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Inactivation of Fecal Bacteria in Drinking Water by Solar Heating

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We report simulations of the thermal effect of strong equatorial sunshine on water samples contaminated with high populations of fecal coliforms. Water samples, heavily contaminated with a wild-type strain of *Escherichia coli* (starting population = 20×10^5 CFU/ml), are heated to those temperatures recorded for 2-liter samples stored in transparent plastic bottles and exposed to full Kenyan sunshine (maximum water temperature, 55°C). The samples are completely disinfected within 7 h, and no viable *E. coli* organisms are detected at either the end of the experiment or a further 12 h later, showing that no bacterial recovery has occurred. The feasibility of employing solar disinfection for highly turbid, fecally contaminated water is discussed.

A primary concern of people living in developing countries throughout the world is that of obtaining clean drinking water. In many places, this problem is made harder by the fact that many of the available water sources are unpotable without some form of treatment. The most common water treatment techniques are not always available to the local population since the practice of chopping down trees for firewood to boil water has been discouraged in many countries for environmental health reasons and the cost of chlorination may be considered prohibitive. It has been suggested that solar energy might have a role to play in improving water quality in those regions that enjoy a hot, sunny climate (1, 2, 4, 9–11, 14–16). Our group has been studying the possibility of using solar energy to improve the quality of water samples stored in ordinary 2-liter transparent plastic bottles placed in direct sunlight. We have concentrated on this technique in particular because we consider a low running cost to be a primary requirement and the bottles, which are usually treated as litter, are easily obtained.

Much of the published research has focused on the antibacterial role that solar UV radiation (200 to 400 nm) plays in solar disinfection of drinking water (2, 4, 5, 10, 15) while the thermal contribution has not been fully investigated (3, 15). This work was carried out to determine the antibacterial effect of the elevated temperatures that are established within water samples contained in ordinary transparent plastic soft drink bottles when placed in direct sunlight in equatorial climates. Since water retrieved from many water sources in hot, arid climates is often highly turbid, we have focused our study on the thermal contribution of the incident solar radiation to the disinfection process. Measurements recorded in our laboratory have shown that, in water samples with turbidities higher than 200 nephelometric turbidity units (NTU), less than 1% of the total incident UV light penetrates further than a depth of 2 cm from the surface (12) and thus can't be expected to have a significant germicidal effect beyond this distance in the liquid volume.

MATERIALS AND METHODS

Field measurements. Initial solar exposure of water samples took place at Esonorua in the Kenyan Rift Valley (latitude, 1° 29′S; longitude, 36°38′E). The water samples were stored in 2-liter, uncolored transparent plastic (polyethylene terephthalate) soft drink bottles that were collected locally. The label was removed from each bottle, and the bottle was washed and rinsed with filtered and chlorinated water from a standpipe source in Nairobi. The sample bottles were filled at about 8:00 each morning with water taken from the Rolkeju Ngarengiro River, which flows through Esonorua and is the main water source for the local community. Water turbidity was measured in NTU with a standard turbidity tube (DelAgua; manufactured by the Robens Institute, Guildford, United Kingdom). The sample bottles were placed on their sides in direct sunshine. Control samples in similar containers were placed in the shade, usually under a tree. Solar power was measured with an optical power meter (Coherent 200 series) which is sensitive over the 0.3-μm (UVB) to 10.2-μm (mid-infrared) range of the electromagnetic spectrum. Water temperature measurements were made with thermocouple-based digital thermometers (Checktemp 2; Hanna Instruments) which had been calibrated against a standard Type J Iron-Constantan reference thermocouple. Temperature and solar power measurements were recorded hourly over the course of each bottle exposure, which usually lasted from early morning to sunset. Microbiological analysis of the water performed by the authors at the site with dipslides coated on one side with tryptic soy agar (total aerobic count) and on the other with violet red bile glucose agar (count of members of the family *Enterobacteriaceae*) (HYCHECK dipslides for members of the *Enterobacteriaceae*; manufactured by Difco Laboratories Ltd.). More accurate counts were obtained by sending the water samples to the National Reference Laboratory in Nairobi for analysis. There, the water was analyzed by the most probable number standard coliform test recommended by the United Kingdom Department of Health (6). These analyses showed the water to be highly contaminated with fecal bacteria with an average population of 8.6×10^3 (95% confidence interval, 5.3×10^3 to 1.2×10^4) CFU/ml.

Laboratory temperature simulations. Since precise measurements of the effect of solar heating on bacterial populations in drinking water were not possible on-site in Kenya, the solar thermal effect was simulated in our laboratory in Ireland. The temperature of a 300-ml sample of water within the sample bottle was measured with a platinum resistance thermometer sealed inside a silicone pad immersed in the water. This thermometer was calibrated against a standard Type J Iron-Constantan reference thermocouple. The output from the thermometer controlled the mains power to a standard 2.2-kW domestic fan heater via a temperature-controlled relay system (Omron E5CS-X Multi-Range Temperature controller in series with a 10-A, 250-V alternating current power relay). Air heated by the fan was directed onto the sample bottle, which stood inside a rectangular housing that was open on the side facing the fan. The housing trapped the heated air and thus assisted in the heating process. The sample temperature was adjusted hourly to match those temperatures recorded on-site under Kenyan field conditions and listed in Table 1.

Bacterial preparation and enumeration. A wild-type strain of *Escherichia coli*, isolated from the stool of a Maasai child living in Esonorua, was inoculated into 25 ml of sterile nutrient broth (Oxoid CM67) and incubated overnight at 37°C. The culture was washed the following morning to completely remove any nutrients. To do this, the culture was transferred into a sterile universal container and centrifuged at 3,000 rpm ($855 \times g$) for 10 min. The supernatant was then discarded, and the pellet was resuspended in 20 ml of high-pressure liquid chromatography (HPLC) analytical reagent (Analar)-grade sterile water. This washing procedure was repeated three times. Finally, the pellet was resuspended in 8 ml of sterile water, to form the stock solution with a presumed concentration of 10^7 CFU/ml. A viable bacterial count of this prepared stock was performed by the Miles and Misra drop count technique (13). Stock prepared in this manner consistently produced a viable count of approximately 10^7 CFU/ml.

Test samples of varying turbidities with bacterial concentrations of 10^5

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TABLE 1. Profiles of solar power and water and air temperatures for three sets of solar disinfection experiments carried out in the Kenyan Rift Valley

Measurement set, date, and turbidity	Exposure time (h)	Solar power (mW/cm^2)	Shaded air temp (°C)	Water temp (°C)
Minimum water temp, 13 August 1994, 200 NTU	0	65	16.9	19.5
	1.1	3	17.8	22.2
	1.75	13	18.0	21.6
	2.6	16	18.5	23.5
	3.6	89	18.7	24.1
	4.2	16	19.0	29.2
	4.75	16	19.4	30.2
	5.75	45	19.6	30.5
	6.25	76	19.8	35.2
	7	13	20.3	36.3
	8	6	20.0	34.3
	8.5	48	20.0	34.0
	10.1	—[a]	18.4	28.7
Intermediate water temp, 17 August 1994, 150 NTU	0	35	26.2	29.5
	0.9	80	27.1	34.0
	1.9	76	28.2	40.1
	2.6	8	28.0	40.3
	3.4	16	28.9	40.3
	4.2	70	30.4	41.0
	4.9	57	32.0	45.6
	5.9	11	30.3	43.3
	7	—	—	35.0[b]
	8	—	—	30.0[b]
	9	—	22.0	26.0
	—	—	—	—
	—	—	—	—
Maximum water temp, 25 February 1995, 15 NTU	0	19	25.0	24.2
	1	67	26.3	27.9
	2	41	27.8	32.4
	3	80	30.3	39.4
	4	76	31.6	46.4
	5	76	34.1	51.2
	6	80	33.7	54.2
	7	80	34.8	55.0
	8	76	35.8	54.5
	9	76	33.6	47.7
	10	22	33.4	42.6
	12	41	24.3	34.1
	—	—	—	—

[a] Dashes indicate that no data were recorded for that period.
[b] Interpolated data not recorded in Kenya.

CFU/ml were required to simulate the approximate water conditions encountered in Kenya. Dust and soil collected from around the Esonorua River site were gradually added to 300 ml of HPLC Analar-grade sterile water until the required turbidity of either 12 or 200 NTU was achieved, as measured with the Del Agua turbidity tube described earlier. This solution was then sterilized by autoclaving for 15 min at 120 lb of pressure per in^2. A total of 3 ml of the stock solution was added to 297 ml of the sterile turbid sample to make a test sample with a bacterial concentration of 10^5 CFU/ml. The control solution was prepared in exactly the same manner.

Once prepared, the test samples were placed in a 1.5-liter polyethylene terephthalate bottle and heated according to the temperature profiles recorded in Kenya and listed in Table 1. Temperatures were adjusted on an hourly basis over a period of 8 h. Control samples were kept at room temperature (23 to 25°C). Volumes of 1 ml were taken from the test and control samples at the beginning of the experiment and then every hour. The final volumes were taken 24 h after the start of the experiment. These volumes were diluted in a series of 10-fold dilutions. A 20-μl volume was taken from each dilution and dropped onto a standard plate count agar plate (Oxoid CM463). Each dilution was sampled three times to ensure accuracy. Plates were incubated overnight at 37°C and counted the following day. Only those plates which showed discrete colonies in the drop area, preferably those which gave fewer than 40 colonies per drop, were selected and counted. The count was divided by the number of drops, multiplied by 50 to convert to 1 ml, and then multiplied by the dilution itself to give the number of CFU per ml (13).

RESULTS

Kenyan field measurements. Water samples were exposed to the Kenyan sunshine on a total of 15 occasions in August 1994 and a further 5 times in the latter half of February 1995. A complete range of cloud and sunshine conditions was encountered during these experiments. Solar power levels varied from a maximum of 89 mW/cm^2 (full sunshine) to a minimum of 3 mW/cm^2 (overcast). From the 20 sets of measurements recorded, 3 were chosen to represent the maximum, minimum, and intermediate water temperatures achieved during the solar exposures. The exposure time, solar power levels, shaded air temperatures, and sample water temperatures that were recorded on these occasions are listed in Table 1. The turbidity of the water samples varied between 2,000 NTU (opaque) and 5 NTU (effectively transparent) on a day-to-day basis, depending on prevailing weather conditions and how soon the water was collected after domestic livestock were watered. The highest turbidity encountered was 2,000 NTU, which was recorded on the day after an unseasonable and heavy rain in February

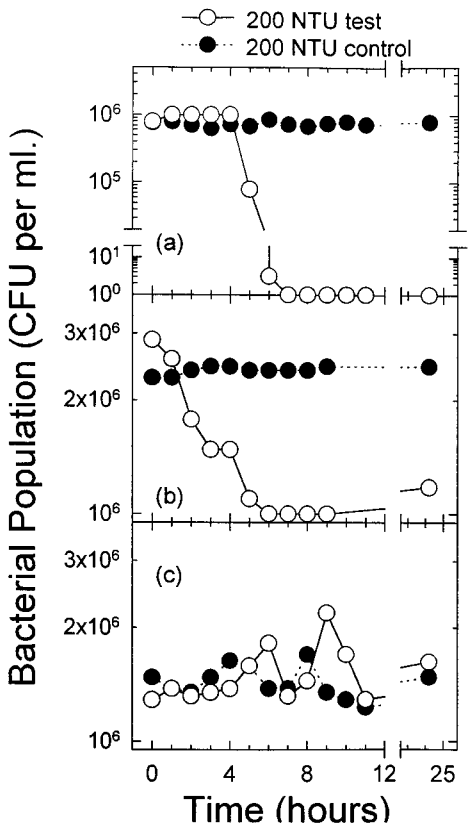

FIG. 1. Effect of solar heating on populations of a wild-type strain of *E. coli*. The water temperature in the test samples simulates the temperature profiles of the measurements listed in Table 1. (a) Maximum temperature data (maximum temperature = 55°C); (b) intermediate temperature data (maximum temperature = 45.6°C); (c) minimum temperature data (maximum temperature = 36.3°C). Filled circles represent control populations, and hollow circles represent test populations, for all sections of the graph.

1995. This unusually high turbidity reading resulted from a higher-than-normal proportion of suspended particulate matter in the sample. Readings taken on the following day showed the turbidity to have fallen to approximately 28 NTU. This maximum turbidity value was judged not typical for the water source (7), and so the next-highest value recorded of 200 NTU was used for the laboratory simulations of high-turbidity conditions.

Laboratory simulations. The three temperature regimens listed in Table 1 were simulated under two different turbidity conditions, yielding six sets of results. The variations in water turbidity produced no significant difference, with essentially both sets of data displaying the same general trends for corresponding temperature regimens. Consequently, only the data for the 200-NTU water samples are given in Fig. 1. The lowest-temperature data (Fig. 1c), which follow the temperature profile of August 13th in Table 1, show no significant reduction in bacterial population. A slight reduction of less than 1 order of magnitude is observed for the intermediate-temperature simulations (Fig. 1b) of the August 17th temperature profile (Table 1). This slight reduction is, however, sustained with no recovery observed 12 h after the simulations were completed. Simulations of the February 25th temperature profile (Table 1) produce a dramatic reduction in bacterial population (Fig. 1a). The viable bacterial count for the test samples shows a reduction of 6 orders of magnitude within 7 h with no corresponding reduction in the control sample count. Samples taken 12 h after the end of the temperature simulation, by which time the water temperature had fallen to 22°C, contained no viable bacteria, showing that bacterial recovery had not occurred.

DISCUSSION

Although the topic of solar disinfection of drinking water has experienced a renewal of interest in recent years, the purely thermal contribution of the solar germicidal action has not been studied in great detail. Wegelin et al. (15) report that the synergism of water temperatures above 55°C enhances the solar fluence germicidal effect by a factor of approximately 2 for *Streptococcus faecalis* and *E. coli* but do not study the thermal effect in isolation. Ciochetti and Metcalf (3) say they regularly recovered unspecified coliforms from water samples heated to temperatures of 50, 55, and 59.5°C in a solar box cooker, although they do not indicate how long the water samples were maintained at these temperatures before being tested for bacteria. Our studies show that water samples, heavily contaminated with *E. coli* (starting population = 20×10^5 CFU/ml) and heated to those temperatures recorded for 2-liter samples exposed to full Kenyan sunshine, are completely disinfected within 7 h. No viable *E. coli* organisms are detected at either the end of the experiment or a further 12 h later, showing that no bacterial recovery has occurred.

Much emphasis has been placed on the role of UV light in the mechanism of solar disinfection of drinking water. Consequently, the need for low-turbidity water samples has been stressed (8, 9). Our results show that solar disinfection is feasible even for high-turbidity (approximately 200 NTU) water that otherwise would not allow incident UV radiation to penetrate very far, provided that the water temperature exceeds 55°C.

Further studies are taking place to characterize the synergistic effect (15) between the thermal and optical processes involved in the disinfection process. Clinical field trials of this technique in the prevention of childhood diarrhea among the Maasai community of southern Kenya are also under way.

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