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ORIGINAL RESEARCH

Evaluation of *Escherichia coli* Inactivation at High Altitudes Using Solar Water Disinfection

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Introduction—Solar disinfection (SODIS) is an effective method for microbiologic inactivation of contaminated water using ultraviolet rays at low elevations. The aim of this study was to determine the effectiveness of SODIS at higher elevations.

Methods—The ability of SODIS to inactivate *Escherichia coli* bacteria was evaluated at an altitude of ≥1600 m using Nalgene bottles, disposable plastic water bottles, and Ziploc plastic bags. Bacterial viability was determined through measurement of colony forming units (CFUs). Decreases in CFUs were determined at each time point relative to those at the baseline, and a multivariable regression analysis was used to assess significant changes in CFUs.

Results—Bacterial CFUs in exposed containers decreased by >5 log after 6 h of exposure to sunlight. In contrast, the CFUs remained nearly unchanged in unexposed containers, showing a mean decrease of 0.3 log. By 2 h, bacterial inactivation at high altitudes was 1.7-fold greater than that at lower altitudes ($P<0.05$). By 6 h, nearly all bacteria were inactivated at high or low altitudes. At 6 h, no statistical difference was observed in the efficiency of inactivation between elevations. Compared with Nalgene bottles, plastic bottles had a 1.4-fold greater decrease in CFUs ($P<0.05$). No statistical difference in bacterial inactivation was found between plastic bottles and plastic bags.

Conclusions—At high altitudes, SODIS is an effective method for inactivating *E coli*. Further research investigating other microorganisms is warranted to determine whether SODIS is suitable for disinfecting contaminated water at high altitudes.

Keywords: SODIS, drinking water, UV disinfection, water safety, global health, disaster medicine

Introduction

Water can be disinfected through several effective methods: heat, filtration, chemical treatment, and ultraviolet (UV) light. The use of natural sunlight for solar disinfection (SODIS) of contaminated water effectively inactivates many microorganisms, including bacteria, viruses, protozoa, and fungi.[1-4] The Wilderness Medical Society's clinical practice guidelines for water disinfection have assigned Grade 1B evidence (strong recommendation with moderate-quality evidence) for full sunlight exposure for at least 4 h in clear plastic bottles to significantly decrease microorganism contamination.[3]

Water disinfection using the SODIS technique is established in lower-altitude (LA) environments (<2500 m). The SODIS technique uses a reflective surface with clear bottles and requires UV-A and UV-B radiation to inactivate microorganisms. Because the optical energy of

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solar radiation decreases with increasing latitude from the equator, the efficiency of SODIS is maximal between the latitudes of 60°N and 45°S.[5,6] In settings in which clouds block sunlight from reaching the water or the water is cloudy, SODIS may not work. Therefore, the effectiveness of the SODIS technique may be limited to specific geographic and environmental conditions.

More than 140 million people live in high-altitude (HA) environments (>2500 m), and an additional 35 million people visit HA regions each year.[7,8] Less atmospheric density at HAs enables greater amounts of UV radiation to reach the surface, thus suggesting that SODIS should be effective at high elevations. Further testing of SODIS at HAs is required to determine whether it can be used for disinfection at varying elevations.

In this study, the effectiveness of SODIS at HAs was evaluated using *Escherichia coli* as a model bacterial contaminant of water. The effectiveness of SODIS was evaluated using World Health Organization (WHO) standards,[9] for which an outcome of a ≥4-log decrease in the number of viable *E coli* within 6 h of UV exposure is considered effective.

Methods

We conducted an experimental study using the SODIS technique at 3 study sites. The LA site was in Aurora, Colorado, at the University of Colorado Strauss Health Sciences Library, Anschutz Medical Campus, located at 1655 m above sea level. HA testing was performed in Leadville, Colorado, at 3101 m above sea level and in Evergreen, Colorado, at Mount Evans at 3329 m above sea level. Solar disinfection was tested on 6 d between August 25, 2021 and October 3, 2021. Weather and ambient temperature specific to the site locations were recorded from The Weather Channel website. Daily surface solar radiation levels specific to experimental sites were recorded from the National Renewable Energy Laboratory's Solar Radiation Research Laboratory baseline measurement system based in Golden, Colorado.[10]

The study was designed as previously described,[4] with modifications in container types described herein. We used *E coli* as a model fecal indicator because it is a commonly used laboratory model.[11] *E coli* K-12 (MG1655) was inoculated into water in various containers: 1.2-L freezer Ziploc bags (Racine, WI) made of low-density polyethylene plastic; standard 355-mL plastic water bottles made of bisphenol A-free polyethylene terephthalate, a plastic typically used for commercially bottled drinking water; and 946-mL clear Nalgene (Rochester, NY) water bottles made from Tritan copolyester, a bisphenol A-free plastic.

Figure 1. Experimental setup with Nalgene bottles, plastic water bottles, and a plastic bag placed on a reflective tarp and exposed to the sun during a lower-altitude trial.

E coli K-12 (MG1655) was grown on a solid medium comprising Luria broth (LB) and 2% agar and cultured overnight at 37°C. A single bacterial colony was used to inoculate 5 mL of LB, and this was incubated at 37°C for 18 h. The culture was diluted to approximately 1×10^6 colony forming units (CFUs) mL^{-1} in sterile water, according to the optical density determined based on absorbance at 600 nm. The actual CFUs were determined later by plating on LB agar plates and counting the colonies after incubation at 37°C for 18 h.

All containers were filled with 500 mL of sterile water at room temperature (25°C) containing bacteria at 1×10^6 CFUs mL^{-1}. The containers were aerated by shaking for 20 s and then placed in a plastic foam box. The containers were stored at room temperature for 2 h inside or at room temperature during the 2-h travel time to the study site. The samples were exposed to the sun from 0900 through 1500 on each experimental day. At the site, the containers were placed horizontally on a 1.5×2.1-m^2 space blanket (Grabber Outdoors, Dalton, GA) on a grass field. The bottles were placed on the reflective side of the blanket and exposed to direct and reflected sunlight for 6 h (Figure 1). To avoid shadows, containers were placed away from buildings or trees. Control containers were placed at the same elevation in a plastic foam box in the shade to block visible and UV light.

The abundance of *E coli*, as measured using CFUs, was determined by diluting and plating of a 100-μL sample from the control and UV-exposed containers at each time point. Containers were shaken at each time point before sample collection and before being placed back onto the space blanket. The 100-μL aliquots were immediately placed into 15-mL plastic conical tubes containing 0.9 mL of LB; 4 additional 10-fold serial

Table 1. Experimental conditions

Trial	*Date*	*Location*	*Elevation*	*Weather description (UV index)*	*Time of exposure*	*Ambient temperature (°C)*	*Mean bottle temperature±SD (°C)*
Low 1 (LA1)	August 25, 2021	Aurora, Colorado 39°44'49" N 104°50'12" W	1655 m (5430 ft)	Sunny (9)	0900 (0 h)	20	[a]
					1000 (1 h)	[a]	[a]
					1100 (2 h)	22	[a]
					1300 (4 h)	25	[a]
					1500 (6 h)	30	[a]
Low 2 (LA2)	September 25, 2021	Aurora, Colorado 39°44'49" N 104°50'12" W	1655 m (5430 ft)	Sunny (6)	0900 (0 h)	10	16±0.3
					1000 (1 h)	16	27±5.4
					1100 (2 h)	21	28±2.1[b]
					1300 (4 h)	26	39±5.6[b]
					1500 (6 h)	29	39±9.1[c]
Low 3 (LA3)	October 3, 2021	Aurora, Colorado 39°44'49" N 104°50'12" W	1655 m (5430 ft)	Sunny with sparse clouds (6)	0900 (0 h)	9	26±0.7
					1000 (1 h)	19	19±1.5
					1100 (2 h)	24	22±1.5
					1300 (4 h)	27	29±3.6
					1500 (6 h)	29	34±7.6
High 1 (HA1)	August 26, 2021	Leadville, Colorado 39°15'08" N 106°17'37" W	3101 m (10,174 ft)	AM sun, PM cloudy with storms (9)	0900 (0 h)	4	19±1.8
					1000 (1 h)	[a]	[a]
					1100 (2 h)	7	19±0.9
					1300 (4 h)	10	21±2.5
					1500 (6 h)	13	[a]
High 2 (HA2)	August 28, 2021	Evergreen, Colorado 39°40'23" N 105°32'31" W	3329 m (10,921 ft)	Sunny with sparse clouds (9)	0900 (0 h)	17	20±1.2
					1000 (1 h)	19	24±3.9
					1100 (2 h)	21	27±8.4
					1300 (4 h)	23	32±7.8
					1500 (6 h)	24	[a]
High 3 (HA3)	September 26, 2021	Evergreen, Colorado 39°40'23" N 105°32'31" W	3329 m (10,921 ft)	Partly cloudy (6)	0900 (0 h)	10	25±4.6
					1000 (1 h)	18	27±3.2
					1100 (2 h)	19	4±3.4
					1300 (4 h)	15	8±5.1
					1500 (6 h)	16	[a]

HA, high altitude; LA, low altitude; UV, ultraviolet.
[a]Temperature not recorded.
[b]Container temperature was recorded in 6 out of 9 containers.
[c]Container temperature was recorded in 8 out of 9 containers.

dilutions were performed. A 1-mL sample from each dilution was used to inoculate LB agar plates, which were stored in the dark throughout the rest of the experiment. After being transported to the laboratory, the plates were incubated for 24 h at 37°C. For each sample, the altitude, date, time of the experiment, and ambient outside temperature were recorded. For 2 of 3 trials at the LA site and for all 3 trials at the HA sites, the water temperature inside each container was recorded using an infrared digital thermometer with a targeting laser (Klein Tools, Lincolnshire, IL).

Variables used in the analysis included time, altitude, container type, ambient temperature, and water temperature during the experiment. The CFU analysis was performed after linear transformation of $\log_{10}$-based values. The decrease in CFUs was calculated as the fold change in CFUs at a given time relative to baseline CFUs (1, 2, or 6 h). Changes in CFUs were summarized as mean±SD. Multivariable linear regression models were used to adjust for water temperature and container type. Results were evaluated at a significance level of 5%, and analyses were performed using R, version 4.1.1 (Vienna, Austria).

Results

Across experiments, the weather condition was mainly sunny; however, some days were partly cloudy (Table 1). The ambient temperature was cooler at the HA sites. The

Table 2. Change in log colony forming unit concentrations at each hour for exposed and nonexposed containers

Change in log CFU over time	*Exposed (n=36)*	*Not exposed (n=18)*
	1 h	
Mean±SD	3.24±1.60	−0.01±0.33
	2 h	
Mean±SD	4.04±1.09	0.18±0.45
	6 h	
Mean±SD	5.12±0.66	0.31±0.50

CFU, colony forming unit.

mean daily direct surface solar radiation levels at the HA locations ranged from 7 to 7.5 kW m^{-2} d^{-1}, whereas those at the LA location ranged from 6.5 to 7 kW m^{-2} d^{-1}.[10] Our analysis included 54 samples of *E coli*. HA and LA locations were assessed on different days. On each experimental day, 9 samples were tested (3 control and 6 experimental). Although a <0.5-log decrease occurred in control containers, the concentration of viable *E coli* in all exposed containers decreased by >4 logs within 4 h of exposure time (Table 2 and Figure 2).

Compared with the control containers, the UV-exposed containers showed a greater decrease in viable *E coli* at all the time points (Figure 2; Table 3, Models 1−3). At 6 h, a 4.8±0.18 greater decrease was observed in the exposed containers than in the control containers ($P<0.05$). Among the exposed containers, HA was associated with a greater decrease in *E coli* concentration than LA at 1 (estimate, 1.7±0.40 greater, $P<0.05$) and 2 h (estimate, 1.09±0.30 greater, $P<0.05$) (Table 3, Models 4 and 5). The difference in bacterial inactivation at 6 h between HA and LA was not significant.

Beyond altitude, container type affected the efficiency of SODIS. Plastic bottles led to greater decreases in *E coli* concentrations than Nalgene bottles at all time points (Table 3). Solar disinfection performed similarly in plastic bags and plastic bottles (Table 3). Bottle temperature was recorded in 45 of 54 containers (45°C at 0 h, 36°C at 1 h, 42°C at 2 and 4 h, and 17°C at 6 h). Multivariable regression (Models 1−6) showed significantly fewer CFUs at all time points in exposed containers than in nonexposed containers after controlling for bottled water temperature, thus demonstrating that bacterial inactivation by UV radiation can be achieved independently of water temperature (Table 3).

Discussion

Solar disinfection has been used to improve water quality by inactivating pathogens at low altitudes.[1,4,6,12] Solar disinfection inactivates bacteria, viruses, and protozoa through heating of water and causing UV-mediated DNA damage to microorganisms.[13] Responses to UV exposure vary among bacteria, viruses, and protozoa because of their different cellular compositions; for example, some protozoans' thick-walled, chitinous cysts confer resistance to disinfection by UV radiation. The efficiency of SODIS functions primarily through UV-A radiation (315−400 nm) and UV-B (280−315 nm) radiation.[6] Ultraviolet-A radiation damages microorganisms via formation of reactive oxygen species,[6] whereas UV-B causes endogenous damage to RNA and DNA.[13] On a typical cloudless day in the summer at the equator, the Earth's surface receives an irradiance of approximately 1120 W m^{-2}, and the value decreases with increasing distance from the equator.[6] The UV index increases by 4% to 9% for each 1-km increase in elevation.[14] The UV radiance also varies by time of year: the northern hemisphere experiences the lowest values in December and January and the highest values in June.[15]

We observed that most bacterial inactivation occurred within 2 h of exposure (Figure 2) compared with Beattie et al,[16] who reported that most bacterial inactivation occurred after the first 2 h. In contrast to reports in the literature, our study did not show a significant increase in water temperature, whereas other studies[16,17] have reported a rapid increase in water temperature with the use of parabolic reflectors rather than flat reflectors. Together, these findings suggest that the type of surface reflector affects the amount of energy input required to raise water temperature. Temperature can act synergistically with SODIS in inactivating *E coli*, at both high temperatures exceeding 45°C (113°F)[6] and potentially low temperatures. A recent study indicated a synergistic effect below 10°C (50°F).[12] The water temperatures in our experiment were between 10 and 45°C, thus indicating that temperature played relatively less of a role in our experimental system. Our data showed that a synergistic effect with temperature is not often achieved during exposure unless modifications are made for thermal enhancement.

This study was conducted using materials generally available to people in multiple settings, including people recreating in the mountains, away from sophisticated water disinfection, and people living in rural or HA regions that lack developed water systems. Our results indicated that plastic bottles outperformed Nalgene bottles in the first few hours of exposure, in agreement with published comparisons of various container types.[18-21] Polyethylene terephthalate plastic bottles are thinner than Nalgene bottles and, consequently, may allow more UV radiation to reach the water and enable greater microbial inactivation. Additionally, the use of a reflective surface, such as the space blanket used here

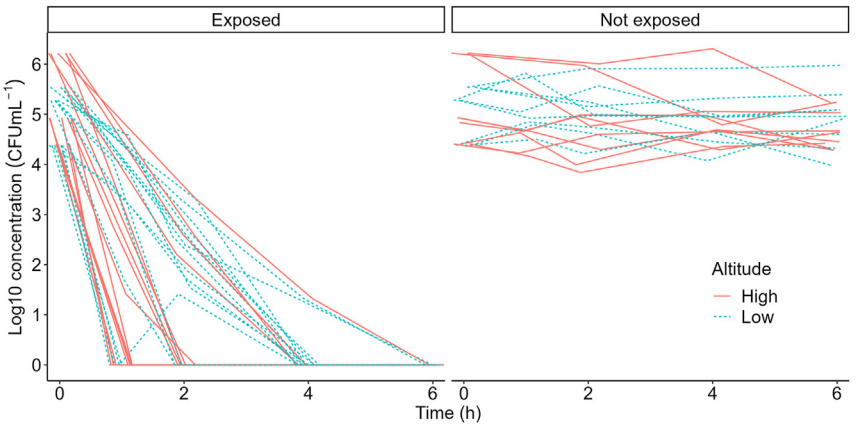

Figure 2. A, Sunlight exposure decreased the *Escherichia coli* concentrations to 0 CFU mL^{-1} in all exposed containers over 6 h. B, However, the *E coli* concentrations in unexposed containers were stable over 6 h. The trends were similar in containers at higher altitudes (Leadville, Colorado, and Evergreen, Colorado) and lower altitudes (Aurora, Colorado). CFU, colony forming unit.

and in other studies,[22] enhances the efficiency of SODIS.[6,23]

A similar approach to SODIS is SteriPEN, a handheld device that is placed into a container and uses UV radiation to inactivate microorganisms. It requires only 1.5 min, probably because it is placed directly into the water and uses UV-C at 254 nm.[24] Ultraviolet-C radiation is more effective than UV-A and UV-B in inactivating pathogens; however, UV-C is typically filtered out by the ozone layer and is a smaller contributor to microbial inactivation by SODIS.[25]

Although the US Environmental Protection Agency (EPA) guidelines require a 6-log decrease in culturable bacteria for microbiologic water purifying systems,[26] WHO requires a 4-log decrease.[9] In our test of the effectiveness of SODIS, we observed at least a 4-log decrease in *E coli* at both HA and LA, regardless of container type, thereby demonstrating that in our experimental system, SODIS met the WHO standards but not the EPA standards. These data indicate that SODIS is effective at elevations as high as 3300 m. Although our data showed statistically greater inactivation of *E coli* at HAs between 0 and 2 h, no difference was observed in the efficiency of inactivation over the entire time course. These observations indicate that SODIS is comparably effective at HAs and LAs.

Limitations

The main limitation of our study was the use of only *E coli* as a model organism to investigate the SODIS method. Water gathered in settings without adequate purification systems may contain other bacteria, viruses, and protozoa, each of which responds differently to SODIS. Unlike the sterile water in this study, water collected from the natural environment contains nutrients and other particulates that might potentially shield pathogens from UV light. In addition, the presence of microparticles in water can alter the absorption of UV light and limit the effectiveness of SODIS. Glass containers were not studied because they are more difficult to transport than plastic containers (heavier and more prone to breaking, etc). Glass might have a different UV absorption capacity, thus affecting the efficacy of SODIS. Future studies could apply a predictive model that quantitatively estimates the amount of radiation transmitted through various container types to better understand the differences in the efficiency of bacterial inactivation.[27] Another limitation of this study is that our experiments were conducted on only 1 overcast day. We also used a reflective space blanket to increase UV exposure of the bottles, which could have affected our results compared with placing these on open, bare ground. Finally, we did not collect experimental site-specific solar radiation data.

Conclusions

Our study demonstrated that the SODIS technique significantly decreases viable *E coli* concentrations at LAs and HAs by at least 4 logs within 6 h. Solar disinfection–mediated bacterial inactivation is comparably effective at HAs and LAs and is more efficient in plastic bottles. The effectiveness of SODIS at HAs for other bacteria, viruses, and protozoa requires further testing. Future investigation of the clinical effectiveness of SODIS at HAs is needed to inform guidelines and recommendations for safe water drinking policies.

Table 3. Estimated log differences in the reduction in log *Escherichia coli* concentration (estimate±SE)

	Time frame		
Full multivariable models	*Model 1: 0 to 1 h*	*Model 2: 0 to 2 h*	*Model 3: 0 to 6 h*
Exposed (ref=control)	3.25±0.34[a]	3.86±0.23[a]	4.82±0.18[a]
High altitude (ref=low altitude)	1.22±0.32[a]	0.84±0.22[a]	0.19±0.17
Container type (ref=plastic bottle)			
Nalgene	−1.41±0.39[a]	−0.68±0.26[a]	−0.14±0.21[a]
Bag	0.18±0.39	−0.20±0.26	−0.08±0.21
Multivariable models within exposed	Model 4: 0 to 1 h	Model 5: 0 to 2 h	Model 6: 0 to 6 h
High altitude (ref=low altitude)	1.68±0.40[a]	1.09±0.30[a]	0.12±0.23
Container type (ref=plastic bottle)			
Nalgene	−1.90±0.49[a]	−0.81±0.37[a]	<0.01±0.28
Bag	0.34±0.49	−0.11±0.37	<0.01±0.28

ref, reference.

[a]Statistically significant $P<0.05$.

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