Bioremediation of Turbid Surface Water Using Seed Extract from *Moringa oleifera* Lam. (Drumstick) Tree

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ABSTRACT

An indigenous water treatment method uses *Moringa oleifera* seeds in the form of a water-soluble extract in suspension, resulting in an effective natural clarification agent for highly turbid and untreated pathogenic surface water. Efficient reduction (80.0% to 99.5%) of high turbidity produces an aesthetically clear supernatant, concurrently accompanied by 90.00% to 99.99% (1 to 4 log) bacterial reduction. Application of this low-cost *Moringa oleifera* protocol is recommended for simplified, point-of-use, low-risk water treatment where rural and peri-urban people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water. *Curr. Protoc. Microbiol.* 16:1G.2.1-1G.2.14. © 2010 by John Wiley & Sons, Inc.

Keywords: *Moringa oleifera* • turbidity • natural coagulant • coagulation • flocculation • household water treatment • developing countries

INTRODUCTION

In poor countries throughout Africa, Asia, and Latin America, approximately 1.4 billion rural and peri-urban people (Chen and Ravallion, 2008) live deplorably in extreme poverty (<$1.25 U.S./day), struggling day-to-day to survive. The majority of this population rely almost exclusively upon traditional sources of highly turbid and untreated pathogenic (viral and bacterial) surface water for their domestic water needs. Since time immemorial, the indigenous method employing crushed seed powder from the pan-tropical *Moringa oleifera* (MO) tree has resulted in an effective natural flocculating agent, providing a low-cost household (point-of-use) solution to the critical need for potable water in rural riparian communities.

A literature review indicates that *Moringa oleifera* seeds coagulate 80.0% to 99.5% turbidity (surrogate for suspended fine particles) and color (surrogate for natural organic material), efficiently leading to a aesthetically clear supernatant. As a safer indicator, this was concurrently accompanied by a 90.00% to 99.99% bacterial load reduction (fecal coliforms), with bacteria concentrated in the sedimented sludge (Madsen et al., 1987). *Moringa* flocculants released from the crushed seed kernels have been characterized as basic polypeptides with a molecular weight between 6 and 16 kDa (Jahn, 1988) and an isoelectric pH of 10 to 11 (Folkard and Sutherland, 2001). They bind suspended particles in a colloidal suspension, forming larger sedimenting particles (flocs) that include pathogenic microorganisms (Madsen et al., 1987). These findings highlight the importance of widespread dissemination of this rudimentary water treatment protocol (Basic Protocol 2 and Support Protocol 1) among the rural and peri-urban marginalized poorest of poor communities, contributing to improving equity, social justice, and the overall quality of life through the provision of potable water.

CAUTION: The relationship between infectious turbidity dosage (pathogens attached to suspended particles) and host susceptibility, especially among the malnourished and

Current Protocols in Microbiology 1G.2.1-1G.2.14, February 2010
Published online February 2010 in Wiley Interscience (www.interscience.wiley.com).
DOI: 10.1002/9780471729259.mc01g02s16

vulnerable (children under 5), is a complicated and critical factor that contributes to the likelihood of acquiring a waterborne illness, i.e., diarrhea, a leading cause of extreme child morbidity and mortality in the developing world. Rudimentary *Moringa oleifera* usage can produce potable water of higher quality than the original source, but is unable to guarantee 100% complete viral and/or bacterial elimination immediately after treatment or storage. If at all possible, an additional low-cost treatment process is recommended (e.g., see Support Protocols 2, 3, 4, and 5).

CAUTION: Since chlorine-based disinfectants react with natural organic material, exerting a chlorine demand (precursor to the emergence of chlorination by-products, i.e., carcinogenic trihalomethanes), the use of MO crude extract in conjunction with primitive application of chlorine-based disinfectants is not recommended.

STRATEGIC PLANNING

There are three overall strategic points of planning to consider.

Seed Powder or Seed Cake?

Moringa oleifera seeds contain 35% to 40% oil by weight (Levicki, 2005), and can first be processed to yield a high-quality edible vegetable oil "high in oleic acid" (Sutherland et al., 1994) that resists rancidity (Fahey, 2005). Commonly referred to as Ben oil, its potential value within the household for cooking, smoke-free lamp fuel, and the manufacturing of soap should be examined. In addition, a little coating of oil atop exposed water containers will help destroy mosquito larvae, and thus reduce the threat of malaria and other deadly insect-borne diseases. Of particular importance, the seed cake (presscake) remaining after oil extraction still retains the active flocculating properties for removing turbidity, without diminished efficacy.

Since the oil possesses local market value, adoption of the presscake as a coagulant is overwhelmingly advantageous when compared to the use of seed powder. Therefore, if possible, it is recommended that the household oil-extraction method described in Basic Protocol 1) be implemented beforehand, to capture value-added commodities.

MO Dosage Rates?

To treat surface water, the equivalent weight of presscake or seed powder required to make up a crude extract solution is dependent upon the turbidity. Table 1G.2.1 provides an overall general (rough) guideline for dosage rates. In light of logistical complications within a primitive household level of action, i.e., the inherent variability of raw water conditions and dosage performance, it is recommended that simple village jar test procedures be undertaken to determine the best clarification dose (see Support Protocol 1).

Table 1G.2.1 MO Dosage Rates (Seed Cake or Seed Powder)[a]

Raw water turbidity (NTU)	Dose range (seeds/liter)	Dose range (mg/liter)
<50 NTU (low)	1 seed/4 liters	50 mg/liter
50-150 NTU (medium)	1 seed/2 liters	100 mg/liter
150-250 NTU (high)	1 seed/liter	200 mg/liter
>250 NTU (extreme)	2 seeds/liter	400 mg/liter

[a] As a general rule of thumb, one shelled seed (~200 mg) is used to treat 1 liter of very turbid surface water (Doerr, 2005). Note that MO is not an effective coagulant for low-turbidity (<50 NTU) water.

Turbidity Performance Considerations

Turbidity is the measure of presence of fine negatively charge particulate matter suspended in water (cloudiness), usually reported as nephelometric turbidity units (NTU), determined by measurements of light scattering and reflection rather than from beams transmitted in straight lines. MO seed powder as a clarifying agent has an important limitation, i.e., unsuitability for low-turbidity waters <50 NTU (Dorea, 2006). “This may be due to the low molecular weight of the coagulant and the patch mechanism of charge neutralization and floc formation that forms smaller and light flocs” (Bratby, 2006). This is an important consideration, for example, if one is contemplating using MO as a turbidity-removing agent before the SODIS disinfection method. (Yazdani, 2007).

Alternatively, the seeds are highly effective in the removal of turbidity from surface waters with medium and high to extreme initial turbidities, i.e., >15 NTU to 10,000 NTU. (Jahn, 1988). This fact is of paramount importance, especially in the highly turbid conditions experienced throughout the rainy (monsoon) season. It has been experienced that the “level of polyelectrolyte present in the kernels is substantially less during the wet season”; therefore, it is recommended that the seeds “should be harvested during the dry season only” (Fuglie, 2000).

HOUSEHOLD SEED OIL EXTRACTION

BASIC PROTOCOL 1

Within households, value-added oil can easily be extracted from MO seed. The following procedure is adapted from Price (1985).

Materials

Moringa oleifera Lam. mature fruit pods
Boiling water

Household spice crusher (grinding stone or mortar)
Skillet
Pot
Skimmer utensil (e.g., ladle)
Cotton filter cloth
Clean containers

1. Harvest mature (brown) fruit pods from tree.

2. Crack fruit pods along seam and pluck out seeds.

   Depending on pod size, each fruit may contain 5 to 20 seeds.

3. Remove the seed coat and the wings from the white/yellowish seed kernels (cotyledons).

   Remove (do not use) discolored or soft seeds (Jahn, 1988).

4. Roast the seed kernels in a skillet.

   Take care not to burn the seed kernels.

5. Mash the seed kernels thoroughly.

   Use a mortar or grinding stone for crushing.

6. Place the seed cake in boiling water for a total of 5 min.

7. After boiling, strain liquid through a cotton filter cloth into a clean container. Retain the seed cake from the cotton cloth.

   After oil extraction, the seed cake has all the coagulating properties to clarify raw surface water.

8. Dry seed cake (presscake) for 2 to 3 days in direct sunlight.

9. Leave liquid in container overnight.

10. Next day, skim off the oil that has risen to the surface.

11. Filter the oil through a cotton cloth and store in a clean container.

    This will remove the protein content upon which bacteria feed. The viscosity of the oil can be improved by heating before filtering.

12. Store dried seed cake.

    Seed cake has a 1 month efficiency shelf life if stored in air-tight plastic bags in a cool, dry place.

SUPPORT PROTOCOL 1

SIMPLE VILLAGE JAR TEST PROCEDURES

Village health workers or other trained community leaders will determine the correct dosage of seed powder or presscake to be used domestically by carrying out the following village jar test. Briefly, it is described as follows. A series of three samples of water are placed in jars and dosed with a range of *M. oliefera* coagulant concentrations, e.g., 100, 200, and 400 mg/liter, and are stirred vigorously for ~1 min. This is followed by gentle stirring for 5 min, after which the samples are allowed to stand and settle for 60 min. The samples are then examined for color and turbidity, and the lowest dose of coagulant that gives satisfactory clarification of the water is noted by the naked eye. There is no set schedule for undertaking village jar test procedures; they should be done according to the changing seasonal turbidity levels, i.e., drought versus rainy season.

Materials

Moringa oleifera Lam. mature fruit pods or presscake (Basic Protocol 1)
1 liter clean or previously clarified water
2 to 3 liters raw source water

Household spice crusher (grinding stone or mortar)
0.8-mm mesh (e.g., tea strainer)
Muslin or cotton cloth
One 12-in. ruler
One black marker pen
One pair of scissors or a knife
Five clean and transparent (not colored) plastic (soda) bottles (~591 ml size), including bottle caps
One water storage container, e.g., plastic bucket, ~20 liters (5 gallons)
Three people to stir one soda bottle each at the same time
Three paddle-type utensils, e.g., spoon, ladle, spatula, or fork

1a. *If using presscake prepared in Basic Protocol 1:* Proceed directly to step 4.

1b. *If preparing powder fresh from seeds:* Proceed with step 2.

2. Harvest mature (brown) fruit pods from tree.

3. Crack fruit pods along seam and pluck out seeds.

   Depending on pod size, each fruit may contain 5 to 20 seeds.

4. Dry seeds and seed cake (presscake) for 2 to 3 days in direct sunlight.

   Dried seeds and presscake have a 1 month efficiency shelf life if stored in air-tight plastic bags in a cool, dry place.

5. After 2 to 3 days of outside drying, carefully remove the seed coat and the wings from the white/yellowish seed kernels (cotyledons).

Remove (do not use) discolored or soft seeds (Jahn, 1988).

6. Using a mortar or a grinding stone, pound and crush the white seeds kernels (from step 4) or solid residue after household oil extraction (seed cake; see Basic Protocol 1) thoroughly into a fine powder.

Traditional techniques employed for the production of maize flour would be ideal for this particular stage.

7. Sieve the seed or presscake powder through 0.8-mm mesh or similar, e.g., a tea strainer.

The fine powder has a 1 to 2 month shelf life if stored in air-tight plastic bags in a cool, dry place.

8. Using the ruler and the black marker pen, measure and mark 3 in. up from bottom on all five plastic (soda) bottles (∼591 ml size), and also measure and mark 6 in. up from bottom on three of the soda bottles.

3 in. is approximately the 250 ml (8 fl. oz.) level.

9. Using scissors or knife, cut completely around the 6-in. mark on the three soda bottles, discarding the bottle tops.

Keep all bottle caps to be used as measuring tools.

10. Transfer ∼2.5 g of stored, finely crushed white MO powder (the equivalent of 12 seeds; may be estimated as two heaping teaspoons or the content of two rounded soda bottle tops) into a clean plastic (soda) bottle.

11. Add a small amount of clean or clarified water (∼100 ml) to the MO seed powder, and shake vigorously to form a paste.

The paste can now be diluted to the required dosing strength to form the stock solution.

12. To prepare a 250-ml (8 fl. oz.) stock solution, dilute the 100 ml of paste to the required strength by pouring an additional 150 ml of clean or clarified water into the plastic soda bottle.

The stock solution extract is prepared at 10,000 mg/liter (1%) by dissolving 2.5 g MO powder in 250 ml (8 fl. oz.) clarified water. Dosage change equals 40.0 mg/liter for each milliliter of stock solution added to raw water in step 18.

13. Seal the 591-ml soda bottle by screwing on bottle cap.

14. Vigorously shake the soda bottle for 5 min to obtain a good water extract and allow to stand for 10 min.

When MO powder is mixed with water, it yields a solution that reacts as a natural cationic polyelectrolyte (Sutherland et al., 1994).

15. Filter insoluble material from the crude extract through a tea strainer (ideally with an additional muslin or cotton filter cloth over the tea strainer) into a clean plastic soda bottle.

The stock solution is now ready.

Crude extract has a 24-hr efficiency. It is best to prepare fresh each day (Katayon et al., 2004).

16. Using water storage container, collect 2 to 3 liters of raw surface water.

17. Fill three soda bottles (from step 9) with an equal volume amount of raw surface water to the marked (3 in.) 250-ml (8 fl. oz.) level.

18. Measure and add the following soda bottle cap volume dosages of stock solution individually to each of the three 250-ml (8 fl. oz.) raw surface water–filled water bottles:

Bottle #1, coagulant dose of 100 mg/liter (2.5 ml stock solution = half a bottle cap);
Bottle #2, coagulant dose of 200 mg/liter (5.0 ml stock solution = one full bottle cap);
Bottle #3, coagulant dose of 400 mg/liter (10.0 ml stock solution = two full bottle caps) of a 1.0% (w/v) crude *Moringa* extract solution.

The actual dosages may be different due to local conditions; the dose ranges shown in Table 1G.2.1 are given as a guide only; concentration dosages between 0.5% and 5% (w/v) can be tested (Schwarz, 2000) to determine optimal performance for the raw surface water in question.

19. Each of the three persons now begin to stir the raw water rapidly using a paddle utensil, e.g., spoon, ladle, spatula, or fork, maintaining fast mixing for 60 sec.

The fast mixing allows coagulation to take place. The suspended particles (turbidity), which previously repelled each other, are now attracted to one another or to the positively charged stock solution added, to be transformed into aggregates. This "process occurs very quickly, in a matter of fractions of a second" (Kalibbala, 2007).

20. After 30 sec, immediately initiate regular slow, gentle mixing (15 to 20 rotations per min), which should be maintained for 5 min.

The slow mixing allows flocculation to take place. As the particles are attracted to each other, larger particles (flocs) are formed, which will be allowed to settle (sedimentation) or be filtered out.

21. Allow the three 591-ml sized containers to sit undisturbed for 1 hr to allow larger flocs to settle.

If clarification is proceeding rather slowly, let container sit undisturbed for 2 hr.

22. Using the naked eye, determine which soda bottle out of the three has the preferred level of residual turbidity.

The soda bottle with the least turbidity will have the optimal coagulant dosage to be used.

BASIC PROTOCOL 2

CLARIFICATION OF TURBID SURFACE WATER USING SEED EXTRACT FROM *M. OLEIFERA*

No matter what the technology is used for treating water, they each have to overcome the hurdle of removing turbidity from the source water. It is a crucial step towards water treatment. Little has been done to define, optimize, and standardize protocols for their use. Furthermore, there appears to be little current effort to encourage or disseminate such treatment. The following protocol will overcome these short-falls.

This standard method for water clarification is performed with locally available materials and may be modified according to specific local conditions.

Materials

Moringa oleifera Lam. mature fruit pods or presscake (Basic Protocol 1)
1 liter clean or previously clarified water
20 liters (5 gallons) raw source water

Household spice crusher (grinding stone or mortar)
One clean and transparent (not colored) plastic (soda) bottle (∼591 ml size), including bottle cap

0.8-mm mesh (e.g., tea strainer)
Muslin or cotton filter cloth
One paddle-type utensil, e.g., spoon, ladle, spatula or fork
One designated water collection container, e.g., plastic bucket or clay vessel, ~20 liters (5 gallons)
One designated safe-storage water container, e.g., plastic bucket or clay vessel, ~20 liters (5 gallons)

NOTE: The following protocol is designed to treat 20 liters (~5 gallons) of highly turbid (150 to 250 NTU) raw surface water (see Table 1G.2.1).

1a. *If using presscake prepared in Basic Protocol 1:* Proceed directly to step 4.

1b. *If preparing presscake powder fresh:* Proceed with step 2.

2. Harvest mature (brown) fruit pods from tree.

   It has been experienced that the "level of polyelectrolyte present in the kernels is substantially less during the wet season"; therefore, it is recommended the seeds "should be harvested during the dry season only" (Fuglie, 2000).

3. Crack fruit pods along seam and pluck out seeds.

   Depending on pod size, each fruit may contain 5 to 20 seeds.

4. Dry seeds and seed cake (presscake) for 2 to 3 days in direct sunlight.

   Dried seeds and presscake have a 1 month efficiency shelf life if stored in air-tight plastic bags in a cool, dry place.

5. After 2 to 3 days of outside drying, remove the seed coat and the wings from the white/yellowish seed kernels (cotyledons).

   Remove (do not use) discolored or softened seeds (Jahn, 1988).

6. Using a mortar or a grinding stone, pound and crush the seeds kernels (from step 4) or solid residue after household oil extraction (presscake; see Basic Protocol 1) thoroughly into a fine powder.

   Traditional techniques employed for the production of maize flour would be ideal for this particular stage.

7. Sieve the seed or seed cake powder through 0.8-mm mesh or similar, e.g., a tea strainer.

   The fine powder has a 1 to 2 month shelf life if stored in air-tight plastic bags in a cool, dry place.

8. Transfer ~4 g of finely crushed white MO powder or seed cake (the equivalent of 20 seeds; may be estimated as four heaping teaspoons or the contents of four rounded soda bottle tops) into one clean and transparent (not colored) plastic (soda) bottle (~591 ml size).

9. Add a small amount of clean or clarified water (~100 ml) to the MO powder and shake vigorously to form a paste.

   Crude extract has a 24-hr efficiency. It is best to prepare fresh each day (Katayon et al., 2004). The paste can now be diluted to the required dosing strength to form the solution.

10. To prepare a 250-ml (8 fl. oz.) suspension, dilute the 100 ml of paste to the required strength by pouring an additional 150 ml of clean or clarified water into the plastic soda bottle.

11. Seal the 591-ml soda bottle by screwing on a bottle cap.

12. Vigorously shake the soda bottle for 5 min to obtain a good water extract and allow to stand for 10 min.

    When MO powder is mixed with water, it yields a solution that reacts as a natural cationic polyelectrolyte (Sutherland et al., 1994).

13. Filter the milky extract solution through a tea strainer (ideal to also have additional muslin or cotton filter cloth over the tea strainer to remove insoluble materials) into the designated 20-liter (~5 gallon) collection container of turbid water to be treated.

    The remaining insoluble seed material needs to be cleaned from the stock solution container after each batch mixing.

14. Using the paddle-type utensil, e.g., spoon, ladle, spatula, or fork, begin to stir the raw water rapidly; maintain rapid mixing for 30 sec.

    The fast mixing allows coagulation to take place. The suspended particles (turbidity), which previously repelled each other are now attracted to one another or to the positively charged stock solution added, to now be transformed into aggregates. This "process occurs very quickly, in a matter of fractions of a second" (Kalibbala, 2007).

15. After 30 sec, immediately initiate slow, gentle mixing (15 to 20 rotations per min), which should be maintained for 5 min.

    The slow gentle mixing "accelerates the electrostatic flocculation" to take place and allows the larger particles (flocs) to "condense the contaminants" (Bergman and Arnoldsson, 2008).

16. Cover and allow the 20-liter (5 gallon) container to sit undisturbed for 1 hr to permit the settling of larger flocs.

    This allows sufficient time for the sedimentation of larger flocs—those aggregates which are heavier, and settle out to the bottom of container at a faster rate due to gravity.

    If clarification is proceeding rather slowly, either because the container was moved or shaken, or the water is cooler (Ndabigengesere and Narasiah, 1996) due to seasonal change, let the container sit undisturbed for 2 hr.

17. After 1 to 2 hr, when satisfactory filtrate water has been determined, decant or siphon the clear supernatant water and filter through a clean cloth into the designated safe-storage container. Be careful to not disturb the sludge on the bottom of the first container.

    CAUTION: *Rudimentary Moringa oleifera usage can produce potable water of higher quality, but is unable to guarantee (100%) virus- and/or bacteria-free water immediately after treatment or storage. If possible, an additional treatment process is recommended (see, e.g., Support Protocols 2 to 5).*

    After several hours of storage, temperature-induced bacterial regrowth will be experienced within the storage container. To minimize this, either consume the water shortly after treatment or initiate more frequent treatment (8 hr) of smaller amounts of water, rather than storing large (20-liter) containers for longer periods, e.g., 24 hr.

    The remaining precipitated sludge at the bottom of the container is biodegradable and therefore environmentally safe (Taley, 2007), but only after being exposed to the sun for several days to destroy contained pathogenic particles within the sludge. In the meantime, handle the sludge carefully (Ives and Jahn, 1994).

    Interestingly, a recent study on the sludge effect of the seed-derived peptide termed Flo indicates that it mediates bacterial disinfection, being able to kill antibiotic-resistant bacteria, including several human pathogens (Suarez et al., 2005).

18. If necessary, provide additional treatment of clarified water.

It is beyond the scope of this protocol to explain in depth the multiplicity of additional low-cost household water treatment technologies that may be suitable under primitive conditions. To stimulate the reader's interest in further exploration, some low-cost suggestions are briefly described (see Support Protocols 2, 3, 4, and 5).

It is important to be mindful that additional water treatment procedures require additional financial, technical, and education resources and, therefore, increase the complexity; they may not be available within the limited means of the household.

For further guidance about additional water-treatment options, the World Health Organization provides the following report entitled Managing Water in the Home: Accelerated Health Gains from Improved Water Supply. It is available for download at the following URL: http://www.who.int/water_sanitation_health/dwq/wsh0207/en/.

ADDITIONAL TREATMENT OF CLARIFIED WATER

No matter the technology used for treating water, all such methods have to overcome the hurdle of removing turbidity from the source water, which is a crucial step in water treatment. *M. oleifera* can be utilized to pretreat water (as described in Basic Protocol 2) to reduce turbidity prior to implementation of additional multi-carrier treatment technologies such as those described below in Support Protocols 2, 3, 4, and 5.

SUPPORT PROTOCOL 2

Treatment by Biological Sand (Biosand) Filtration

For further aesthetic reduction of turbidity and the quantity of microorganisms (parasites, protozoal cysts, and bacterial pathogens) and, in some situations, for removal of chemical contaminants (e.g., arsenic), biosand filtration (*UNIT 1G.1*) is recommended.

SUPPORT PROTOCOL 3

Disinfection by Natural pH Shift

Low-cost water disinfection by decreasing water pH with the use of naturally acidic solutions, such as lime juice, are a traditional point-of-use treatment for inactivating certain pathogens, i.e., *V. cholerae*. The World Health Organization has reported that adding lime juice to water (1% to 5% final concentration) to lower pH levels below 4.5 reduced *V. cholerae* by >99.999% in 120 min (Sobsey, 2002).

SUPPORT PROTOCOL 4

Disinfection by Solar Pasteurization

Water disinfection by solar pasteurization has a synergistic effect (radiation and thermal), killing 99.9% of pathogens when temperatures rise above 56°C. For further in-depth details about solar pasteurization, visit the Solar Cooking International Web site: *http://solarcooking.org*.

SUPPORT PROTOCOL 5

Disinfection by Chlorination

Chlorination is a well-proven technique, but it also has its own unique challenges. For instance, waterborne pathogens such as *Cryptosporidium* and *Giardia* have high resistance to chemical disinfectants, such as chlorine. Also, it is well established that people dislike the strong odor and disagreeable taste associated with free chlorine. Most importantly, due to the necessity of continuously repurchasing a consumable product, at-risk households will abandon treating water when financial resources are unavailable.

CAUTION: Since chlorine-based disinfectants react with natural organic material, exerting a chlorine demand which is the precursor to the emergence of chlorination byproducts, e.g., carcinogenic trihalomethanes, the use of MO crude extract in conjunction with primitive application of chlorine-based disinfectants is not recommended.

COMMENTARY

Background Information

Coagulation, flocculation, and sedimentation with *M. oleifera* are the key steps in this simple water-treatment protocol that has been used by indigenous cultures for centuries for the significant removal of color and particulate matter, including indicator bacteria and other microorganisms.

Description of Moringa oleifera

M. oleifera, a tree native to northern India (southern foothills of the Himalayas), is the most studied of the 14 species in the genus *Moringa*. It is cultivated widely throughout many countries of Africa, Asia, and Latin America. *M. oleifera* grows rapidly from seed and cuttings, even in marginal soils, to 3 meters (9 feet) in height, with flowers and fruit (drumsticks), within the first year. It is hardy enough to be drought resistant, but it is not tolerant to frost and is susceptible to wind damage.

The *M. oleifera* species is not only important for water treatment, but the tree has many other uses as well. It is one of the world's most valuable plants in terms of humanitarian benefit and nutritional value—almost every part (except for the trunk and root) of *M. oleifera* can be consumed as food.

"The immature green pods, called 'drumsticks' are probably the most valued and widely used part of the tree. They are widely consumed in India, are generally prepared in a similar fashion to green beans, and have a slight asparagus taste. The seeds are sometimes removed from more mature pods and eaten like peas or roasted like nuts. The flowers are edible when cooked, and are said to taste like mushrooms" (*http://en.wikipedia.org/wiki/Moringa_oleifera*).

According to Badruddoza (see Internet Resources), "*Moringa* leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas, and. . .the protein quality of *Moringa* leaves rivals that of milk and eggs . . .. . ." In fact, the nutritional properties of *Moringa* are now so well known that there seems to be little doubt of the substantial health benefit to be realized by consumption of *Moringa* leaf powder in situations where starvation is imminent.

M. oleifera "has been used successfully to combat malnutrition among infants and women of childbearing age. In Africa, breastfeeding nursing mothers have been shown to produce far more milk when they add *Moringa* leaves to their diet and severely malnourished children have made significant weight gains when caregivers add the leaves to their diets" (*http://en.wikipedia.org/wiki/Moringa_oleifera*).

The tree's most unique property is the ability of its dry, crushed seeds and seed presscake, which contain polypeptides, to serve as an effective and low-cost traditional source of natural coagulant for removing turbidity and reducing bacterial and viral contamination from drinking water in rural households. Systematic research has shown that *M. oleifera* seeds are an effective water clarification agent across a wide range of various colloidal suspensions. When mixed with water, the crushed seed powder or residue presscake yields water-soluble organic polymers, also known as natural cationic (net positive charge) polyelectrolyte (Marobhe, 2008).

The actual *Moringa* flocculants are basic polypeptides with a molecular weight between 6 and 16 kDa; Jahn, 1988) and an isoelectric pH of 10 to 11 (Folkard and Sutherland, 2001). The natural polyelectrolytes released from the crushed seed kernels function as natural flocculating agents, binding suspended particles in a colloidal suspension, forming larger sedimenting particles (flocs). Pathogenic microorganisms are generally attached to the solid particles, and treatment employing *M. oleifera* seed or presscake can remove 90.00% to 99.99% of indicator (fecal coliforms) bacterial load (Madsen et al., 1987).

M. oleifera should not be regarded as a panacea for reducing the high incidence of waterborne diseases. However, it can be an important, sustainable, and affordable method towards reduction, and can also improve the quality of life for a large proportion of the poor by providing extra income and food. The amazing thing about *M. oleifera* is that it propagates exactly where clean water is needed the most—Africa, Asia, and Latin America. The challenge for us is to recognize, promote, and adopt proven indigenous methods that remove the most turbidity at the lowest cost, producing water of low risk for marginalized households.

Vernacular names

The scientific name of this species is *Moringa oleifera* Lamarck (*M. oleifera* Lam.). *M. oleifera* is known by several common names in different countries. A list of over 400 vernacular names in various languages is provided by Trees for Life International at the following link: *http://www.treesforlife.org/our-work/our-initiatives/moringa.*

Chemical composition and toxicology

"Chemical analysis found the seed to contain 34.1% protein, 15% carbohydrates and 15.5 % lipids" (Olayemi and Alabi, 1994). *M. oleifera* "seeds contain seed oil and a germicide. The smell-and-taste effect on the water is very small, even for the maximum *Moringa* seed dose (200-250 mg/l)" .... " In the low range of optimal doses there is no germicide effect and there are no risks from toxic substances." (Ives and Jahn, 1994).

Affordability

M. oleifera trees can be readily propagated, and a coagulant can be made readily available at the household level, with seeds produced within the first year. Initial setup and protocol application using common household equipment would be minimal. The only consumable items would be the actual MO seeds, which have the additional benefit of providing value-added consumables if marketable oil is extracted (see Basic Protocol 1). After oil production, the residual presscake solid can still be utilized for its clarification properties, so the coagulant is obtained at extremely low or zero net cost. The sweat equity cost of using this method would come from gathering and preparing the seeds and possibly oil extraction.

Research

"*Moringa* preparations have been cited in the scientific literature as having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic, and hypoglycemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis, and even reduction of *Schistosoma cercariae* titer." (Dewan Md. Badruddoza; see Internet Resources). The next requirement needed is the implementation of a multi-year health impact study to determine *M. oleifera* acceptability and effectiveness in reducing waterborne infectious disease.

Nancy Jotham Marobhe has stated that "It is now up to the governments in poor countries to first recognize and duly support the initiatives of the poor and yet disadvantaged population segments. This will need commitments of countries to strengthen the natural water coagulation technology in a holistic approach and to support these initiatives including empowering and enabling local scientists and technologists to build up the natural coagulation technology that will suit the local requirements and situation based on scientific knowledge available" (Marobhe, 2008).

Critical Parameters

There is evidence to suggest that performance of same-species seeds can differ significantly based solely on location of cultivation (Narasiah et al., 2002). This fact would highlight the importance of initiating jar test procedures to confirm the correct dosage for reducing turbidity (see Support Protocol 2).

Toxicity

An extensive literature review has concluded that aqueous MO seed or presscake extract presents low toxicity, particularly at the low doses required for water treatment.

Popular beliefs

The use of the *M. oleifera* protocol may have overwhelming humanitarian benefits, but may be unacceptable to the local populace simply because of strongly held beliefs, e.g., that "more than three *Moringa* trees in a house is a source of misfortune, because it will increase poverty and cause death in the family" (Diouf et al., 2007). Such cultural sensitivities have to be taken into account before field implementation of *M. oleifera*.

Other species

M. oleifera is the most widely distributed, well known and studied species of the family Moringaceae because of its water-purifying capabilities. The African Moringa, *M. stenopetala*, is less widely distributed than *M. oleifera*, but there is evidence that *M. stenopetala* is a more effective water clarifier than *M. oleifera* (Jahn, 1988).

Anticipated Results

The performance efficiency is better with moderately to heavily turbid water than less turbid water, and is comparable to that of inorganic coagulants (alum), especially for highly turbid waters. In addition, with *M. oliefera* there is no effect on the pH, alkalinity, or conductivity of the treated water (Bergman and Arnoldsson, 2008). The technique highlighted should result in a turbidity reduction of 80.0% to 99.5%, paralleled by a primary bacterial reduction of 90.00% to 99.99% (1 to 4 log units) obtained within the first 1 to 2 hr of treatment. The bacteria are concentrated in the coagulated sludge at the bottom of the container. After several hours of storage, temperature-induced bacteria regrowth will occur within the storage container.

The *M. oleifera* protocol can produce potable water of higher quality that the original source, but is unable to guarantee (100%) virus- and/or bacteria-free water immediately after treatment or storage. An additional disinfection process is required to meet such stringent requirements. Still, it has to be recognized that such low-cost efficiency is of significant importance to people relying almost exclusively upon untreated surface water for their drinking water needs.

Note that categories for efficacy in microbial reduction are estimated by order-of-magnitude ($\log_{10}$) reductions of waterborne microbes. The categories are low ($<1 \log_{10}$ or $<90\%$ reduction), moderate (1 to 2 $\log_{10}$ or 90% to 99% reduction), and high ($>2 \log_{10}$ or $>99\%$ reduction). See Sobsey (2002).

Time Considerations

Using the specific *M. oleifera* protocol for coagulation, flocculation, and sedimentation (Basic Protocol 2) would take ~1.5 hr. Additional overnight time is required to implement Basic Protocol 1, and Support Protocol 1 requires 2 to 3 hr.

Literature Cited

Bergman, M. and Arnoldsson, E. 2008. Assessment of Drinking Water Treatment using *Moringa oleifera* Natural Coagulant—A Minor Field Study in Maputo, Mozambique. Masters Thesis. Water Resources Engineering, Lund University, Lund, Sweden.

Bratby, J., 2006. Coagulation and Flocculation in Water and Wastewater Treatment, 2nd ed. IWA Publishing, London.

Chen, S. and Ravallion, M. 2008. The Developing World is Poorer than We Thought, But No Less Successful in the Fight Against Poverty. The World Bank Development Research Group. Washington, D.C.

Diouf, M., Gueye, M., Faye, B., Dieme, O., and Lo, C., 2007. The commodity systems of four indigenous leaf vegetables in Senegal. *Water SA* 33:343-348.

Doerr, B., 2005. *Moringa* Water Treatment: ECHO Technical Note. Education Concerns for Hunger Organization (ECHO), North Fort Myers, Fla.

Dorea, C.C. 2006. Use of *Moringa spp.* seeds for coagulation: A review of a sustainable option. *Water Sci. Technol. Water Supply* 6:219-227.

Fahey, J.W. 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *J. Phytochem.* 47:123-157.

Folkard, G. and Sutherland, J. 2001. The Use of *Moringa oleifera* as a Natural Coagulant for Water and Wastewater Treatment. Department of Engineering, University of Leicester, U.K.

Fuglie, L.J. 2000. New Uses of Moringa Studied in Nicaragua. ECHO Development Notes #68. Educational Concerns for Hunger Organization (ECHO), North Fort Myers, Fla.

Ives, K. and Jahn, S.A.A. 1994. Coagulation and Flocculation. Small Community Water Supplies: Technology, people and partnership TP40_14: 296-312. IRC International Water and Sanitation Centre (IRC), The Hague, The Netherlands. *http://www.irc.nl/page/37789*.

Jahn, S.A.A. 1988. Using Moringa seeds as coagulants in developing countries. *J. Am. Water Works Assoc.* 80:43-50.

Kalibbala, H.M. 2007. Application of Indigenous Materials in Drinking Water Treatment. Royal Institute of Technology. Stockholm, Sweden.

Katayon, S., Megat Mohd Noor, M.J., Asma, M., Thamer, A.M., Liew Abdullah, A.G., Idris, A., Suleyman, A.M., Aminuddin, M.B., and Khor, B.C. 2004. Effects of storage duration and temperature of *Moringa oleifera* stock solution on its performance in coagulation. *Int. J. Engin. Technol.* 1:146-151.

Levicki, K. 2005. A Catchment to Consumer Approach to Rural Water Resource Assessment: Baseline Study and Safe Drinking Water Supply Strategy for Orongo Village, Lake Victoria Basin, Kenya. Royal Institute of Technology. Stockholm, Sweden.

Madsen, M., Schlundt, J., and Omer, E.F. 1987. Effect of water coagulation by seeds of *Moringa oleifera* on bacterial concentrations. *J. Trop. Med. Hyg.* 90:101-109.

Marobhe, N.J.M. 2008. Water Supply in Tanzania and Performance of Local Plant Materials in Purification of Turbid Water. Ph.D., Thesis. University of Stockholm, Sweden.

Olayemi, A.B. and Alabi, R.O. 1994. Studies on traditional water purification using *Moringa oleifera* seeds. *African Study Monographs* 15:135-142.

Narasiah, K.S., Vogel, A. and Kramadhati, N.N. 2002. Coagulation of turbid waters using *Moringa oleifera* seeds from two distinct sources. *Water Sci. Technol. Water Supply* 2:83-88.

Ndabigengesere, A.I. and Narasiah, K.S. 1996. Influence of operating parameters on turbidity removal by coagulation with *Moringa oleifera* seeds. *Environ. Technol.* 17:1103-1112.

Price, M. 1985. The Moringa Tree. ECHO Technical Note (revised 2000 by Kristin Davis). Educational Concerns for Hunger Organization (ECHO). North Fort Myers, Fla.

Schwarz, D. 2000. Water Clarification using *Moringa oleifera*. Gate Information Service, Eschborn, Germany.

Sobsey, M.D. 2002. Managing Water in the Home: Accelerated Health Gains from Improved Water Supply, prepared for the WHO Water Sanitation and Health Programme, Geneva, Switzerland.

Suarez, M., Haenni, M., Canarelli, S., Fisch, F., Chodanowski, P., Servis, C., Michielin, O., Freitag, R., Moreillon, P., and Mermod, N., 2005. Structure-function characterization and optimization of a plant-derived antibacterial peptide. *Antimicrob. Agents Chemother. Am. Soc. Microbiol.* 49:3847-3857.

Sutherland, J.P., Folkard, G.K., Mtawali, M.A., and Grant, W.D. 1994. *Moringa oleifera* as a Natural Coagulant. Affordable Water Supply & Sanitation: Proceedings of the 20th WEDC Conference, Colombo, Sri Lanka.

Taley, S. 2007. *Moringa oleifera* for Water Purification—a Traditional Boon for Rural Peoples. Stockholm Water Symposium, Workshop Poster. Stockholm, Sweden.

Yazdani, I. 2007. High Density Polyethylene (HDPE) Containers as an Alternative to Polyethylene Terephthalate (PET) Bottles for Solar Disinfection of Drinking Water in Northern Region, Ghana. Masters Thesis, Massachusetts Institute of Technology. Cambridge, Mass.

Key References

Jahn, 1988. See above.

Madsen et al., 1987. See above.

Dorea, 2006. See above.

The three references above provide practical information on Moringa and the treatment of water in developing countries.

Suarez, M., Entenza, J.M., Doerries, C., Meyer, E., Bourquin, L., Sutherland, J., Marison, I., Moreillon, P., and Mermod, N. 2003. Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities. *Biotechnol. Bioeng.* 81:13-20.

Suarez et al., 2005. See above.

The two references above describe leading-edge research.

Internet Resources

http://www.deutsch-aethiopischer-verein.de/Gate_Moringa.pdf

Gate Information Service. Provides the most comprehensive concise overview of water clarification using Moringa oleifera.

http://www.treesforlife.org/our-work/our-initiatives/moringa

Trees for Life International. A plethora of information including free access to Moringa books, posters, PowerPoint presentations and educational materials.

http://www.solutionexchange-un.net.in/environment/cr/res31010701.pdf

Eric Lemetais's comprehensive but brief overview of M. oleifera, including map, photographs of Moringa seeds, and before and after depictions of water treatment.

http://www.moringanews.org/index.html

Moringa News. An international network of people interested in M. oleifera. Their Web site offers reliable information and a platform to exchange knowledge.

http://www.lboro.ac.uk/well/resources/technical-briefs/60-water-clarification-using-moringa-oleifera-seeds.pdf

WELL: Resource Center Network for Water Sanitation and Environmental Health, Loughborough University, Leicestershire, U.K. This technical brief gives an overview of the application of an indigenous, naturally derived coagulant, namely seed material from the multi-purpose tree Moringa oleifera Lam. (M. oleifera) which offers an alternative solution to the use of expensive chemical coagulants.

http://www.echotech.org/network/modules.php?name=News&file=article&sid=863

Educational Concerns for Hunger Organization (ECHO). Provides basic overview of steps for water treatment with M. oleifera.

http://www.unep.or.jp/ietc/Publications/techpublications/TechPub-8a/natural.asp

United Nations Environment Programme. UN's Moringa Technical Brief.

http://www.farmradio.org/english/radio-scripts/54-11script_en.asp

Farm Radio International. This script for radio broadcasters in developing countries is about using seeds from the Moringa tree (Moringa oleifera) to clean dirty water.

http://tilz.tearfund.org/Publications/Footsteps+11-20/Footsteps+20/Moringa+oleifera+A+multipurpose+tree.htm

Tearfund International. Briefly describes M. oleifera multiple uses including household water treatment.

http://www.atatwork.org/page/363

AT@Work. This toolkit at link above explains the principles of doing business in poverty-stricken (less than $1 a day) areas, offers a step-by-step business development approach, and provides practical tools, tips and background information.

http://www.ifh-homehygiene.org/2003/2library/low_res_water_paper.pdf

Household Water Storage, Handling and Point-of-Use Treatment Report. A review commissioned by Internal Scientific Forum on Home Hygiene. This PDF paper reviews the range of simple, low-cost physical and chemical treatment methods and systems for safe water collection, handling, and storage, along with the evidence which shows the extent to which water can be the source of disease outbreaks, and how point-of-use treatment and safe water storage can reduce the burden of diarrheal and other waterborne diseases.

http://books.nap.edu/openbook.php?record_id=11763&page=247

National Academies Press. Moringa chapter from Lost Crops of Africa: Volume 2. Great descriptive overview of M. oleifera.

http://davesgarden.com/guide/pf/showimage/20749

Excellent photographs of mature Moringa seeds within the fruit pod. Other general photographs of nice quality are included.

http://www.moringanews.org/documents/WaterGB.pdf

An illustrative depiction of preparing Moringa oleifera seeds for water treatment. Though outdated, it explains the major steps nicely.

http://www.nesc.wvu.edu/pdf/dw/publications/ontap/2009_tb/jar_testing_DWFSOM73.pdf

National Environmental Services Center. Nice technical brief about jar testing. Includes "how to build a simple jar tester" schematic.

http://www.amazines.com/article_detail.cfm/592163?articleid=592163&title=Moringa%2CSajina%2Cphytichemicals%2Cplant%2Cextract

Article by Dewan Md. Badruddoza: Moringa oleifera: Its Phytochemicals and Their Usage. Crop Protection and Toxicology Laboratory, Dept. of Zoology, University of Rajshahi, Bangladesh.

http://en.wikipedia.org/wiki/Moringa_oleifera

Wikipedia entry for Moringa oleifera, accessed Nov. 24, 2008.