminary tests. The flasks were stirred for 10 sec, 30 sec, 1 min, 5 min, and 10 min at 240 rpm and left to settle for 12 hours or until NTU readings of 25 or less were obtained. Statistics were run on the results of the six reps.

Results

Three of the four clay types responded favorably to the moringa treatment (Table 2). Moringa caused clay particles to coagulate, flocculate, and settle more quickly and effectively than gravity alone (compare to the no-moringa control group). Bentonite showed no response whatsoever to moringa. Among the three clays that responded to moringa treatment, the highest TRE_{100} was with five minutes of moringa stir time (Table 2 & Figure 1). Stirring moringa an additional five minutes, for a total of 10 minutes of stir time, did not yield significantly lower final NTUs. In fact, extra stirring resulted in longer settling times to reach <25 NTU. Stirring moringa for one minute or less did produce final NTUs below the 25 NTU threshold, however, significantly more time was required for the clay particles to settle out. Five minutes of moringa stir time thus achieved the clearest water in the shortest amount of settling time.

Table 2. Average settling times to 25 NTU, final turbidities, total reduction %, and TRE₁₀₀ across four clay types at various moringa stir times.

		Moringa Stir Times					
	No Moringa	10s	30s	- 1m	5m	10m	
Settling Time Avg (h)							
Bentonite	12.0	12.0	12.0	12.0	12.0	12.0	
Green Sea	7.9	7.9	5.8	5.6	2.8	3.0	
Kaolin	9.5	9.5	5.5	5.3	3.1	3.4	
Rhassoul	8.9	8.9	7.3	7.3	5.0	5.7	
Residual Turbidity (NTU)							
Bentonite	265.3	241.5	258.2	257.7	261.7	258.7	
Green Sea	86.5	20.6	21.7	20.2	20.0	19.8	
Kaolin	441.2	19.9	22.4	22.5	19.7	20.4	
Rhassoul	217.5	22.3	22.4	23.0	21.8	19.1	
Reduction (%)							
Bentonite	69.8	72.3	70.4	70.3	69.9	70.4	
Green Sea	86.1	96.7	96.5	96.8	96.8	96.9	
Kaolin	49.5	97.7	97.4	97.4	97.7	97.7	
Rhassoul	74.4	97.4	97.4	97.3	97.4	97.8	
TRE ₁₀₀							
Bentonite	0.5	1.0	0.6	0.6	0.5	0.6	
Green Sea	12.4	15.6	25.7	27.6	72.8	62.1	
Kaolin	-0.9	10.9	28.4	29.5	62.2	54.6	
Rhassoul	6.1	12.5	19.0	19.4	32.9	28.8	

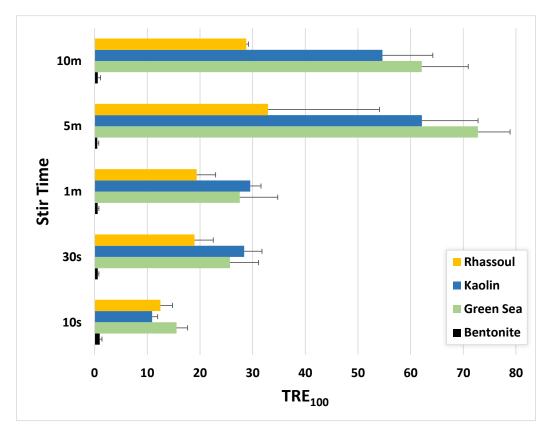


Figure 1. Average TRE₁₀₀ across four clay types at various moringa stir times.

BOTTOM LINE

The moringa method could be a useful tool in Cambodia for preparing dirty water for use in household ceramic filters. Before expectations are communicated to locals, however, tests should be run in different ecological zones of the country to confirm moringa effectiveness with different soils. We did not transport clay from Prasat Trew pits back to the U.S., though the result of the single field trial in Cambodia was most encouraging. The moringa worked quickly to floc and settle the suspended sediment, leaving a column of very clear water above the precipitate. We used four different industrial clays in our experiments because we assumed, correctly as our experiments demonstrated, that not all clays bind to moringa particles in a way that effects flocculation and precipitation.

As different clays do not exhibit the same response to moringa treatment, not all clays will respond to the same moringa dosage. It will be necessary to test for ideal dosages in different soil regions of Cambodia to ensure that you are maximizing your moringa seed resources.

Crushed seed powder should work fine. It is not necessary to go through the extra steps of creating a moringa extract solution.

Based on the results of our experiment, five minutes of stir time should optimize your effort. You could spend less time stirring but the time spent waiting for turbidity to reach <25 NTU will increase. Time spent stirring beyond five minutes will yield diminishing returns.

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Next Steps

As mentioned above, **OPTIMAL MORINGA DOSAGES** may vary from locale to locale. The powder is your power. You should use no more than is necessary for each batch of water. Moreover, excessive dosing can actually be counterproductive as particles will begin to repel one another because of a shift from neutral charge to *positive* charge. See Moringa Magnetism on page 2.

To determine optimal moringa concentrations,

- 1) Set up three 0.5 L (16 oz.) bottles of dirty water, 4/5 full.
- 2) In one of them add 25 mg of moringa seed powder, in one add 50 mg, in one add 100 mg.
- 3) Shake all of the bottles at the same time for 5 minutes.
- 4) Start a stopwatch and leave the bottles to settle.
- 5) Do a visual check of turbidity levels every 10 minutes for one hour. The bottle that achieved the most clarity in the fastest time is your optimal dosage.
- 6) Repeat this experiment three times to get an average and strengthen the trustworthiness of your results.

Several parameters for optimizing the moringa method have been established by our research for volumes of water in the 1-liter size class. **UPSCALING** our moringa method for preparing 227-liter (50-gallon) drums of water is a logical next step. At least four logistical hurdles will need to be jumped with production at this scale.

- Stirring moringa powder into a full barrel of turbid water at 240 rpm for 5 minutes will pose a significant physical challenge. Peddle paddles bicycles linked to paddles in the barrel could be a solution.
- Siphoning the cleaned water from the barrel will require a spigot to be placed at a strategic height on the barrel. Tests should be run to determine the average height of the precipitate after settling and the spigot must be placed sufficiently above this height so that sludge is not drawn into the siphon. Nor should the spigot be placed so high that much good water is left in the barrel.
- A method will need to be developed for efficiently disposing of the sludge at the bottom of the barrel after each use. Thankfully, the sludge is not toxic it is merely soil and moringa seed juice. Perhaps cleaning is as simple as installing a second spigot under the center of the base of the barrel, adding some pit water, and stirring the watered-down sludge to keep the solids suspended while it drains.
- Long term storage of large quantities of moringa-treated water may not be possible due to secondary bacterial growth in the treated water. Moringa can be an effective bactericide, but it has its limits. If the active components in moringa are used up and the remaining sugars and particulates in the clarified water are left for days without additional treatment, bacteria colonies could resprout in the moringa treated water. If long term storage of large quantities of clean water is a goal, then the water should first be put through final filtering and/or sterilization before storage.

The Sweetwater team would be pleased to assist with guidance for experimental design and troubleshooting throughout the upscaling process if our assistance is desired.

SECTION III

- 1) Final notes
- 2) Moringa cultivation
- 3) Useful links

Final Notes

Some researchers suggest harvesting seeds during the dry season because the water cleaning compound in moringa is more abundant in the seed than in the wet season.

If you research the moringa method further, you will encounter some studies that assert that *Moringa stenopetala* is more effective for water treatment, but this species is native to the horn of Africa, not Cambodia. Although it *could* possibly be cultivated in Cambodia, it probably *should* not be attempted.

People accustomed to drinking untreated and contaminated water should be able to consume water treated only with moringa without much trouble, though further filtering/sterilization is recommended if it is available. Others should not depend solely on moringa treatment except in a pinch. Additional treatment to remove microbes should be employed, such as ceramic filtering, solar sterilization, chlorination, etc.

Our estimates for the number of trees needed to supply a family and community with water for a year are rough averages. Young trees and poorly-performing mature trees might produce 8,000 seeds per year whereas healthy mature trees can produce well over 20,000 seeds a year. Calculations also depend on whether you assume the average person drinks 0.5 gal/day or 1.0 gal/day. There is typically a wide gulf between what is recommended for daily consumption and what is practiced. The numbers used in our calculations are well within the ranges found in diverse sources of moringa information.

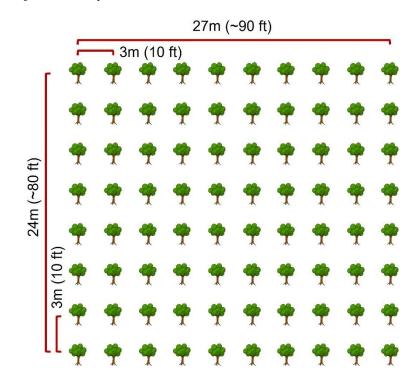
For the sake of brevity, this report is not an exhaustive description of our experiments. The bibliography, notes on moringa cultivation, and so forth, are all a very small sample of the full body of information published on amateur websites, as technical notes from professional organizations, and peer-reviewed scientific papers. We intend that what is conveyed in this report will be sufficient to give you a robust start. We will soon be submitting a manuscript for publication in a peer-reviewed journal that contains all of the technical minutia. If you want the details or a copy of our full bibliography we will be happy to share them with you.

Moringa has the potential to undergird local economic diversification initiatives in addition to its service as a water pretreatment agent. Because there are several steps from tree to pretreated water, there are several niches for entrepreneurs to fill with their labor. The farmer grows the tree and sells the seeds. The processor crushes the seed and sells the powder. The water handler manages the barrels, treats unfiltered water with moringa, and stores filtered water for customers. In addition, moringa products such as dried leaf powder, can be sold locally or exported to larger markets. There are at least two businesses in Siem Reap that sell leaf powder, oils, and other moringa goods (see links below).



Moringa Cultivation

Moringa oleifera is native to Cambodia and is adapted to the extremities of the dry season by virtue of its long taproot. Inundation can result in root rot, so effort should be made to raise the tree's feet out of low spots using raised beds. Seed production is the priority for water clarification and the optimal spacing for moringa seed production is 3 m (10 ft) according to several sources. Based on approximate drinking water consumption, average seed production per tree, etc., a community of 6 households (30 people) should cultivate 70-80 trees to produce enough seed powder to pretreat highly turbid water for one year. If a plot is dedicated to moringa, the configuration below would require 7,200 ft², or roughly 1/6 acre (0.067 ha). This graphic is offered only to kickstart perspective regarding land requirements, not to recommend a particular layout.



A few assorted notes on moringa cultivation:

- Moringa's resiliency to drought means less water demand during the critical dry season.
- Moringa can grow up to 5 m (16 ft) in its first year, but it serves little purpose if left to do so. Pruning off the lead when the tree is 60 cm tall as well as strategic pruning of branches will encourage bushy branching and higher seed production. Details can be found in this <u>Fuglie and</u> <u>Sreeja article</u>, also linked below. We strongly recommend you read this helpful write-up before committing to moringa cultivation.
- Goats, cattle, and other livestock will heavily graze moringa they can reach. This may be to your advantage if you have an integrated livestock operation due to the many vitamins and minerals found in moringa. Trees designated for seed production should be protected by living fences or other barriers.

Links Pertaining to Cultivation

- This article by Fuglie and Sreeja is a pithy, condensed, one-stop shop for all the essentials of moringa cultivation. Start here. <u>https://moringafarms.com/growing-it/cultivation-moringa/</u>
- This clearinghouse of moringa information from the Educational Concerns for Hunger Organization (ECHO) contains files and links to everything you will likely ever need to successfully cultivate moringa. https://www.echocommunity.org/en/resources/c9f55480-Ifa0-464c-8bfa-6899aa3f88cf

Selected Useful Links

- Introduction to F-sand filters. For a deep dive into the next generation of moringa-based water clarification technology, check out the work of Dr. Velegol. <u>https://www.echocommunity.org/en/resources/79a33ca4-e00e-4ce8-bb2d-fd2ed28b5eb2</u>
- Moringa sellers in Siem Reap. <u>https://www.splemoringa.com/</u> <u>https://baca-villa.com/</u>
- ECHO Asia Impact Center represents a resource for information and training similar to Farming God's Way. They are located in Chiang Mai, Thailand. <u>https://echonet.org/our-work/regional-impact-centers/asia-impact-center/</u>
- Build your own Secchi tube for measuring turbidity. This paper is clear and informative. <u>http://serresconseil.com/WASH/Watsanmissionassistant/mainSpace/files/Turbidity-Myre_Shaw.pdf</u>



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