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Evaluation of the natural coagulant *Moringa oleifera* as a pretreatment for SODIS in contaminated turbid water

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ABSTRACT

Solar Disinfection of water (SODIS) is a treatment method that traditionally exposes low turbidity water filled in clear bottles to direct sunlight up to 6 h. Typically, water should have turbidity lower than 30 NTU before solar exposure; however turbidities of water sources in communities vary and can reach higher than 200 NTU. In order to reduce turbidity, flocculating agents like Moringa oleifera (Moringa) may be used. In this study we assess the efficacy of Moringa to clear turbid water as a pretreatment for SODIS. We initially evaluate two preparations– powdered seeds and an aqueous filtrate of the seeds, to determine if these can benefit SODIS in turbid, *E. coli* contaminated water (Experiment 1). We show that powdered Moringa seeds reduce turbidity best and that SODIS treatment of highly turbid water was effective regardless of reduced turbidity. Overnight, however; a bio-active sludge layer formed. We then determined if 24 h Moringa pretreatment and decanting can maintain water quality over an extended period (Experiment 2). After 24 h Moringa treatment showed a 2.1 log reduction in *E. coli*, increasing following SODIS (6-log) *E. coli* without nightly recovery or sludge formation. Untreated turbid controls showed SODIS disinfection after 6 h direct sunlight; however, nightly regrowth and sludge layer formation occurred by 48 h. These results suggest that SODIS is capable of inactivating bacteria in highly turbid water at 6 h; however, active biofilm sludge layers formed by 48 h. We conclude that, for longer term water storage, we find a combination of Moringa seed powder pretreatment prior to SODIS to be optimal.

1. Introduction

Solar water disinfection (SODIS) is a method that relies on the bactericidal properties of solar radiation to disinfect contaminated water. Water to be treated is filled into transparent containers such as polyethylene terephthalate (PET) beverage bottles and exposed to sunlight for up to 6 h (McGuigan et al., 1998). SODIS has been proven effective on a wide range of water borne disease associated pathogens including *E. coli*, *E. faecalis*, *Shigella dysenteriae*, etc. (Berney et al., 2006; Keogh et al., 2015; McGuigan et al., 2012). Field data in various countries, from urban slums in India to rural communities in Kenya have shown health benefits and protection from conditions such as cholera and diarrhea following consumption of SODIS treated water (Conroy et al., 2001; du Preez et al., 2011; Rose et al., 2006). While a proven and cost-effective method of treating water in poor and rural communities, the efficacy of SODIS is dependent on factors including the source water's turbidity (Sommer et al., 1997). Turbid water contains dissolved and suspended organic materials which are assumed to block the efficient penetration of sunlight through the water volume (Joyce et al., 1996; Sommer et al., 1997). It is hence recommended that the turbidity of water to be treated by SODIS not exceed 30 NTU (Meierhofer and Wegelin, 2002). However, besides being microbiologically contaminated, the turbidity of unimproved water sources can rise higher than 200 NTU depending on factors such as weather, time of collection and the surrounding environment (Joyce et al., 1996). Methods to reduce turbidity can include filtration, gravity settling and coagulation by chemical or natural flocculants such as those produced by the seeds of the *Moringa oleifera (Moringa)* tree.

The seeds of Moringa, a tree which grows across the tropical belt, contain a potent natural coagulant which has long been utilized by indigenous communities to clarify muddy water before human use (11). The water soluble extract of the seeds contains a cationic protein which

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has been proven to have a dramatic coagulation effect on suspended and dissolved particles in highly turbid water (Ndabigengesere et al., 1995). The costs and irregular accessibility that can accompany the use of mechanical filtration apparatuses and chemical coagulants like alum make *Moringa* an attractive method of clarifying turbid water. Treatment with *Moringa* has a minimal effect on the pH, alkalinity and conductivity of the water being treated (Ndabigengesere and Narasiah, 1998). Extracts of the seeds have also been shown to have anti-microbial properties on a number of pathogenic bacteria including *E. coli* (Fernandes Vieira et al., 2010; Jabeen et al., 2008). The majority of people dependent on unimproved water sources are concentrated in rural communities of Sub-Saharan Africa and parts of Asia (16) and moreover, there is a sizable overlap between these countries and ones where *Moringa* is or can be effectively cultivated (17). Apart from the seeds' water clarifying properties, the trees are a source of leaves, vegetable oil and pods suitable for animal and human consumption and can also be grown as fences and windbreakers around properties (Lea, 2010). Taken together, these factors make *Moringa* a useful asset in resource limited communities as it can provide beneficial uses on several levels.

Providentially, a number of countries dependent on unimproved water sources also lie in a broad belt around the equator which is the region that receives high solar radiation around the year and is thus thought to be most suitable for SODIS (Meierhofer and Wegelin, 2002). Wilson and Andrews examined the use of *Moringa* in combination with SODIS to treat low turbidity (~1.7 NTU), highly coloured water. They reported a 1 log reduction in inoculated *E. coli* due to the coagulation step alone. Following 6 h of SODIS treatment, there was an overall inactivation of bacteria with no regrowth of *E. coli* detected after dark storage (Wilson and Andrews, 2011). Given that source water turbidities in communities can increase to above 200 NTU, in this study we evaluated the effectiveness of *Moringa* flocculation and sunlight as a pretreatment option for SODIS in highly turbid, coliform contaminated water. In our first experiment, we compared the action of powdered *Moringa* seeds and seed filtrate to determine which preparation gives better turbidity removal. Along with addition of *Moringa,* we chose to simultaneously expose the water to sunlight as part of pretreatment postulating that this would have an added microbicidal effect on the bacterial concentration while coagulation was achieved. Since communities may consume SODIS treated water several h after solar exposure (Asiimwe, 2013; Vivar et al., 2017), our second experiment aimed to evaluate long term efficacy and regrowth in SODIS treatment of turbid water and pretreated water.

2. Methods

2.1. Experiment 1: Examination of the flocculation action of Moringa powdered seeds

2.1.1. Turbid water preparation

Deionized water was mixed with commercially available terracotta modelling clay (Jovi, Spain) to produce water of desired turbidity 200 ± 5 NTU. Prepared turbid water was sterilized; filled into new, clean 1.5 L polyethylene terephthalate (PET) bottles and turbidity measured just before coagulant addition and solar exposure. Turbidity was measured using a digital turbiditimeter -HI98703 Precision Turbidity Portable Meter (Hanna Instruments, USA).

Moringa powder (MO_powder) preparation - The brown seed coats were removed and the white kernels crushed in a mortar and pestle. The resultant white seed powder was sieved through a tea strainer and the fine seed fraction kept in an air-tight container until needed.

Moringa filtrate (MO_filtrate) preparation - The coagulant was prepared based on the Basic Protocol outlined by Lea (2010). The concentrations of seed powder to be used for our 1 L reaction bottles was determined by a preliminary flocculation assay and the filtrate was passed through a filter paper instead of a muslin cloth. Briefly, for making the MO_filtrate, batches of fine seed powder were each agitated in 10 mL of sterile water for 5 minutes. The suspensions were allowed to stand for 10 minutes before being filtered.

2.1.2. Bacterial culture

Bottles were inoculated with *E. coli* strain ATCC 25922 from stock cultures made from 1 CFU grown in 15 mL of Luria broth (LB) nutrient medium (Sigma–Aldrich, USA) incubated overnight at 37 °C with constant agitation under aerobic conditions. Appropriate dilutions were made directly into the bottles to achieve the initial bacterial concentration of 10^5 CFU mL^{-1}.

2.1.3. SODIS set up

A preliminary bench top *Moringa* flocculation assay was carried out to determine the minimum concentration of coagulant that would reduce the turbidity of 200 NTU water to below 30 NTU. For *Moringa* filtrate (MO_filtrate), 300 mg of the powdered seeds was needed per litre of turbid water. For the Moringa powdered seed (MO_powder) this was found to be 200 mg/L.

12 clean 1.5 L PET bottles were filled with 1 L of sterile, 200 NTU turbid water and inoculated with *E. coli* (~2×10^5 CFU/mL). Coagulant addition groups with solar exposures in triplicate and one indoor control each are summarized in Table 1.

Samples were taken for microbial analysis at 0 and 6 h after the start of solar exposure. Turbidity measurements were carried out 0, 6 and 24 h after the start of exposure. Hourly temperature was recorded using standard mercury thermometers.

2.2. Experiment 2: Effect of a 24 h Moringa seed powder treatment prior to SODIS

2.2.1. Bacterial culture

Bottles were inoculated with *E. coli* strain ATCC 25922 from stock cultures of 1 CFU grown in 15 mL of Luria broth (LB) nutrient medium (Sigma–Aldrich, USA) and incubated overnight at 37 °C with constant agitation under aerobic conditions. Appropriate dilutions were made directly into the bottles to achieve the initial bacteria concentration of 10^6 CFU mL^{-1}.

2.2.2. Pretreatment

Preparation of *Moringa* seed powder and bacterial enumeration were same as in Experiment 1. Briefly, clean 1.5 L PET bottles (n = 3, 1 indoor control) were filled with 1.15 L of sterile, 200 NTU turbid water and inoculated with *E. coli* strain ATCC 25922 (~2×10^6 CFU/mL). *Moringa* seed powder (200 mg/L) was added to each bottle and agitated several times. Test bottles were allowed to settle undisturbed overnight with solar exposure for the first 6 h. The indoor control was held in the dark at room temperature (21 °C).

2.2.3. Solar exposure

Following the MO_powder 24 h pretreatment, the clear supernatant layer was decanted into fresh, clean bottles (MO_decant). At the same time, 1 L of 200 NTU water bottles (n = 3, 1 indoor control) were inoculated with *E. coli* (~2×10^6 CFU/mL).

Table 1
Experiment 1 - Demographic data for Hourly solar radiation (HSR), Total accumulated solar energy dose (TAE), temperature and turbidity over the 6 h of solar exposure.

Experiment 1 (day 1: 0–6 h)			
TAE	13064.58 kJ/m^2		
Highest HSR	742 W/m^2 at 3 h		
Mean temperature (°C)	*Moringa* seed powder (MO_powder)	*Moringa* seed filtrate (MO_filtrate)	Turbid control No coagulant
Solar exposure	36.3	37.6	37
Indoor Controls	21.5	21.5	21.5