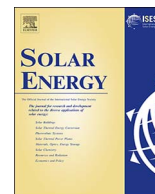


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Evaluation of the natural coagulant *Moringa oleifera* as a pretreatment for SODIS in contaminated turbid water



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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 micro-biologically 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.

Provisionally, 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 turbidimeter -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* ($\sim 2 \times 10^5$ 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 ($\sim 2 \times 10^6$ 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* ($\sim 2 \times 10^6$ 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)	<i>Moringa</i> seed powder (MO_powder)	<i>Moringa</i> seed filtrate (MO_filtrate)	Turbid control No coagulant
Solar exposure	36.3	37.6	37
Indoor Controls	21.5	21.5	21.5

These, along with the MO_decant bottles were set up in an area of direct sunlight for 48 h and corresponding indoor control was kept in the dark.

Samples were taken at 0, 6, 24, 30 and 48 h after the start of solar exposure. Turbidities and bacterial cell number was recorded by drop plating as described above. Hourly temperature during solar exposure (0–6 h and 24–30 h) was also recorded. Temperature was measured using a hand-held infrared thermometer (RadioShack, USA). Turbidity was measured using a digital turbidimeter -HI98703 Precision Turbidity Portable Meter (Hanna Instruments, USA).

2.3. Solar radiation

Experiment 1 was carried out on the 30th of October 2015. Experiment 2 was carried out on November 27th, 2015. Solar radiation data was obtained from the Meteorological Directorate, Ministry of Transportation, Kingdom of Bahrain (Latitude 26°16'N, Longitude 50°39'E). The below equation was used to calculate the total accumulated solar energy dose received per unit of illuminated surface where t_n is the experimental time for n -sample and \overline{SR}_{n-1} is the average solar radiation measured during the period (t_n to t_{n-1}). Accumulated dose was calculated as $\sum_n \overline{SR}_{n-1} \cdot (t_n - t_{n-1})$ in accordance with previous publications (Keogh et al., 2015).

2.4. Bacterial enumeration

Colony counts were carried out using 10-fold serial dilutions of the water samples in LB broth. Volumes of 10 μ L in triplicate were drop plated on Luria agar (Sigma–Aldrich, USA). Colony forming units (CFU) were counted after overnight incubation at 37 °C. Number of CFUs was divided by the number of drops plated and then adjusted according to the dilution to convert to CFU/mL (Miles et al., 1938).

3. Results

3.1. Experiment 1

The demographic characteristics of hourly solar radiation (HSR), total accumulated solar energy dose (TAE) and temperature changes for exposure during Experiment 1 are summarized in Table 2. The radiation profile over the 6 h of exposure was maintained above 500 W/m² for around 5 out of 6 h. There was no marked difference in temperatures between the three conditions during the sunlight exposure time. The addition of the seed powder showed a clear turbidity reduction and over 24 h brought average turbidity of the supernatant to 28.5 NTU (~85% reduction). There was an overall ~47% reduction in turbidity (201–109 NTU) over 24 h under coagulation with the seed filtrate. The supernatant turbidity of the outdoor control bottles that had no coagulant treatment had fallen to 121.3 NTU due to gravity settling alone (Fig. 1).

SODIS caused significant decrease in bacterial concentrations in *Moringa* treated and untreated turbid water following 6 h of sunlight exposure whereas the indoor controls that had received no sunlight had an increased bacterial load in the supernatant.

Table 2
Setup of coagulant addition groups with and without solar exposure.

	Solar Exposure	Indoor control
MO_powder	<i>Moringa</i> fine seed powder added to turbid water + sunlight exposure.	<i>Moringa</i> fine seed powder added to turbid water + dark storage.
MO_filtrate	<i>Moringa</i> seed filtrate added to turbid water + sunlight exposure.	<i>Moringa</i> seed filtrate added to turbid water + dark storage.
No coagulant	Untreated turbid water + sunlight exposure	Untreated turbid water + dark storage

In the case of the MO_powder + SODIS group, the supernatant layer showed > 3 log decrease in microbial concentration (Fig. 2) but the settled sludge layer was heavily contaminated (data not shown). The turbid water solar exposure bottles that had no coagulant added showed no viable bacteria in the supernatant at the end of the 6 h solar exposure period (Fig. 3). There was no stable sludge layer formed in the MO_filtrate and No coagulant bottles at Hour 6 hence samples were not collected from this fraction. We categorized the sludge layer formed under each of the 3 coagulant addition groups after overnight settling as loose (+), medium (++) or heavy (+++) based on its ability to remain at the bottom of the bottles upon tilting without re-mixing into the supernatant layer. For the MO_filtrate and No coagulant bottles a loose layer of settled particulate matter was observed (+). The sludge layer formed in the MO_seeds group was thicker and stable upon tilting (+++).

3.2. Experiment 2

The demographic characteristics of hourly solar radiation (HSR), total accumulated solar energy dose (TAE), turbidity and temperature changes for exposure during Experiment 2 are summarized in Table 3. The radiation profile over the 6 h of exposure was maintained above 500 W/m² for around 4 h out of 6. The turbid SODIS group had slightly higher average temperature over the 2 day time period compared to the clear MO_decant group.

The MO_decant clear water poured into fresh bottles after 24 h of *Moringa* powder pretreatment showed an ~87% decrease in turbidity (26 NTU, average of $n = 3$). The turbidity remained below the 30 NTU recommended SODIS limit for the duration of exposure. The turbidity of the non-pretreated turbid water was maintained at ~200 NTU.

The clear decanted layer also showed an overall 2.1 log reduction from the initially inoculated bacterial load; the indoor MO_decant control showed a 1 log reduction. Turbid water controls that received no pretreatment showed no detectable bacteria (6 log reduction) at Hour 6 but nightly regrowth was detected up to Hour 48. The pretreated water had an overall 6 log decrease in bacterial concentration and showed no nightly recovery of bacteria for up to 48 h following SODIS (Fig. 4). The indoor, No-coagulant control did not show any appreciable decrease in bacterial load over the 48 h.

4. Discussion

SODIS in 1–2 litre PET bottles has typically been used to treat clear water and this study aimed at investigating the effect of a natural coagulant *Moringa* to reduce turbidity prior to SODIS disinfection. *Moringa* seeds are an attractive natural turbidity removal alternate to membrane filtration devices and chemical coagulants like alum in that apart from removing the need for purchasing synthetic coagulants and mechanical setups, this method is already known and practiced in several communities throughout Asia and Africa. Considering the simplicity and cost effectiveness of *Moringa* for turbidity removal and SODIS for water disinfection, these methods could form an effective way of families in resource poor settings to obtain drinking water on a day to day basis.

In our first experiment, the *Moringa* seed powder proved more effective at reducing water turbidity than the seed filtrate which has conventionally been studied in laboratory based coagulation experiments (Ndabigengesere and Narasiah, 1998; Ndabigengesere et al., 1995; Wilson and Andrews, 2011) (Fig. 1). Apart from achieving overall ~85% reduction in turbidity, the powder had a shorter preparation time and fewer steps than were required for the filtrate. The seed filtrate did not remove turbidity as well as was seen in previous studies (Sarpong and Richardson, 2010) as well as in our preliminary tests. This might possibly be due to the shape and dimensions of the 1.5L PET bottle (which are the preferred vessels for SODIS) differing from the laboratory beakers used in the benchtop tests. Following overnight

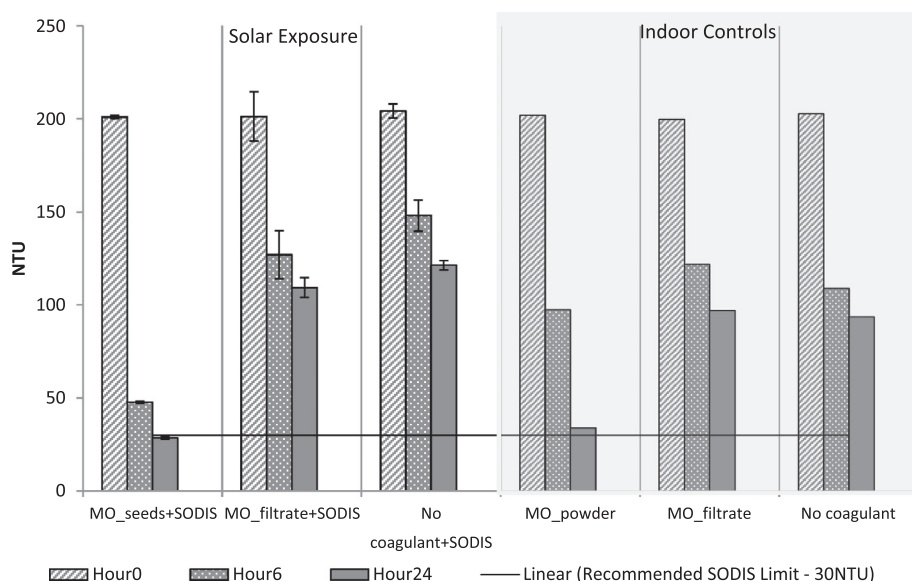


Fig. 1. Changes in average water turbidities with and without the addition of coagulant post 6 h SODIS. Each bar in the unshaded region indicates the average (n = 3) of supernatant turbidities from bottles placed in sunlight. Each bar in the grey shaded region indicates indoor, dark controls. The horizontal line represents the recommended limit for water turbidity in SODIS (30 NTU). MO_powder- Fine seed powder used as coagulant. MO_filtrate-Seed filtrate used as coagulant.

MO_powder	MO_Filtrate	No coagulant
Supernatant layer	Supernatant layer	Supernatant layer
28 NTU	109 NTU	121 NTU
Settled layer (+++)	Settled layer (+)	Settled layer (+)

Fig. 2. Experiment 1 – Overnight flocculation and accompanying turbidity changes with and without the addition of *Moringa* coagulant. The MO_powder treated water has a well settled sludge layer (+++) stable and clearly visible. The MO_filtrate and No coagulant groups have a loose and unstable layer of settled particulate matter (+).

settling, the thin particulate layer formed in the filtrate treated and the ‘No coagulant’ bottles was unstable and mixed back into the supernatant layer when bottles were tilted. In contrast, the powder forms a well settled layer of sludge at the base of the pretreatment bottles and due to this; we found there was minimal back mixing when decanting the supernatant layer into fresh bottles in Experiment 2 (Fig. 5).

In Experiment 1, 6 h microbial analysis of the bottles that had seed powder added indicated inoculated bacteria had settled into the sludge layer and solar radiation had reduced the fraction of viable cells left in the clear supernatant. In bottles exposed with seed filtrates as well as those without coagulant addition, we observed no viable bacteria in the supernatants following 6 h of SODIS, with water having turbidity significantly above the recommended 30 NTU SODIS threshold (Meierhofer and Wegelin, 2002) (Figs. 1 and 3). This is similar to extended exposure, strong sunlight SODIS results in turbid water reported elsewhere (Kehoe et al., 2001; Vivar and Fuentes, 2015) and is a result that merits further study as it might be that the inactivation of pathogens in turbid water (> 30 NTU) is possible within the timeframe of a conventional SODIS run (5–6 h) and

without the need for harsh radiation and temperature exposure. From our results in Experiment 1, it would appear there is no total benefit to pre-treating water to remove turbidity prior to SODIS as no viable bacteria were seen in turbid water post 6 h of solar exposure.

However, our samples were drawn from the supernatant without agitation so as to accurately gauge the decreasing turbidity and 24 h after the start of solar exposure, we did observe a loose, unstable layer of settled particulate matter at the base of the bottles (Fig. 2). This could potentially shield bacteria from being inactivated by sunlight by absorbing or scattering UV rays thus hindering the effective penetration of radiation (Qualls et al., 1983). This layer of organic matter might also provide the means for injured cells to recover and multiply during the night. In several communities, the practice is to expose the water early in the morning and then collect and consume the water from the SODIS treated bottles the next day while setting out the fresh batch of bottles for exposure (Asimwe, 2013; McGuigan et al., 2011). This indicates that the need for pretreatment may arise in communities where turbid SODIS treated water is used the day after exposure. Hence, in

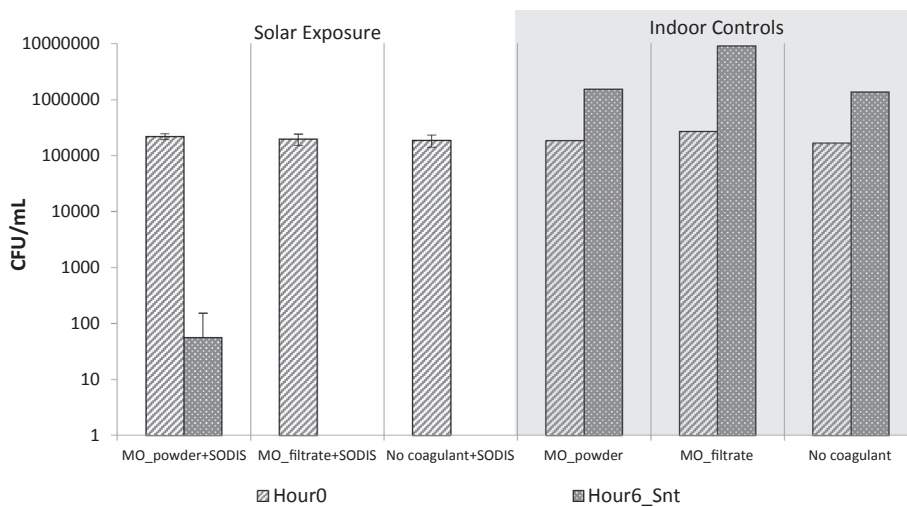


Fig. 3. Changes in viable *E. coli* concentrations in bottles over time with and without the addition of coagulant, post 6 h SODIS. Each bar in the unshaded region indicates the average CFU/mL (n = 3) from supernatants from bottles placed in sunlight. grey shaded region indicates the concentration in indoor, dark controls. MO_powder- Fine seed powder used as coagulant. MO_filtrate- Seed filtrate used as coagulant.

Table 3
Experiment 2 - Demographic data for hourly solar radiation (HSR), total accumulated solar energy dose (TAE), temperature and turbidity over the hours of solar exposure.

Mean temperature (°C)	Decanted pretreated water	Turbid control
Solar exposure	33.5	34.5
No solar exposure	21.2	21.2
Turbidity NTU (24 h)	27.8	199.5
Turbidity NTU (30 h)	26.6	202.3

Experiment 2, we sought to look for and compare the regrowth characteristics of bacteria in *Moringa* pretreated and non-pretreated turbid water. Given that the seed powder showed better turbidity removal compared to the filtrate, we decided to use this preparation for coagulation in the second experiment. In particular, we wanted to gauge whether a decanting step following *Moringa* pretreatment would benefit SODIS treatment by removing the settled particulate layer seen in Experiment 1 which could potentially harbor viable bacterial cells causing overnight regrowth.

In Experiment 2, we observed a 2.1 log reduction in bacterial concentrations owing to the combined seed powder addition and solar exposure pretreatment step. The control which received the *Moringa* powder but no sunlight showed a 1 log reduction which is in line with the decrease reported elsewhere through *Moringa* addition (Wilson and Andrews, 2011).

At the end of 6 h of SODIS, samples were collected after agitation.

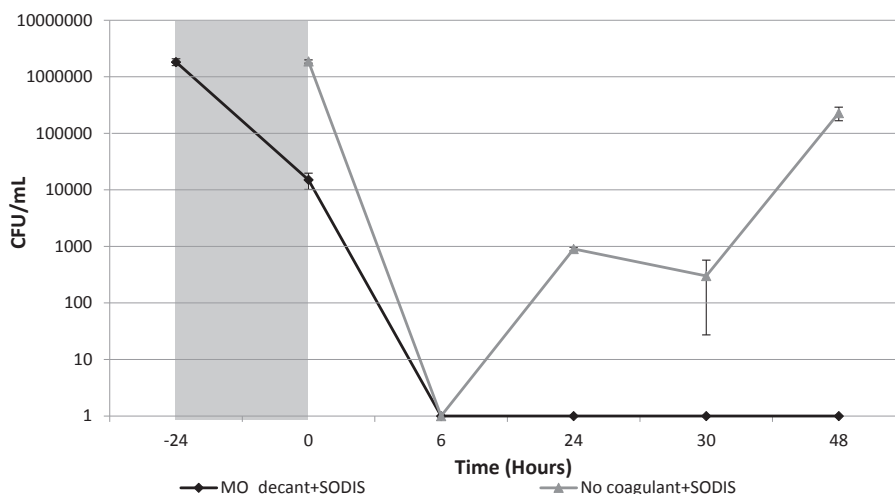


Fig. 4. Experiment 2 - Changes in viable *E. coli* concentrations over pretreatment and 2 days of SODIS. Grey shaded area represents pretreatment time (-24 to 0 h). The unshaded area represents outdoor exposure times (sunlight exposure: 0–6 h & 24–30 h). The black curve represents *E. coli* CFU/mL in turbid water pretreated with *Moringa*. The grey curve represents changes in *E. coli* CFU/mL in turbid water. MO_decant - Clear water decanted following *Moringa* pretreatment. No coagulant - Untreated turbid water.

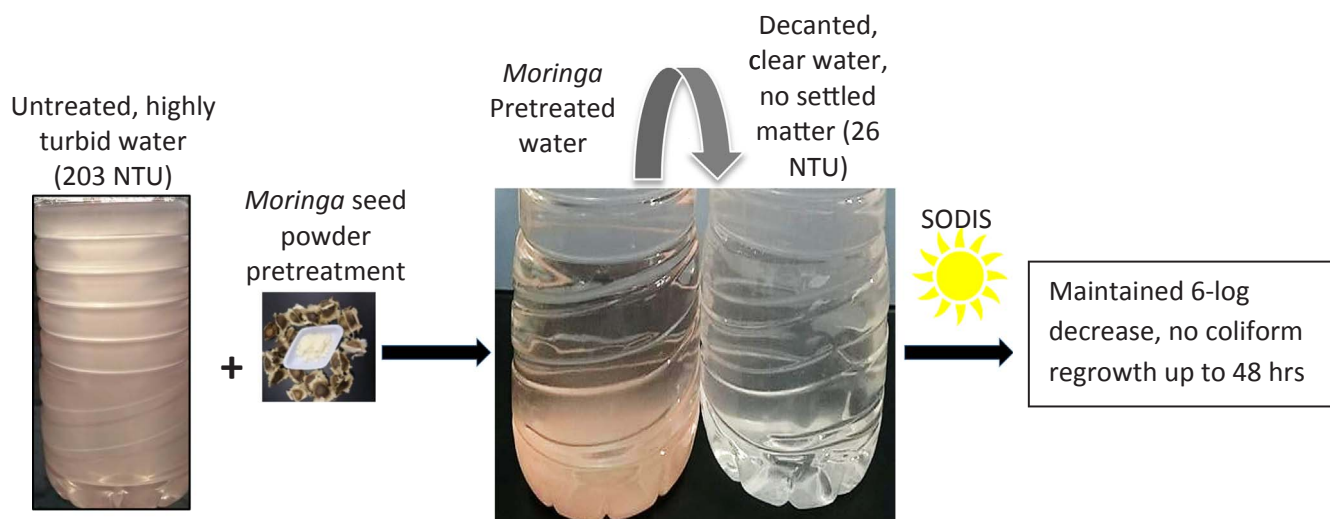


Fig. 5. Experiment 2 – Clear supernatant obtained after *Moringa* flocculation and decanting into fresh bottles. Following 24 h of untreated turbid water (203 NTU, $n = 3$) settling with *Moringa* seed powder, the clear supernatant layer was decanted into fresh bottles (26 NTU, $n = 3$) before solar exposure. The decanted water showed no sludge formation.

for a full 6 h. The clear water layer decanted following pretreatment with *Moringa* showed no settled particulate matter and no nightly regrowth of bacteria indicating total inactivation (Figs. 4 and 5).

Based on the data from the indoor controls, all of which showed viable bacteria after prolonged contact with *Moringa*, we did not observe any antimicrobial action due to the addition of *Moringa* seed powder or the filtrate (Fig. 2). Previous studies have described the biocidal properties of the seeds but have done so at much higher concentrations (extracts were prepared from up to 200 grams of seeds/litres of solvent) than those we used in our coagulation (Fernandes Vieira et al., 2010). These studies have also used various extraction and purification protocols to concentrate the seeds' antimicrobial agent rather than simple addition of freshly ground seed powder directly into contaminated water as we have done (Jabeen et al., 2008).

Communities vary and field studies in populations dependent on turbid water sources are needed before establishing the feasibility of *Moringa* seeds as a pretreatment option for SODIS. It should be noted that the addition of any steps to a water treatment method poses risks of affecting user compliance due to the added time and effort involved. Previous work that considered *Moringa* as a pretreatment for SODIS concluded that any time gained from clarifying the water before SODIS was lost due to the time needed to complete coagulation to a satisfactory level before initiating solar exposure. With pretreatment performed on the morning of any given day of SODIS treatment, the preparation of the coagulant and the coagulation steps together may take up to 3 h of the day apart from the 6 h required for SODIS (Wilson and Andrews, 2011).

The addition of *Moringa* seed powder over 24 h followed by a decanting step prior to SODIS gave best turbidity removal results and also prevented regrowth after SODIS treatment. Overall, our results are encouraging as we show the use of the seed powder is an effective method of obtaining low turbidity water as well as enhancing the long term efficacy of SODIS. In communities dependent on turbid water, where *Moringa* trees are available or can be conveniently planted, the powdered seeds can provide an excellent means of clarifying turbid water before disinfection by SODIS. Both these methods are sustainable and cost effective and can thus significantly contribute to fulfilling the day to day water requirements of users in the field.

5. Conclusions

- *Moringa* seed powder reduces turbidity better than seed filtrate; bringing turbidity to below the 30 NTU recommended SODIS level.

- SODIS is effective in turbid water above 30 NTU up to 6 h; however, nightly recovery of bacteria can occur.
- Pretreatment of turbid water by solar exposure and settling with *Moringa* seed powder for 24 h followed by a decanting step prior to SODIS produces clear water and this removes the risk of regrowth.
- The use of the seed powder as a pretreatment helps enhance the efficacy of SODIS and may benefit communities dependent on turbid water sources as the quality of treated water is sustained over several days.

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