

Impact of a natural coagulant pretreatment for colour removal on solar water disinfection (SODIS)

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ABSTRACT

Solar water disinfection (SODIS) is the process of treating microbiologically contaminated water in clear plastic bottles through exposure to sunlight. One of the major limiting factors of this treatment is source water quality. This work investigates the impact of source water colour on SODIS efficiency and evaluates a natural coagulant for colour removal. The ability of *Moringa oleifera* seed emulsion to both clarify and decolourize source waters was investigated as a coagulation pretreatment for SODIS. This coagulant reduced the colour by more than two-thirds and achieved up to 1-log₁₀ bacterial removal (90%). The combined *Moringa oleifera* coagulation-SODIS treatment sequence was tested in highly coloured natural source water and was found to reduce the sunlight exposure time required by up to 2 hours. However, despite being an effective clarification and decolouring process, the pretreatment may not shorten the overall treatment time because of its own labour and time requirements, potentially decreasing the treatment compliance rates. In addition, while total coliform and heterotrophic bacteria regrowth was observed during overnight storage of the treated water, no *Escherichia coli* regrowth was found to occur.

Key words | drinking water, *E. coli*, *Moringa oleifera*, point of use, pretreatment, solar disinfection

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INTRODUCTION

In regions throughout Africa, Asia and Latin America people living in extreme poverty in rural and peri-urban communities are often forced to rely on pathogenic surface water sources for their domestic water. Surviving through subsistence living, little can be spared for obtaining safe potable water. Under such circumstances, low-cost household (point-of-use) water treatment solutions are vital for the general health and well-being of a community. One of these household treatments is solar water disinfection (SODIS), whereby contaminated water is treated in discarded plastic bottles through exposure to sunlight (Sobsey 2002; Sobsey *et al.* 2008; Rosa & Clasen 2010). Although there is very little cost associated with this treatment technique, its efficacy is dependent on numerous factors such as: availability of discarded bottles, appropriate weather conditions and the source water quality.

Poor source water quality (i.e. highly turbid and/or coloured water) can render solar disinfection ineffective as

a treatment process (Joyce *et al.* 1996). In response to the issue of source water quality dependence, the Swiss Federal Institute for Aquatic Science and Technology (EAWAG) and other SODIS researchers (McGuigan *et al.* 1998) have recommended applying a SODIS-pretreatment such as: sedimentation (overnight settling), pre-filtration or the application of a coagulant such as alum or a natural flocculating agent. The aim is to reduce the turbidity level to below 30 NTU (nephelometric turbidity unit) to increase penetration of sunlight further into the water, and to remove particle-associated pathogens. However, particles are not the only impediments to solar penetration. Dissolved material, such as that which is perceived as colour, also absorbs significant amounts of sunlight. Such dissolved material must be precipitated before it can be removed either by settling or by filtration.

While suspended particles may settle out of the water matrix if the water is allowed to sit undisturbed for a

period of time (i.e. overnight), little dissolved organic matter (colour) will be removed through sedimentation. Alternatively, pre-filtration has the potential to remove both suspended solids as well as some dissolved organic matter depending on the type of filter media used. However, often dissolved components, such as natural organic matter (NOM), will pass through the filter resulting in a coloured filter effluent. This suggests that when dealing with a highly coloured source water, a coagulation pretreatment may be the best option for removing colour prior to disinfecting the water via SODIS.

The limited accessibility and high costs associated with conventional coagulants (alum, ferric chloride) have led to the use of locally available natural coagulants, such as *Moringa oleifera* (Moringa) seeds, as a point-of-use treatment in developing nations. Moringa seed is native to the sub-Himalayan areas of Agra and Oudh in north-western India, and is now cultivated worldwide throughout the semi-arid tropical regions surrounding the equator (Jahn 1986).

This research aims to evaluate the use of a natural coagulant as a pretreatment for SODIS, and confirm whether this coagulation-SODIS treatment sequence should be recommended as a household water treatment in the field. Although the ability of the Moringa seed coagulant to aid in the removal of suspended particles is well known, testing has been done to estimate its ability to also remove colour and UV-absorbing components, such as dissolved organic matter (DOM), from water because of their additional impact on sunlight transmission and SODIS efficiency (Muela *et al.* 2000; Cantwell *et al.* 2008).

METHODS

Natural source water

The natural source water was obtained from a pond in the Muskoka District Municipality of Ontario, Canada. This water was chosen based on its relatively high amount of colour (239 to 297 Pt-Co units). Water was obtained on 27 June 2010, and transported to a laboratory refrigerator where it was stored at 4°C until tested. The experiments were performed within 3 weeks of collecting the source water.

Although Moringa seed emulsion solids (MSES) has previously been used for turbidity removal, in order to more clearly determine its impact on colour removal, tests reported here were done on a low turbidity, high colour natural water. Tests done with high turbidity waters may be found in a report by Wilson (2010).

Coagulant preparation

Moringa oleifera (Moringa) seeds were sourced from an American seed supplier (Seedman.com, Gautier, MS) who distributes Moringa seeds from India. The procedure for processing the seeds was adapted from Samia al Azharia Jahn's publication on African natural coagulants (Jahn 1986). This literature describes the traditional use of the seeds as a coagulant in Sudanese communities who source their drinking water from the Nile River. The main deviation in this research from the procedure presented by Jahn (1986) is in filtering the coagulant suspension through filter paper prior to coagulant dosing, as opposed to using cloth or a mesh filter as would be used in the field.

The brown seed coat of the Moringa seeds was removed and the white kernels were dried overnight in a desiccator. The dried seeds were crushed using a mortar and pestle, and added to 10 ml of deionized water per seed crushed (10 ml is approximately equal to 1 soup spoon). This mixture was stirred for 30 min and then filtered through coarse (P8) filter paper (Fisher Scientific, Ottawa, Ontario). The filtrate coagulant suspension contained 3.5–5.5 g l⁻¹ of total solids (Moringa seed) and had a pH of 4. A fresh batch of coagulant was prepared the day before each experiment and stored at 4°C overnight.

Jar tester and PET bottle preparation

Discarded 500 ml PET (polyethylene terephthalate) drinking water bottles, without scrapes or dents, were collected. These bottles, as well as the jar tester (Phipps & Bird, Richmond, VA) beakers and paddles were filled with 100 mg l⁻¹ sodium hypochlorite (Sigma-Aldrich Inc., St Louis, MO) solution and soaked overnight for disinfection purposes. They were then thoroughly rinsed with deionized water (5 rinses) immediately prior to being filled with the source water. The same beakers and water bottles were used

repeatedly for each experiment, with the disinfection procedure conducted prior to each experiment.

Bacterial preparation

An *Escherichia coli* culture (ATCC[®] 11229) was grown from a seed culture purchased from Cedarlane Laboratories (Burlington, Ontario) and stored at -80°C with 10% sterile glycerol according to Standard Method 9224-B (*Standard Methods* 2005). This specific strain of faecal coliform indicator microorganism had been used in previous Canadian SODIS studies (Shah *et al.* 1996; Hirtle 2008). The evening prior to an experiment a portion of the *E. coli* stock culture was thawed and cultured in a 1:10 ratio of stock culture to tryptic soy broth (TSB) for 16 h at $37 \pm 0.5^{\circ}\text{C}$. It was assumed that these cells had reached their stationary growth phase after 16 h (personal communication with M. Mesquita, 2009, University of Waterloo, Ontario). The cells were washed in sterile phosphate buffer saline (PBS) solution (centrifuged at $10,000 \times g$ for 10 min) three times before being added to specific source waters.

Bacterial enumeration

The microbial content of water samples was analysed by the membrane filtration method using differential coliform (DC) agar (according to Ontario Ministry of the Environment Method #E3407 and USEPA Method #1604 [USEPA 2002]) in order to monitor the *E. coli* and total coliform (TC) concentrations. The standard pour plate method using R2A agar (Becton, Dickinson and Company, Sparks, MD) was used for measuring the heterotrophic plate count (HPC) bacteria concentrations. Tenfold sample dilutions were made into sterile PBS (in duplicate) in order to plate concentrations within the target range of 10 to 100 colony forming units (CFUs) per plate of DC agar and 20 to 200 CFU per plate of R2A agar. The duplicate DC and R2A agar plates were cultured for approximately 22 and 36 h, respectively, at $37 \pm 0.5^{\circ}\text{C}$ before enumeration and averaging. On the DC agar plates the *E. coli* coliforms were observed as blue dot colonies whereas other coliforms were grown as orange/red colonies. The TC concentrations were determined by summing the *E. coli* and 'other' coliform counts together. All dilutions and plating procedures

were conducted inside an operating biosafety cabinet (Contamination Control Inc., Kulpsville, PA), following standard sterile techniques, in order to prevent sample contamination.

Experimental setup

A preliminary coagulation experiment determined the optimal coagulant dose, which would result in the largest decrease in colour (measured at 455 nm) without adding more organic carbon than necessary, to be 250 mg l^{-1} of MSES. A fresh batch of coagulant suspension was prepared and the source waters to be pretreated were dosed at the optimal amount of 250 mg l^{-1} of MSES.

Six waters were tested during each experiment, and the experimental design is outlined in Table 1. The experiment was repeated on 10, 14 and 22 July 2010. The solar irradiance experienced during the SODIS treatment was measured at hourly intervals during the experiments on 10 and 22 July using a thermopile sensor (200–2,000 nm) with a IL1700 radiometer (International Light Technologies, Peabody, MA).

Waters A, B, C and D were pretreated using the Moringa coagulant (250 mg l^{-1} of MSES). Waters A and B are duplicate waters containing only naturally present microorganisms native to the source water, while waters C and D are duplicates containing cultured *E. coli* (cultured to its stationary growth phase in TSB and washed in sterile PBS solution) along with the natural organisms. Waters E and F are the same initial waters as A/B and C/D, respectively, but were not pretreated with coagulation/flocculation/sedimentation (CFS). E and F were included as control waters in order to make a comparison between waters that had and had not been pretreated prior to SODIS.

Table 1 | Experimental plan for combined coagulation-SODIS treatment sequence

Water	Coagulant dose (mg l^{-1})	Main organism type	Approx. initial [<i>E. coli</i>] (CFU/100 ml)
A & B	250	Natural coliforms	2.2×10^2
C & D	250	Natural coliforms + cultured <i>E. coli</i>	3.6×10^7
E	0	Natural coliforms	2.2×10^2
F	0	Natural coliforms + cultured <i>E. coli</i>	3.6×10^7

The waters were characterized by measuring the UV absorbance (254 nm; 1 cm pathlength; Hewlett Packard Diode Array Spectrophotometer, Model 8452A, Mississauga, Ontario), the total organic carbon (TOC) concentration (O-I Corporation Analytical TOC Analyzer, Model 1030, College Station, TX), the turbidity (HACH laboratory 2100N turbidimeter, Loveland, CO) and the colour (455 nm; HACH DR/2500 Spectrophotometer, Loveland, CO). The absorbance spectrum of the initial waters, pretreated waters and post-SODIS waters were measured using a Lambda 25 UV/Vis Spectrometer (Perkin Elmer instruments, Shelton, CT).

Jar test experiments

The CFS treatment is based on the ASTM D2035-08 standard (Standard Practice for Coagulation-Flocculation Jar Test of Water) as well as the traditional methods reported by Jahn (1986) and Folkard *et al.* (1999).

Immediately prior to initiating the CFS pretreatment the source waters were characterized by measuring each water's UV₂₅₄ absorbance, turbidity, pH, TOC and *E. coli* concentration. The waters were then measured into each jar tester beaker (the volume being 2 l minus the volume of coagulant to be added). Based on the total solids concentration of each batch of coagulant the appropriate volume of coagulant suspension was measured out.

Similar to the jar test experiments conducted by Ndabigengesere & Narasiah (1998), the pre-measured coagulant was added to each source water beaker and allowed to stir rapidly at 90 rpm for 3 min. At this point the stirring speed was reduced to 20 rpm and allowed to continue stirring slowly for 30 min. The stirring was then stopped, the paddles raised and the water was allowed to settle, undisturbed, for 2 h. Preliminary experiments demonstrated that after 2 h of settling time the water quality parameters had reached a relatively stabilized state where there was no significant change in the turbidity or absorbance (Wilson 2010).

Solar disinfection experiments

Following 2 h of settling time the six waters were each decanted from the jar tester beakers and placed in three prepared PET water bottles. One bottle of each different test water was included in three sets of water bottles

which are herein referred to as 'light bottles', 'dark bottles' and 'temperature bottles' for the bottles exposed to the sunlight, the bottles kept in the dark and the bottles used for measuring the water temperature in the sunlight, respectively.

Light bottles and temperature bottles were taken to the roof of the Galbraith Building (St George Campus, University of Toronto, Ontario) and placed horizontally on a southward-facing corrugated metal sheet at approximately 10.00 a.m. for SODIS treatment. The dark bottles were stored inside a closed cardboard box in the laboratory. Samples were taken before coagulation and after 0, 1, 2, 3, 4 and 6 h of sunlight exposure, and then after a 24 h regrowth period (18 h in the dark at room temperature).

After 6 h of exposure, the bottles were brought down from the roof and were placed inside a closed box (with the dark bottles) overnight at room temperature. The following morning, 24 h after the SODIS treatment was initiated, a 24 h sample was taken from each of the light and dark bottles to measure the extent of regrowth of *E. coli*.

Samples for microbial analysis were plated on differential coliform (DC) agar (according to MOE Method #E3407) to monitor the *E. coli* and TC concentrations. The samples were also plated on R2A agar (Becton, Dickinson and Company, Sparks, MD), using a standard pour plate method, in order to monitor the HPCs.

Quality control

These experiments, which looked at the impact of the treatment sequence on natural and cultured bacteria in natural source water, included two positive controls: one with only natural bacteria, and the other with natural bacteria plus cultured *E. coli*. These controls were not treated with CFS pretreatment, and only with 6 h of SODIS. The experiments were completed three times to examine the reproducibility of the coagulation-SODIS results.

The reported detection limit for the DC agar enumeration method is 1 CFU per sample volume or dilution tested (USEPA Method #1604; Brenner *et al.* 1993). Since the largest sample volume filtered through the membrane filter was 100 ml, *E. coli* and TC concentrations measured to be below the 1 CFU/100 ml detection limit have been reported as the detection limit as instructed in USEPA

Method #1600 (USEPA 2005). Furthermore, since the largest volume plated with R2A agar was 1 ml, the HPC detection limit would be 100 CFU/100 ml, and any concentrations measured below this limit have been reported as the detection limit. The log reductions reported only account for concentrations measured above or equivalent to the reported detection limit concentration.

RESULTS AND DISCUSSION

Water quality impact of CFS pretreatment

Water quality parameters measured before and after the CFS pretreatment, and the corresponding percentage change, are shown in Table 2. A source water with a relatively low turbidity and high colour was used in order to examine the colour removal achieved by the coagulation pretreatment. As a result, little turbidity reduction was achieved; however, the colour and UV₂₅₄ absorbance were reduced by 67% and 17%, respectively. The TOC concentration was increased considerably (by more than 400%) which was expected based on preliminary experiments and studies by Narasiah *et al.* (2002) and Okuda *et al.* (2001).

The absorbance spectrum of a typical natural water sample, before coagulation, after the CFS pretreatment and after prolonged storage with sunlight exposure, is shown in Figure 1. Considering that irradiation with wavelengths <320 nm are largely filtered out in the atmosphere or by the UV stabilizers in the plastic bottle (McGuigan *et al.* 1998; Wegelin *et al.* 2001), the wavelength ranges of main concern are in the UV-A and visible regions. Despite

having considerably increased the amount of absorbance in the UV-C region via coagulant addition, the absorbance of the pretreated water in the UV-A region is approximately 36% ± 3% less than that of the initial water. It is suggested that the decrease in the absorbance spectra following the CFS pretreatment is due to prolonged storage and further sedimentation of the flocculated coloured NOM in the water during the solar exposure time, and is not due to the SODIS treatment itself.

Solar irradiance and water temperature

The solar irradiance measured on the roof where SODIS was conducted during the 10 and 22 July experiments is shown in Figure 2. The total amount of solar light is indicative of the amount of UV-A and visible irradiation available for disinfection and therefore is one of the main factors influencing SODIS efficiency. While the solar irradiance measured on 10 July was consistently high (between 748 and 863 W m⁻²), the weather experienced on 22 July was affected by sporadic clouds which resulted in solar irradiance ranging from 330 to 770 W m⁻². Although the solar irradiance was not measured on 14 July (because of technical difficulties with the radiometer), qualitative weather observations reported for that day were similar to those reported for 22 July.

The temperature of the waters was monitored during each experiment and only reached a temperature greater than 45°C during one sampling time (2.00 p.m., 10 July, 46°C). At temperatures below 45°C, the synergistic effects between pasteurization and UV inactivation on the overall disinfection are reported to be minimal (McGuigan *et al.*

Table 2 | Average water quality changes before and after applying the coagulation pretreatment

	Parameter	Before coagulation	After coagulation	% change
Unfiltered	Colour ₄₅₅ (Pt-Co units)	268 ± 29	90 ± 12	-67
	UV ₂₅₄ absorbance (cm ⁻¹)	0.772 ± 0.042	0.641 ± 0.031	-17
	TOC (mg l ⁻¹)	18.4 ± 0.3	99.6 ± 0.26	441
	SUVA ₂₅₄ (mg l ⁻¹ cm ⁻¹)	0.042 ± 0.006	0.006 ± 0	-85
	pH	6.3 ± 0.2	5.3 ± 0.3	-17
	Turbidity (NTU)	1.7 ± 0.5	1.9 ± 1.7	17
Filtered	Colour ₄₅₅ (Pt-Co units)	237 ± 51	45 ± 3	-81
	UV ₂₅₄ absorbance (cm ⁻¹)	0.699 ± 0.036	0.578 ± 0.022	-17
	TOC (mg l ⁻¹)	18.5 ± 0.4	96.4 ± 0.4	424
	SUVA ₂₅₄ (mg l ⁻¹ cm ⁻¹)	0.038 ± 0.003	0.006 ± 0	-84

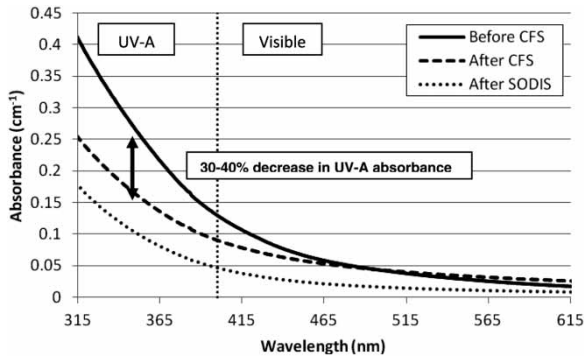


Figure 1 | Typical absorbance spectra of a natural water, before and after CFS and after sunlight exposure and prolonged storage time, in the UV-A and visible regions (315–615 nm range).

1998). Waters that had not been pretreated, and therefore had a higher colour (206–297 Pt-Co units at 455 nm; unfiltered) and UV absorbance (0.596–0.670 at 254 nm; unfiltered) were raised to temperatures approximately 1°C higher than those waters which had been pretreated and had a 66% lower colour.

Combined treatment sequence inactivation

The *E. coli*, TC and HPC removal and inactivation curves of the four different waters tested (A/B, C/D, E and F) in the three separate experiments (10, 14 and 22 July) are presented in Figure 3. Waters containing naturally present *E. coli*, TC and HPC bacteria that were pretreated with CFS prior to SODIS (waters A and B) are presented in the graphs on the left. Waters containing cultured and naturally present *E. coli*, TC and HPCs that were pretreated with CFS prior to SODIS (waters C and D) are shown in the graphs on the right. Cultured *E. coli* was added to natural source water, already containing

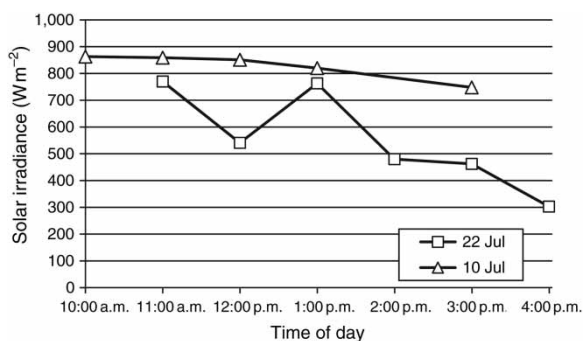


Figure 2 | Solar irradiance measured during SODIS.

native bacteria, in order to increase the *E. coli* concentration by at least 10^7 CFU/100 ml and, in doing so, the disinfection potential of 6 h of sunlight could be better estimated.

Finally, control waters E and F, which are identical to waters A/B and C/D, respectively, but were not pretreated with coagulation/flocculation (i.e. sedimentation only), are presented in each of the respective graphs to provide some context for comparison. Since waters A and B are duplicates of the same water (they were the same initial water and underwent the same treatment processes), their results have been arithmetically averaged for each experiment and are shown in the figures as an average line. The same arithmetic average lines are shown for waters C and D. Since there were no duplicates of waters E and F, these graphs do not contain any average lines since only one set of data was available per experiment.

E. coli are assumed to be a subset of total coliforms, which is a subset of HPC bacteria. To provide some context as to the concentration levels of the three organism categories, the natural *E. coli* makes up 0.05–5% of the natural total coliforms (TCs), while TCs make up 3–40% of the natural HPC organisms. Naturally present *E. coli*, TCs and HPCs were reduced in concentration to levels either below or close to the reported method detection limit when the treatment sequence included the CFS pretreatment. Up to 2 h of sunlight exposure was required for the natural *E. coli*, while up to 3 to 6 h was required for the natural TCs. More than 6 h of sunlight exposure would be required for reducing HPC levels to the detection limit concentration. Cultured and natural *E. coli* reached concentrations below the reported detection limit after 2 to 4 h of sunlight exposure and TCs and HPCs reached concentrations between 1 and 10 CFU/100 ml after 6 h.

The *E. coli* appears to follow first-order disinfection kinetics, while the TCs and HPCs tend to follow characteristic decelerating rate kinetics (Emerick *et al.* 1999) resulting in a tailing region where little further reduction is achieved with additional irradiation. The TC and HPC tailing regions consist of decreasing concentrations within 100 to 1 CFU/100 ml over 4 h of sunlight exposure. The transition between exponential decay and the tailing region occurs between 2 and 4 h of sunlight exposure for TCs and HPCs.

The average reductions (\pm the standard deviation) of the natural and cultured organisms during the CFS or

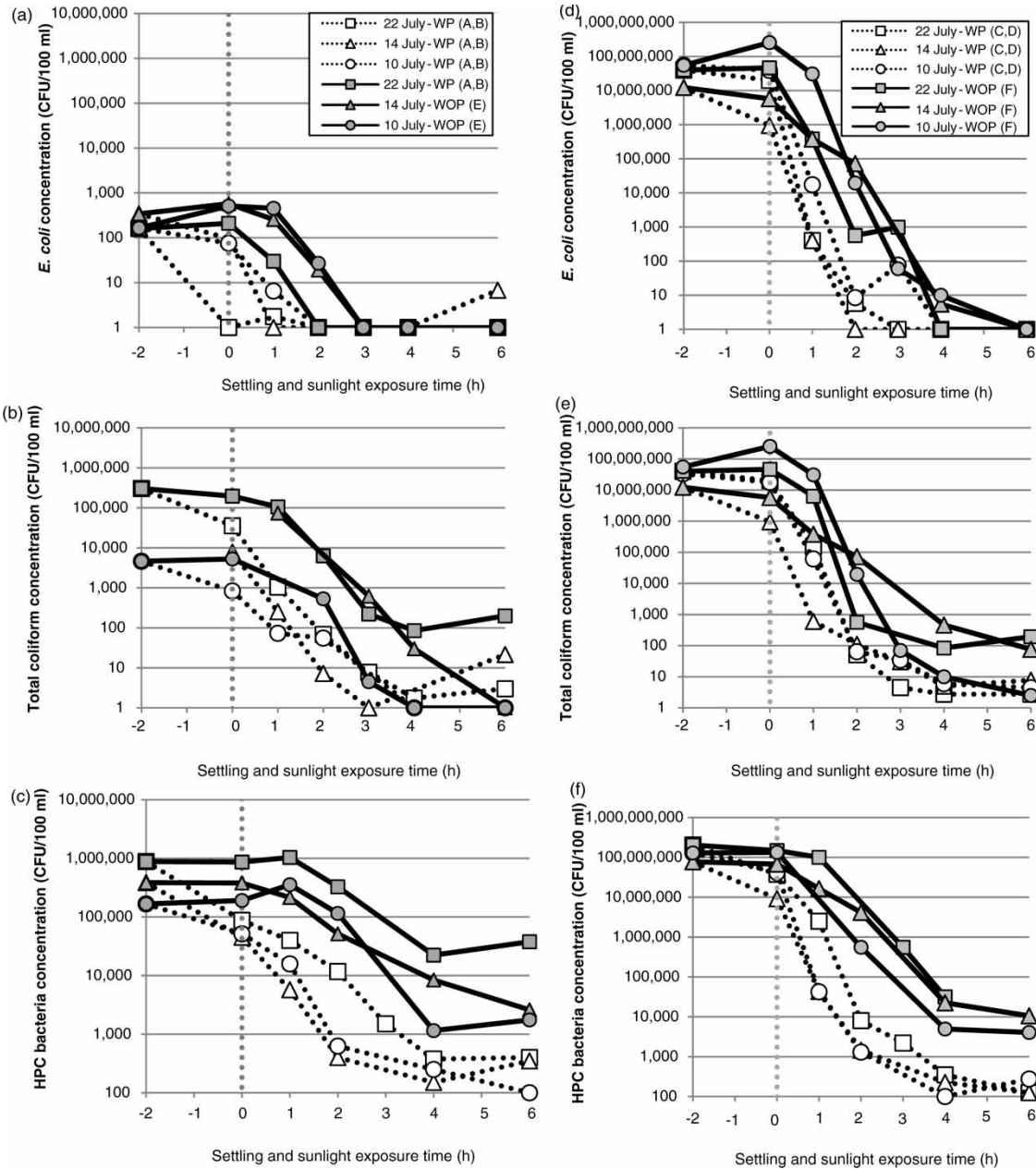


Figure 3 | Removal and inactivation curves during the pretreatment (CFS or sedimentation only; -2 to 0 h) and SODIS (0 to 6 h). Natural bacteria results (for waters A, B and E) (a) *E. coli*, (b) TC, (c) HPC. Natural + cultured bacteria results (for waters C, D and F) (d) *E. coli*, (e) TC, (f) HPC. Waters that were pretreated (dotted line; WP = with pretreatment) and were not pretreated (solid line; WOP = without pretreatment) are presented together for comparison.

sedimentation only pretreatments, and during the 6 h of solar exposure, are summarized in Table 3. The reductions are presented on a logarithmic scale. Additionally, the approximate solar exposure time (at an average irradiance of 670 W m^{-2}) that was required to achieve CFU concentrations below the method detection limit for each tested organism is presented.

Semi-log inactivation curves for the total coliforms are plotted with respect to the cumulative solar irradiance for a typical set of data (22 July experiment) for pretreated (C, D) and not pretreated (F) waters in Figure 4. At low doses of solar irradiation ($<1,500 \text{ W h m}^{-2}$) the inactivation curve follows first-order kinetics, decreasing exponentially,

Table 3 | Log reduction of *E. coli*, TC and HPC by the pretreatment and SODIS (\pm standard deviation), and the approximate sunlight exposure time required to reach concentrations below the detection limit

		Average log reduction achieved by Pretreatment		6 h of SODIS		Sunlight exposure time to reach the detection limit (h)	
		Sed. only*	CFS**	Sed. only	CFS	Sed. only	CFS
Natural	<i>E. coli</i>	-0.3 ± 0.2	1.0 ± 1.0	2.6 ± 0.2	1.0 ± 0.9	2-3	0-2
	TC	0.1 ± 0.2	0.8 ± 0.1	3.9 ± 0.5	3.2 ± 0.8	4->6	3->6
	HPC	0.0 ± 0.0	0.8 ± 0.3	1.9 ± 0.4	2.4 ± 0.3	>6	4->6
Cultured	<i>E. coli</i>	-0.1 ± 0.5	0.5 ± 0.5	7.6 ± 0.8	6.2 ± 0.9	4-6	2-4
	TC	-0.1 ± 0.5	0.6 ± 0.5	6.1 ± 1.7	6.2 ± 1.0	>6	≥ 6
	HPC	0.1 ± 0.1	0.7 ± 0.2	4.0 ± 0.5	5.2 ± 0.3	>6	4->6

*Sed. only = no coagulant added; sedimentation (only) pretreatment applied

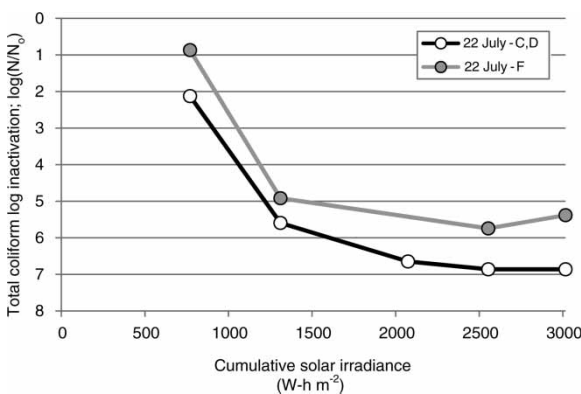
**CFS coagulant dose = 250 mg l⁻¹ total solids

E. coli coliform and total coliform (TC) detection limit: 1 CFU/100 ml

HPC detection limit: 100 CFU/100 ml

but eventually transitions into a tailings region at a cumulative irradiance greater than 1,500 W-h m⁻². These curves demonstrate that a higher log inactivation was ultimately reached by the pretreated waters (C,D). When the log inactivation achieved after 6 h of sunlight was equivalent for pretreated and not pretreated waters, it was found that the pretreated waters achieved this log reduction in a shorter period of time (i.e. with less solar irradiation). The HPC dose-response curves generally mirrored those of the TC curves for each experiment, and therefore have not been included.

The log reductions achieved for the full treatment sequence for each type of bacteria (with or without the CFS pretreatment) are reported in Table 4. The log reductions reported are the sum of the pretreatment + SODIS log reductions reported in Table 3. While there was no

**Figure 4** | Total coliform log inactivation curves with respect to the cumulative solar irradiance for pretreated (C,D) and not pretreated (F) waters on 22 July.

change in the *E. coli* log reductions with the inclusion of the CFS pretreatment, the pretreatment resulted in a slight improvement in the TC log reduction, and a larger increase in the HPC log reduction. This is a collective measure of the log reduction, achieved after 6 hours of SODIS, made regardless of the differences in the rates of inactivation between waters and organism types.

Statistical analysis

In order to evaluate any change in the rate of solar inactivation resulting from the CFS pretreatment, the *E. coli* inactivation was modelled using pseudo first-order kinetics. Waters C, D and F were modelled because they contained a sufficient number of incident organisms. Semi-log plots of the solar disinfection data presented in Figure 3 were generated for waters C, D and F. The inactivation rate constant (K) was determined for each experiment as the slope of

Table 4 | Log reductions achieved by the full treatment sequence without and with CFS pretreatment

		Average log reduction achieved by the full treatment sequence (pretreatment + 6 h of SODIS)	
		Sed. only	CFS
Natural	<i>E. coli</i>	2.3 ± 0.2	2.3 ± 0.2
	TC	3.9 ± 0.9	3.7 ± 1.2
	HPC	1.8 ± 0.4	3.2 ± 0.2
Cultured	<i>E. coli</i>	7.5 ± 0.3	7.5 ± 0.3
	TC	6.0 ± 1.2	6.7 ± 0.5
	HPC	4.1 ± 0.4	5.9 ± 0.3

Table 5 | Chick's law rate constants (K) for the solar inactivation of cultured *E. coli* in waters that have been pretreated with CFS and sedimentation only

Water	Pretreatment	Rate constants (K) (h ⁻¹)			Mean	Std
		22 July	14 July	10 July		
C,D	CFS	3.57	3.19	3.33	3.36	0.19
F	No CFS	2.83	2.06	2.86	2.58	0.45

each semi-log curve prior to the tailings region. Water F was modelled with and without the inclusion of a lag period prior to following first-order rate kinetics. An analysis of variance found that when the lag period was not included, waters C/D and F experienced statistically different rates of solar inactivation. However, when a 15 min to 1 h lag period was accounted for, the difference in the rates of solar inactivation in waters that have and have not been pretreated with Moringa CFS was not statistically significantly ($\alpha = 0.05$). It is presumed that this lag period exists in response to the bacterial shielding and UV resistance effects of the dissolved organic matter (characterized by high colour₄₅₅ and UV₂₅₄ absorbance) and suspended particles within the natural water matrix. Its inclusion reflects the necessity to remove these components in order to achieve microbial reduction with less solar irradiance and in a shorter period of time. The inactivation rate constants (K) where the lag period was accounted for are presented in Table 5.

Regrowth

Although no *E. coli* were found to be present after the 18 h storage period for each experiment conducted, substantial

regrowth of TCs and HPCs was found to occur during (and sometimes before) the 18 h when the bottles were stored in the dark at room temperature. In Figure 3 some TC and HPC bacteria regrowth can also be observed to occur before the end of the sunlight exposure time. It is suggested that any regrowth during the SODIS treatment may be a result of natural cellular repair mechanisms or photo-induced reactivation.

Since the TC regrowth mirrored that of the HPCs, only the TC regrowth is illustrated in Figure 5 and discussed here. Generally the HPCs were found to be present at a concentration 1-log greater than that of the TCs. In waters that had been pretreated (A, B, C and D) TC regrowth was found to occur in the range of a 1.8 to 2.6-log increase. Without the application of the pretreatment, TC regrowth was measured to be in the range of a 0.6 to 3.6-log increase. Therefore, pretreatment aids in improving the stability of the treated water, but not for extended periods of time.

A final consideration regarding regrowth is with respect to the health relevance associated with these different categories of bacteria. While TC and HPC bacteria are observed to regrow, the health concerns associated with their presence are substantially less than those of pathogenic bacteria, such as *E. coli*, which was not observed to regrow.

CONCLUSIONS

If *Moringa oleifera* seeds are available within a community that has poor quality source water, utilizing these seeds

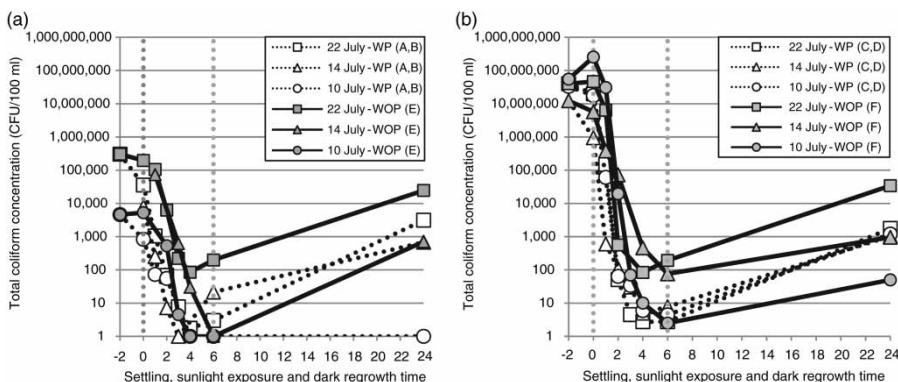


Figure 5 | TC removal and inactivation curves during the pretreatment (CFS or sedimentation only; -2 to 0 h), SODIS (0 to 6 h) and dark storage time (6 to 24 h). (a) Natural bacteria (waters A, B and E), (b) natural + cultured bacteria (waters C, D and F). Waters that were pretreated (dotted line; WP = with pretreatment) and were not pretreated (solid line; WOP = without pretreatment) are presented together for comparison.

as a pretreatment for SODIS is recommended as it will decrease both the colour and turbidity of the water as well as the amount of sunlight required for disinfection. The combined treatment sequence has been shown to be more effective than either treatment individually. However, the time required to perform the Moringa-CFS pretreatment is roughly equivalent to the amount of time saved in sunlight exposure during SODIS. Therefore, the overall treatment time may not be shortened, and the main benefit is improved water clarity. This being said, requiring less sunlight exposure time may allow for disinfection on intermittently sunny days when the CFS pretreatment can be completed before the sunlight is strong enough for SODIS.

The CFS pretreatment was shown to decrease the waters' absorbance in the UV-A region (315–400 nm) by 30 to 40% (depending on the wavelength). While the true colour (filtered measurement at 455 nm) was found to decrease by 81%, the apparent (unfiltered) colour was observed to decrease by 66%. In addition, the coagulation pretreatment alone (no SODIS) was shown to remove up to 1-log of *E. coli* and total coliforms.

CFS pretreated and unaltered (sedimentation only) waters achieved similar log reductions after 6 h of SODIS. However, the log reduction was achieved approximately 2 h faster for all three categories of bacteria monitored in the pretreated waters, possibly because of the waters' lower absorbance of UV-A radiation. Because 2 h were consumed in the CFS pretreatment process the treatment may or may not save the user time. Depending on the user's preference, clarifying highly turbid and/or coloured source waters may or may not be a benefit of this pretreatment, and may determine whether it is appropriate, appealing to the user and successfully implemented.

Whether the water is treated by Moringa-CFS or SODIS or these treatments in combination, there is the potential for regrowth of total coliforms and heterotrophic plate count bacteria if the treated waters are stored for a period of time. Approximately 2-log regrowth was found to occur for both TC and HPC bacteria when kept in the dark overnight; however no *E. coli* regrowth was observed. While regrowth of natural TC and HPC bacteria is less than desirable, there is less health risk associated with their presence relative to that of *E. coli*.

Finally, despite any potential benefits, it should be recognized that introducing additional steps into any treatment method can cause a decrease in compliance rates because the system being promoted is too complicated, is viewed as unnecessary or is not being explained properly. Alternatively, an intervention may be unsuccessful as a result of an unforeseen change in the aesthetics of the finished water that is deemed undesirable by the user (taste, odour, appearance, etc.). In any of these cases, it is recommended that the pretreatment is not incorporated into the method should the disseminators feel that they risk decreasing SODIS compliance as a result of the additional step.

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