Field comparison of solar water disinfection (SODIS) efficacy between glass and polyethylene terephalate (PET) plastic bottles under sub-Saharan weather conditions

J. K. Asiimwe, B. Quilty, C. K. Muyanja and K. G. McGuigan

ABSTRACT

Concerns about photodegradation products leaching from plastic bottle material into water during solar water disinfection (SODIS) are a major psychological barrier to increased uptake of SODIS. In this study, a comparison of SODIS efficacy using glass and plastic polyethylene terephalate (PET) bottles was carried out under strong real sunlight and overcast weather conditions at Makerere University in central Uganda. Both clear and turbid natural water samples from shallow wells and open dug wells, respectively, were used. Efficacy was determined from the inactivation of a wild strain of *Escherichia coli* in solar-exposed contaminated water in both glass and PET bottles. The studies reveal no significant difference in SODIS inactivation between glass and PET bottles (95% CI, $p > 0.05$), for all water samples under the different weather conditions except for clear water under overcast conditions where there was a small but significant difference (95% CI, $p = 0.047$) with less viable bacterial counts in PET bottles at two intermediate time points but not at the end of the exposure. The results demonstrate that SODIS efficacy in glass under tropical field conditions is comparable to PET plastic. SODIS users in these regions can choose either of reactors depending on availability and preference of the user.

Key words | glass, PET plastic, SODIS, solar disinfection, water

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INTRODUCTION

Solar disinfection (SODIS) of drinking water is one of the WHO approved point-of-use household water treatment technologies for drinking water (WHO/UNICEF 2011). It requires that water in transparent containers (usually polyethylene terephalate (PET) plastic bottles) be exposed to direct sunlight for a minimum period of 6 h under clear sky conditions in which time waterborne pathogens are inactivated thus making the water safe to drink (Wegelin *et al.* 1994; Conroy *et al.* 1996). Pathogenic inactivation is due to the synergistic effect of ultraviolet (UV) light and heat produced by solar radiation (McGuigan *et al.* 1998; Berney *et al.* 2006). In developing countries, numerous people are without any access to safe drinking water and childhood diarrhoeal diseases continue to kill infants and children in large numbers (WHO/UNICEF 2009, 2010, 2011). To reduce childhood morbidity/mortality, SODIS would be a realistic and cheap option for provision of safe water in such regions since they receive ample sunlight throughout the year.

Studies have shown that PET bottles are safe for SODIS water treatment under the normal SODIS process (Wegelin *et al.* 2001; Schmid *et al.* 2008). It is also recommended that the bottles be replaced after every 6 months to minimise the effects of bottle ageing (Ubomba-Jaswa *et al.* 2010). However, there are still concerns about the use of PET plastic bottles for SODIS water treatment. Such concerns include health risks associated with plasticisers and other carcinogenic compounds which may leach from the bottles into the

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water (Westerhoff *et al.* 2008). Implementation still encounters these concerns from potential users in the developing world (McGuigan *et al.* 2012). Glass, however, is not subject to photodegradation and can be used for substantially longer periods since it is more resistant to material ageing effects associated with PET plastic.

The main objective of this research was to demonstrate that glass bottles are as effective as PET bottles in terms of microbial inactivation. We compared the dynamics of *Escherichia coli* disinfection observed in 1-litre glass and PET SODIS bottles using real sunlight and natural waters of different turbidity levels under dissimilar weather conditions in central Uganda. No studies have been conducted, to the best of our knowledge, to assess SODIS efficacy of both glass and PET bottles in sub-Saharan tropical field conditions. The results of this study would determine whether to promote the use of glass, PET plastic bottles or both in dissemination of SODIS within the Ugandan context.

MATERIALS AND METHODS

A wild strain of *E. coli* (NL-UGA) isolated from water from Ndagwe sub-county, Lwengo district, Uganda was used as the test organism. The organism was isolated using membrane filtration onto chromogenic media (Conda Pronadisa 1340) in earlier experiments (June–December 2011) by the authors to ascertain drinking water quality from the sub-county. We used the indole test (WHO/UNEP 1996), for confirmation of the organism. The *E. coli* isolate was maintained on nutrient agar slants at 4 °C for later spiking of test water.

Sampling

Ten-litre samples of turbid water from open dug wells (Figure 1(a)) and clear water from shallow wells (Figure 1(b)) were obtained on a weekly basis from Ndagwe sub-county in Lwengo district in the central part of Uganda (latitude 00°24′ S, longitude 31°25′ E and altitude 1,300 m). The samples were transported on ice over a 4 h journey to the Department of Food Technology and Nutrition, Makerere University, in Kampala, Uganda for SODIS treatment and subsequent microbial analysis. Unsterilised natural raw water was used to provide a realistic nutrient environment which would not have been possible using distilled and sterilised water (Joyce *et al.* 1996; Ubomba-Jaswa *et al.* 2009). Turbidity was measured in nepholometric turbidity units (NTU) at the sample collection point using a standard turbidity tube (Del Agua, Robens Institute, Guildford, UK; range 5–2,000 NTU). Two turbidity levels of 5 NTU for clear (shallow well) water and 150 NTU for turbid (open dug well) water were used. Sampling was done early in the morning (7:00–8:00am) before water collection by the community commenced. At this time, open dug well water was unagitated, clear and had an average turbidity of 70 NTU. To obtain a turbidity of 150 NTU, we agitated the water in

Figure 1 | (a) A small girl collects water from a typical open dug well in Ndagwe sub-county, Uganda; (b) water sampling at a typical shallow well in Ndagwe sub-county, Uganda.

the well to increase turbidity and mixed it with the collected 70 NTU water until a turbidity of 150 was achieved. Samples from the shallow well were always at 5 NTU. Sampling was carried out from the same open dug well and shallow well for the duration of this study.

Inoculum preparation

E. coli was grown on nutrient broth (Conda Pronadisa 1216) at 37 °C for 18 h to obtain a stationary phase culture. The cells were harvested by centrifugation (Eppendorf AG 22221, Germany) at 2,000 rpm ($570 \times g$) for 10 min and washed with phosphate-buffered saline. Test water was inoculated aseptically with *E. coli* to give an initial bacterial concentration of 10^6 CFU (colony-forming unit)/100 mL for turbid water and 10^8 CFU/100 mL for clear water. Trial tests had shown that a 10^6 CFU/100 mL starting concentration for clear water would yield undetectable bacterial counts as early as the second hour of bottle exposure. A 10^8 CFU/100 mL concentration was therefore chosen to give more time points of detection for clearer results. The seeded water was asceptically poured into clear 1-litre swing top glass or PET bottles for SODIS treatment. For each sample, two glass and two PET bottles were filled (Figure 2). Before filling, bottles were cleaned with warm soapy water and rinsed twice, first with sterile water and finally with test water.

The sample bottles were then laid horizontally on a raised corrugated iron sheet stand and exposed unobscured, in both sunny and overcast weather conditions for a period of 7 h. Control samples in similar containers were stored at room temperature in a dark cupboard in the laboratory. Solar exposure of samples took place at the Makerere University Department of Food Technology, Nutrition and Bioengineering (latitude 0°21′0″ N, longitude 32°34′3″E and altitude 1,202 m) in Kampala, Uganda. Exposure normally started at 9:00am in the morning and lasted until 4:00pm in the afternoon. Samples of 10 mL for the first 3 h of exposure and 100 mL thereafter were taken for analysis at hourly intervals. The temperature of the water in both test and control samples was measured using a standard mercury thermometer. To prevent cross-contamination, separate thermometers for each sample were used. Ultraviolet light (UVA + UVB) (UVA, ultraviolet light of wavelength 400–315 nm; UVB, ultraviolet light of wavelength 315–280 nm) in W/m^2 was recorded using a UVA + UVB digital UV meter (Solarmeter model 5.0, Solartech Inc, USA) which was sensitive over a UVA-UVB range of 280–400 nm (0–-199.9 mW/cm^2). Both temperature and UV measurements were taken at hourly intervals. The experiments on each sample were replicated four times for both sunny and cloudy/overcast weather conditions.

Figure 2 | Clear shallow well water in PET plastic and swing top glass bottles during exposure to direct sunlight at Makerere University, Kampala.

Bacterial enumeration

E. coli was enumerated on chromogenic medium (Conda Pronadisa 1340) using the standard membrane filtration method (USEPA 2002). Appropriate 10-fold serial dilutions from the 10-mL samples were made. A 1-mL sample from an appropriated dilution was then made up to 100 mL using sterile Ringer's solution and filtered through 0.45-μm pore size and 47-mm diameter (GN-6 Metricel Grid, Gelman Sciences Inc., USA) cellulose nitrate membrane filters. Where dilutions were not made, 100 mL of sample was filtered. Following incubation at 37 °C for 24 h, all violet-dark blue colonies were counted as *E. coli*. Counts were expressed as numbers of *E. coli*/100 mL of water. The detection limit was 1 CFU/100 mL for all water samples.

Data analysis

All samples were analysed in duplicate. Since weather conditions could not be controlled, the best set of results obtained on both predominantly sunny and overcast days

are reported. The data were statistically analysed using paired sample t-tests (SPSS for windows version 116.0, 2007 SPSS Inc.) and graphs were created using Sigmaplot 2000 graphing software. Data points in the graphs represent the four averages of four duplicate plate counts (two from each bottle) used in data analysis and the error bars represent the standard deviations.

RESULTS

Figures 3 and 4 show the different inactivation curves of *E. coli* in water under dissimilar weather conditions. Only data points at T_0 and T_7 for control samples are plotted since there was no significant difference in bacterial concentrations at all times (full data set available upon request). Temperature readings in both glass and PET reactors were similar under all weather conditions.

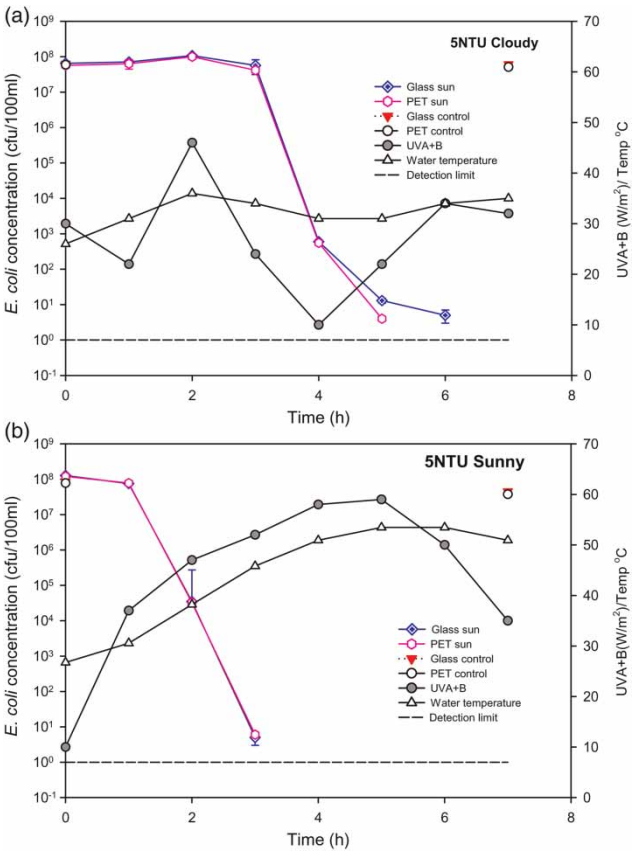

Figure 3 | Inactivation curves of a wild strain of *E. coli* in glass and PET bottles exposed under varying conditions of turbidity and sunlight: (a) clear (5 NTU) water and overcast/cloudy conditions; (b) clear (5 NTU) water and natural full strong sunlight conditions. The dashed horizontal line represents the limit of detection (1 CFU/100 mL). Bacterial plots which end before the 7 h time point indicate that the population has dropped below the limit of detection.

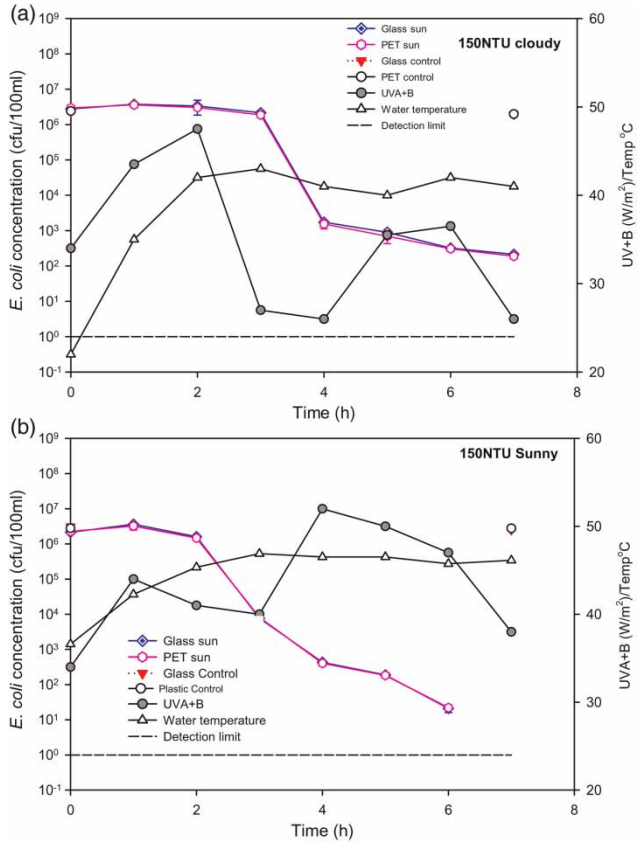

Figure 4 | Inactivation curves of a wild strain of *E. coli* in glass and PET bottles exposed under varying conditions of turbidity and sunlight: (a) turbid (150 NTU) water under natural cloudy/overcast conditions; (b) turbid (150 NTU) water and full strong sunlight conditions. The dashed horizontal line represents the limit of detection (1 CFU/100 mL). Bacterial plots which end before the 7 h time point indicate that the population has dropped below the limit of detection.

Generally, the lag phase of bacterial growth before start of inactivation was shorter in sunny conditions for both clear and turbid water as compared to cloudy conditions. In clear water under sunny conditions, inactivation started after the first hour while in turbid water, bacterial inactivation started after 2 h. In comparison, inactivation for both clear and turbid water under cloudy conditions started after the third hour of exposure with a gradual rate of cell death as compared to the steep drop in bacterial numbers experienced in clear water under sunny conditions.

A wide range of sunshine and cloud conditions were encountered during these experiments. Ultraviolet (UVA + B) light levels ranged from a minimum of 9 W/m^2 in early

morning conditions to a maximum of 60 W/m^2 on completely clear sunny days. The water temperatures ranged from a low of 22 °C (turbid water in cloudy weather) at the start of the experiments to a maximum of 47 °C during maximum sunshine conditions for both turbid and clear water. In clear water under sunny conditions, the average irradiance and temperature recorded was 43.5 W/m^2 and 39.4 °C, while in turbid water under the same conditions, the same recordings were 43.3 W/m^2 and 41.6 °C, respectively. In both cases, the highest temperature recorded was 47 °C. In clear water, temperature was at this peak of 47 °C for 2 h compared to 1 h in turbid water. In comparison, under cloudy conditions, the average irradiance and temperature for clear water was 27.5 W/m^2 and 32.3 °C while that of turbid water was 29 W/m^2 and 38.3 °C.

Statistical analysis showed no significant difference ($p > 0.05$, 95% CI) in bacterial inactivation in all water samples between glass and PET bottles for all weather conditions with the exception of clear water under cloudy conditions. Here, there was a slight statistical difference between glass and PET reactors during the fifth and sixth hours. At these time points, glass had 13 ± 2 CFU/100 mL compared to 4 ± 0.8 CFU/100 mL in PET at T_5 while at T_6 there was 5 ± 1.4 CFU/100 mL in glass compared to undetectable viable counts in PET. However, by the last hour of exposure (T_7), no bacteria were detected in either water sample (detection limit =1 CFU/100 mL). Table 1 shows the statistical analysis results of the water samples under different weather conditions.

During strong sunny conditions, complete inactivation of *E. coli* from a starting concentration of 10^8 CFU/100 mL at T_0 to below limit of detection (1 CFU/100 mL) was achieved within the first 3 h for clear (5 NTU) water representing a reduction of at least 7-log units in bacterial concentration in both glass and PET reactors. In comparison, bacterial inactivation from 10^6 CFU/100 mL to undetectable levels in 150 NTU turbid water under the same weather conditions for both reactors was achieved after a 6 h exposure period representing a 5-log unit reduction value. Bacterial inactivation to below the limit of detection in clear water under overcast weather conditions (Figure 4(b)) was achieved after 6 h of exposure. In comparison, although there was a reduction in bacterial concentration in turbid water under overcast conditions (Figure 4(a)), inactivation to below detectable limits was not achieved by the last hour of exposure T_7. At this time point, bacterial concentration was at 2.12×10^2 and 1.89×10^2 CFU/100 mL in glass and PET bottles, respectively.

Table 1 | Paired sample t-test (95% CI) results of *E. coli* concentrations in solar exposed water (glass and PET) under sunny and cloudy weather conditions for both 5 NTU and 150 NTU water

Weather conditions	Turbidity (NTU)	*p*-value
Sunny	5	0.563
Sunny	150	0.381
Cloudy (overcast)	5	0.047[a]
Cloudy (overcast)	150	0.266

[a]Significant difference.

DISCUSSION

Although a lot of work has been carried out to assess efficacy of SODIS in drinking water treatment, the material of choice has been PET rather than glass mainly because PET plastic is more easily obtained in addition to being more robust, light weight and not prone to breakages (Wegelin *et al.* 2001). Glass, though not frequently used, is inert and less prone to surface scratches (which reduce optical transmission) than PET for SODIS purposes. Results of our study show that *E. coli* inactivation in glass and PET under similar weather conditions is comparable. Our results are in agreement with those of Sommer *et al.* (1997), who report comparable bacterial (faecal coliforms) and viral (*V. cholerae*) inactivation between glass and PET bottles under similar laboratory and field conditions.

One of the frequently identified psychological barriers to SODIS use is that of the potential health risks associated with leaching of chemical compounds from PET bottles (McGuigan *et al.* 2012). Montuori *et al.* (2008), in a study to assess human exposure to phthalic acid and phthalate esters from water packed in PET and glass bottles, reported that the concentrations of phthalates were nearly 20 times higher in sampled PET bottles than in glass bottles. However, the concentration of these phthalates in PET bottles did not present any risk to human health as they contributed less than 0.1% of the maximum allowable US

Environmental Protection Agency (EPA) phthalate reference doses (RfDs). The RfDs estimate daily oral exposure to the human population that is likely to be without appreciable risks of deleterious effects during a lifetime. Shotyk & Krachler (2007) and Westerhoff *et al.* (2008) have also reported leaching of antimony into water packed in PET bottles. In their study of over 132 brands of bottled water from 28 countries, Shotyk & Krachler (2007) report an average antimony leaching of 19% (Canadian) and 90% European brands in water stored over 6 months at room temperature. Westerhoff and colleagues report an average of 13 days and a temperature of 85 °C needed to leach 6 μL or more of antimony, the USEPA maximum allowable level in PET bottled water. All conditions reported in these studies are not typical of the SODIS process. A recent study by Schmid *et al.* (2008) to assess health risks associated with migration of plasticisers and chemical compounds into water exposed in PET bottles under a typical SODIS process reveals no associated human health risks. These authors further reported the maximum concentration levels of di(2-ethylhexyl)adipate (DEHA) and di(2-ethylhexyl)phthalate (DEHP) as 0.046 and 0.71 μg/L, respectively. These values were below the WHO drinking guidelines daily consumption limits of 80 and 8 μg/L for DEHA and DEHP, respectively (WHO 2011). Moreover, both DEHA and DEHP have a short-term low toxicity, and are not genotoxic. They have also been placed in Group 3 by the International Agency for Research on Cancer (IARC), meaning that they are not classifiable as to their carcinogenicity to humans (WHO 2011). Although all these studies show that the level of chemical compounds leaching from PET bottles into water do not pose health risks under proper SODIS conditions, misgivings still linger among some would-be users. This mainly stems from disclaimers made by manufacturers who instruct users not to re-use plastic bottles (McGuigan *et al.* 2012). Glass can therefore be used as an alternative. However, since PET bottles are typically easier to obtain than glass, they should still be promoted for those who are not able to get glass. It is worthwhile to note that the risk of diseases contracted through consumption of microbiologically contaminated water outweigh the perceived risks associated with leaching from PET into solar exposed water.

Ubomba-Jaswa and co-workers (2010) investigated the genotoxicity of solar disinfected water using bottles that were in use for 6 months under strong sunshine conditions in southern Spain. Their recommendation that PET SODIS bottles be replaced every 6 months reflects the duration and limits of their study rather than any indication that genotoxic risks occur after this time. Replacing the bottle after 6 months also helps to avoid the effects of ageing such as scratches which may hinder effective absorption and transmission of UV light hence affecting SODIS efficacy (Ubomba-Jaswa *et al.* 2010). Clearly, glass bottles have an advantage since they do not suffer the effects of ageing and can therefore be used for longer periods. This, in turn, will reduce the cost of water treatment since there would be no need for frequent replacement of bottles unless breakage occurs.

Under strong sunny tropical conditions where high temperatures (>65 °C) can easily be attained during SODIS, PET bottles may not be the container of choice since they are susceptible to deformation unlike glass which can withstand such temperatures. However, glass is more fragile and bulkier than plastic and may prove cumbersome to users, especially in cases where batches of bottles have to be filled every day. In addition, the fact that glass can easily break and therefore cause accidents is a concern, especially in situations where children are the ones responsible for SODIS. In such cases, PET bottles should be encouraged since they pose a minimal risk of accidents.

Finally, glass also may be prone to theft since in most developing countries a financial deposit is made on a glass bottle at the point of purchase (McGuigan *et al.* 2012) such that when a client returns the bottle, this deposit is refunded. Should the bottles be exposed in areas not deemed safe they may be susceptible to theft by individuals who may want to claim this refund. In such cases, PET bottles would be more feasible since there are no financial refunds attached and bottles are normally discarded or put to other use after initial purchase.

It therefore remains the end-user's choice depending on accessibility and cost of either glass or PET bottles, concerns of health risks that may be associated with PET bottles and other factors such as container portability that will determine whether to use PET or glass bottles for SODIS water treatment.

E. coli was chosen as the test organism because of its universal acceptance as an indicator of faecal

contamination of drinking water (WHO/UNICEF 2010). Also in previous studies by the authors (unpublished), *E. coli* proved to be more resistant to SODIS relative to *Enterococcus faecalis* which was also commonly isolated in the same drinking water. These findings were in agreement with other studies which have shown that *E. coli* is more resistant to the bactericidal effect of the sun than other bacteria such as *Campylobacter jejuni, S. epidermidis, Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhimurium* and *Salmonella enteritidis* (Wegelin *et al.* 1994; Kehoe *et al.* 2004; Boyle *et al.* 2008). Consequently, *E. coli* was deemed to be the most suitable indicator of SODIS efficacy in glass and PET bottles for this study.

Two turbidity levels of 5 NTU (shallow well water) and 150 NTU (open dug well water) were used because normal turbidity of the sampled water sources varied at different times of day, with some sources having turbidity levels as high as 400 NTU, especially after heavy rain or intense use (people often wade into open dug wells during the collection process). This phenomenon was mostly encountered with open dug wells during previous research on drinking water quality from the sub-county. The shallow well water was normally at 5 NTU turbidity level. Studies such as those by Joyce *et al.* (1996) and McGuigan *et al.* (1998) have shown that SODIS bacterial inactivation can even occur in turbid water as high as 200 NTU under strong sunny conditions due to heat/temperatures produced by the irradiance of the sun. This is in contrast to other studies which report a water turbidity of 30 NTU as a threshold for SODIS efficacy (Fujioka *et al.* 1981; Meierhofer & Landolt 2009). Joyce *et al.* (1996) were able to show that a wild Kenyan strain of *E. coli* in turbid water (200 NTU) could be inactivated to undetectable levels from a starting concentration of 20×10^5 CFU/mL within 7 h after attainment of 55 °C. The elevated temperatures reached during these strong sunny conditions cause bacterial inhibition through pasteurisation and inhibition of bacterial DNA repair mechanisms (McGuigan *et al.* 2012). It should be noted, however, that water turbidity was not natural and these experiments took place in controlled laboratory conditions which are not typical of field conditions. It was therefore important to also test natural water of higher turbidity under field conditions to cater for those communities that relied on water from open dug wells that were generally very turbid. A turbidity level of 150 NTU was used to represent samples from such wells.

Unlike Joyce *et al.* (1996), in our study, the highest temperature attained in turbid water under sunny conditions was 47 °C and a complete bacterial inactivation was achieved after 6 h. This could have been due to the lower turbidity (150 NTU) which would have reduced the opacity of the water as opposed to the 200 NTU turbidity level of water used by Joyce and colleagues. However, the temperatures recorded in turbid water (150 NTU) under overcast conditions in our study were not high enough to bring about complete inactivation. The highest temperature experienced was 43 °C and this was only for about an hour. It is for this reason that SODIS promoters recommend exposing such water for two consecutive days under extended cloudy conditions (EAWAG 2011). Where possible, highly turbid water can also be filtered to reduce turbidity before SODIS treatment (Sommer *et al.* 1997).

Bacterial inactivation rates in clear water under sunny conditions are in agreement with many other studies (Wegelin *et al.* 1994; Joyce *et al.* 1996; McGuigan *et al.* 1998; Kehoe *et al.* 2001; Ubomba-Jaswa *et al.* 2008) where synergy between temperature (≥45 °C) and optical irradiance (UVA) increases bacterial inactivation. A complete inactivation of *E. coli* was reported to be normally achieved within the first 3 h or less under strong sunny conditions (Boyle *et al.* 2008). In our study, temperatures in clear water under sunny conditions were already at 45 °C by the fourth hour of exposure peaking at 47 °C at hours 5 and 6. Bacterial inactivation in clear water under cloudy conditions to below detection levels was achieved after 6 h of exposure and the highest temperature attained was only 36 °C, well below ≥45 °C needed for thermal and optical synergistic effect hence the longer inactivation time.

CONCLUSIONS

This study has shown that glass bottles are as effective as PET plastic SODIS bottles for inactivating *E. coli* in drinking water in sub-Saharan field conditions. It therefore remains the end-user's choice whether to use glass or PET

bottles depending on factors such as availability, affordability and portability.

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