



# Good optical transparency is not an essential requirement for effective solar water disinfection (SODIS) containers

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## ABSTRACT

The efficacy of 10 L polypropylene (PP) transparent jerry cans (TJCs) to inactivate *E. coli*, MS2-phage and *Cryptosporidium parvum* via solar water disinfection (SODIS) was tested in well water or general test water under natural sunlight. Food-safe PP was used to manufacture the TJCs and a clarifying agent was added to improve optical transparency in the UV–visible range. 10 L PP TJCs and 2 L polyethylene terephthalate (PET) bottles were filled with well water, spiked separately with ( $\sim 10^6$  CFU/mL of *E. coli*,  $\sim 10^6$  PFU/mL of MS2 phage and  $5 \times 10^5$  *C. parvum* oocysts per litre) and exposed to natural sunlight for 6 h. While the 10 L PP TJC prototype had poorer transparency (UV-B 0.001%, UV-A 4.29%, and visible 92% for TJCs without clarifier and UV-B 1.36%, UV-A 8.01%, and visible 90.01% for TJCs with clarifier) than standard 2 L PET (UV-B 0.72%, UV-A 10–85%, and visible 80–90%); log reduction values (LRVs) > 5, 2 and 0.8 for *E. coli*, MS2-phage, and *C. parvum*, respectively, were observed for the TJCs within six hours respectively, which is a minimum standard for drinking water established by the World Health Organisation (WHO). We observed similar inactivation kinetics for all three organisms in PP TJCs and PET bottles despite the poorer optical transparency properties of the SODIS jerry cans. Therefore, for effective SODIS, container optical transparency is not as important as previously believed. We conclude that good visible transparency is not a necessary requirement for containers intended for SODIS use.

## 1. Introduction

In many parts of the world, there is a demand for potable water and people struggle for access to safe drinking water [1]. Many communities

in water scarce areas have no alternative but to rely on contaminated water sources for drinking purposes [2]. Globally, 2 billion people still consume drinking water contaminated with faecal matter of which 2.9 million people are affected annually by waterborne diseases such as

**Abbreviations:** BPA, Bisphenol-A; GTW, General Test water; LRV, Log reduction value; PBS, Phosphate buffer saline; PC, Polycarbonate; PET, Polyethylene terephthalate; PP, Polypropylene; PMMA, Polymethyl methacrylate; ROS, Reactive oxygen species; RT-qPCR, Reverse transcription-qPCR; SODIS, Solar water disinfection; TDS, Total dissolved solids; TJC, Transparent jerry can; TOC, Total organic carbon; TYC, Tryptone yeast glucose; W/out CL, Without clarifier; WCL, With clarifier; WDC, Water dispenser container; WHO, World Health Organization.

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diarrhoea, cholera, dysentery, typhoid, etc. Approximately, 0.83 million deaths occur each year due to diarrheal incidences only where children under five years of age are being more susceptible to these diseases [3]. Solar water disinfection (SODIS) is a point-of-use household water treatment that is employed in resource-poor settings [1,4,5]. The subsequent inactivation of microorganisms occurs due to the effect of solar ultraviolet (UV) radiation and high temperature, which is transmitted through the container wall [6–8].

SODIS typically involves filling of transparent plastic containers with available water, which are then exposed to strong, direct natural sunlight for 6–8 h [1]. The inactivation of microorganisms in SODIS results from both direct and indirect cell damage. Pathogens absorb UV photons that directly damage cellular organelles leading to cell death [9]. Indirect inactivation involves two different mechanisms: (i) indirect endogenous damage, where reactive oxygen species (ROS) such as hydroxyl radicals ( $HO^\bullet$ ), peroxide ions ( $O_2^{\bullet-}$ ), superoxide anions ( $O_2^{\bullet-}$ ), singlet oxygen ( $^1O_2$ ), and hydrogen peroxide ( $H_2O_2$ ) are produced causing oxidative stress; and (ii) indirect exogenous damage where ionic components in water such as sulphates, phosphates, nitrates, nitrites, etc. are responsible for the formation of ROS [9–11]. The damage to the cell structure of pathogens is caused by the absorption of UV-B by enzymes, vitamins, acids, RNA, DNA, proteins, chromophores, etc. and is thought to be the major cause of solar irradiation-induced cell damage [12–15]. Therefore, it is reasonable to expect that the optical transparency of containers might play a significant role in SODIS as both direct and indirect cell damage depends on the presence of solar UV photons in the water [16].

In most SODIS studies, 1.5–2 L PET bottles are typically used and are found to be effective for the inactivation of pathogens [4,17]. With a typical maximum volume of 2 L per bottle, several containers must be treated in parallel to provide sufficient water for most households. This is an obstacle to SODIS uptake [16]. Containers made of different plastic materials such as polypropylene (PP), polyethylene terephthalate (PET), and polycarbonate (PC), inter alia, have been used for SODIS purposes [16].

Many researchers have investigated the microbicidal efficacy of such containers for SODIS purposes and have shown that containers manufactured from transparent PC can inactivate waterborne pathogens. Thus, 19 L PC water dispenser containers (WDC) have shown good SODIS efficacy despite having low UV transmission properties [17]. However, PC is not a suitable material for SODIS containers since it is known for the leaching of bisphenol-A (BPA) into treated water [18]. Transparent 20 L PP buckets manufactured in Malawi demonstrated good SODIS inactivation rates for *Escherichia coli*, MS2-phage, and *Cryptosporidium parvum* [12]. Polo-López et al. demonstrated that UV-B is shown to be more lethal to pathogens than UV-A. PET bottles are entirely opaque to UV-B radiation whereas PP containers can transmit 6% of solar UV-B radiation [12] and provide better disinfection performance results than PET containers.

When larger volume containers are used for SODIS, the effect of physicochemical characteristics of water must be carefully studied [10]. Geographical variations in weather conditions also influence the efficacy of SODIS, and demand a significant safety factor to be added to the typical recommended exposure times that ranges from 6 h on bright days to up to 48 h in cloudy conditions [10]. Irradiance, temperature, and physicochemical characteristics of water to be treated - all have a substantial impact on the pathogen - inactivation efficiency [19]. Therefore, the UV transmission properties of container materials play an important role in SODIS, as the process is mainly driven by UV photons transmitted through container walls [10]. In addition, the response of different pathogenic species varies according to the wavelength of photons absorbed by the microorganisms [16].

The experiments reported in this article were part of a larger project whose objective was to develop transparent 10 L SODIS containers. Initial prototypes were manufactured and were observed to be more

translucent than transparent. An attempt to improve transparency was made by adding an optical clarifying agent to the polymer prior to the moulding process. SODIS efficacies of both prototypes of transparent jerry cans TJC (with clarifier (WCL) and without clarifier (W/out CL)) to inactivate microorganisms (*E. coli*, MS2 bacteriophage and oocysts of *C. parvum*) were tested and compared against 2 L PET bottles under natural sunlight using well water in laboratory settings in India (CSIR-NEERI, Nagpur, 21.1°N, 79.0°E), and in Spain (PSA, Almeria, 37.0°N, 2.3°W; University of Santiago de Compostela, 42.8°N, 8.5°W). The germicidal efficacy of these SODIS TJC after prolonged exposure to outdoor conditions at one of the facilities in Southern Spain (PSA) for 9 months was also assessed.

## 2. Materials and methods

### 2.1. Transparent PP 10 L TJC

TJCs of 10 L volume were manufactured using food-safe PP via blow-moulding. PP was selected to manufacture these prototype TJCs due to their ease of availability [16], low cost and suitability for blow-moulding. The TJCs were designed with a volume of 10 L and a handle for ease of carrying. To maintain the compactness and durability of the prototype, a container wall thickness of 2–3 mm was used.

A clarifying agent (CL-PPRO, Penn Color, Pennsylvania, United States; 3% by weight of polypropylenePP) was added to the formulation of the PP material to improve the optical transparency of the prototype (TJC WCL) and the disinfection performance was compared with 10 L TJC manufactured without using any clarifying agent (TJC W/out CL) and with standard 2 L PET bottles.

The optical transmittance spectra of the materials of containers of 10 L PP TJCs and 2 L PET bottles were measured, by cutting them into ( $2\text{ cm} \times 2\text{ cm}$ ) pieces, using a UV-Vis spectrophotometer (Evolution 220, Thermo Fisher Scientific, Massachusetts, USA) and are displayed in Fig. 1.

### 2.2. Experimental design

The inactivation efficiency of the TJCs (WCL and W/out CL) was assessed at CSIR-NEERI, Nagpur, India, CIEMAT-Plataforma Solar de Almeria (CIEMAT-PSA), Almeria, Spain, and the University of Santiago de Compostela (USC), Spain.

The following experimental tests were carried out at each location under natural sunlight: (i) *E. coli* inactivation at CSIR-NEERI, (ii) *E. coli*

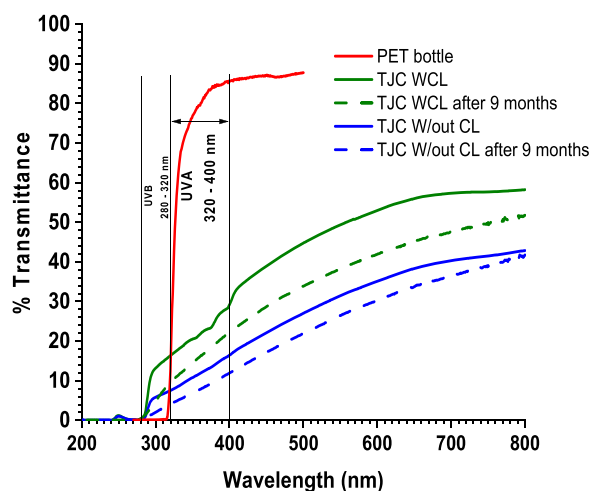


Fig. 1. : Comparison of UV-Vis transmittance from wall pieces of 2 L polyethylene terephthalate (PET) bottle and 10 L polypropylene (PP) transparent jerry cans (TJCs) with (WCL) and without optical clarifier (W/out CL) after 9 months weathered and un-weathered under natural sunlight.

and MS2 phage inactivation at CIEMAT-PSA, (iii) *C. parvum* inactivation at USC, and (iv) ageing of TJC's for 9 months at CIEMAT-PSA. Viral and protozoan studies were only possible in CIEMAT-PSA and USC, respectively. However, *E. coli* inactivation studies were conducted in parallel at both CIEMAT-PSA in Spain and CSIR-NEERI in India. This allowed a comparison of the effect of outdoor natural conditions at both locations, on the SODIS efficiency for *E. coli* inactivation by the designed prototypes in the hope of being able to extrapolate these results obtained for MS2 virus and *C. parvum* oocysts.

The experimental procedure in all cases was as follows: TJC's (WCL and W/out CL) and 2 L PET bottles (as routinely used containers in ordinary SODIS) were filled with water spiked with each microorganism separately ( $\sim 10^6$  CFU/mL of *E. coli*,  $\sim 10^6$  PFU/mL of MS2 bacteriophage and  $5 \times 10^5$  *C. parvum* oocysts per litre) and exposed to natural outdoor conditions for 5–6 h (Fig. 2). Identical controls were stored in the dark. Samples of volume 2 mL for *E. coli* and MS2 bacteriophage and for *C. parvum* 2 L were withdrawn at regular intervals and the number of microorganisms was counted. All SODIS experiments were conducted at least in triplicate from each container to check the variations in the results. The graphed data are the average of replicates, with the error bar indicating standard deviation. Water temperature and intensity of solar UV-A+B radiation and solar UV-A intensity were monitored during the treatment time. At NEERI, temperature was recorded using an Amber Thermometer (Gujrat, India) and a Lutron UV-340A light meter, 290–390 nm, (Taiwan) was used to measure solar UV irradiance. At CIEMAT-PSA, the water temperature was measured by using a digital thermometer (Checktemp, Hanna Instruments, Spain), and a pyranometer (CUV-5, Kipp & Zonen, Netherlands) was used to measure the solar UV irradiance (280–400 nm) expressed in  $\text{W/m}^2$ . At USC, water temperature was monitored by using a Temp 3JKT thermometer (Eutech Instruments Pte Ltd., Singapore) and solar irradiance was measured with a radiometer PMA2100 fitted with a PMA2107 digital non-weighted UV-A + B Sensor (280–400 nm) (Solar Light® Company, Inc, Glenside, Pennsylvania, USA).

The total UV energy dose received per unit of illuminated surface was calculated using Eq. (1), where  $t$  is the experimental time and  $UV(t)$  is the measured solar UV irradiance.

$$UV\text{-Dose} = \int UV(t) dt \quad (1)$$

### 2.3. Water matrices

Actual well - water or general test water (GTW) prepared according to the World Health Organization (WHO) guidelines WHO [3] were used in all SODIS experiments. Actual well water was autoclaved (121 °C for 15 min) to remove any naturally occurring microbial populations. The physicochemical characteristics of the three water matrices used at each

location were similar (Table 1). Therefore, the results obtained can be compared discarding the water matrix as a key influence factor on the microbial inactivation kinetics.

### 2.4. Microbial enumeration and quantification

To evaluate the capability of the SODIS container for disinfection under real sunlight, studies were carried out using bacterial, viral, and protozoan pathogens.

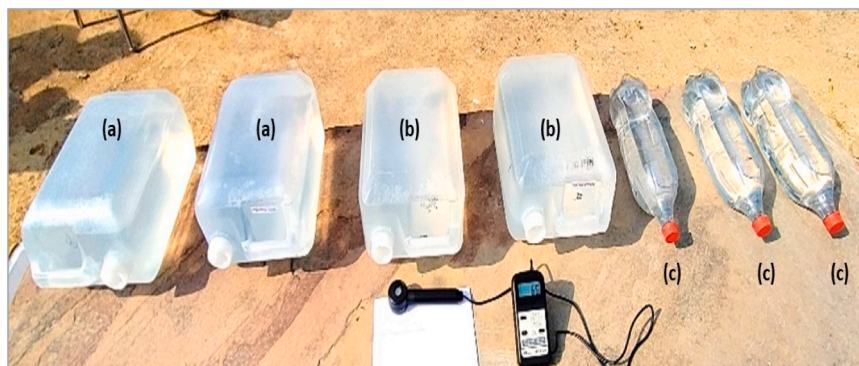
#### 2.4.1. *Escherichia coli*

*E. coli* (ATCC 25922) obtained from preserved pure culture from CSIR-NEERI was used. A single colony of *E. coli* was inoculated in 14 mL sterile Luria Bertani broth (Miller's LB Broth, Hi Media, Mumbai, India) containing following chemicals: Casein enzymic hydrolysate (10 g/L), Yeast extract (5 g/L), Sodium chloride (10 g/L) and final pH (at 25 °C)  $7.5 \pm 0.2$  was then incubated for 18–24 h at 37 °C under continuous agitation. The bacterial suspensions were then centrifuged at 3000 g for 10 min and the pellet obtained was re-suspended in 14 mL sterile phosphate buffer saline (PBS, pH 7.4, Hi Media, Mumbai, India) containing following chemicals: Sodium chloride (7.65 g/L), Disodium phosphate Anhydrous (0.724 g/L) and Dipotassium hydrogen phosphate (0.21 g/L). The bacterial suspension was spiked in autoclaved well water to obtain an initial concentration of  $\sim 10^6$  CFU/mL. Each sample was diluted by up to three-fold serial dilutions and the standard plate count method was used to enumerate bacterial cell reduction on Endo agar media plates (Endo Agar, HiMedia, Mumbai, India) containing following chemicals: Peptone (10 g/L), Lactose (10 g/L), Dipotassium hydrogen phosphate (3.5 g/L), Sodium sulphite (2.5 g/L), Basic fuchsin (0.5 g/L) and Agar (15 g/L). The plates were then incubated for 24 h at 37 °C and the bacterial colonies were counted.

**Table 1**

Characteristics of the water matrices used in the evaluation of the microbial inactivation capability of 10 L polypropylene (PP) transparent jerry cans (TJC's).

Parameters	Unit	NEERI Well-water	PSA Well-water	USC General test water (GTW)
pH		7.3	7.6	7.14
Turbidity	NTU	< 1	< 0.5	< 1
Total organic carbon (TOC)	mg/L	-	< 0.5	3.0
Total dissolved solids (TDS)		-	-	275 $\pm$ 225
Alkalinity (as $\text{CaCO}_3$ )			101.5	100 $\pm$ 20
Chloride ( $\text{Cl}^-$ )		20.0	22.8	-
Sulphate ( $\text{SO}_4^{2-}$ )		11.73	11.75	-
Nitrate ( $\text{NO}_3^-$ )		0.03	0.48	-
Chlorine		No	No	< 0.05



**Fig. 2.** Solar exposure of transparent 10 L polypropylene (PP) transparent jerry cans (TJC's) (a) with optical clarifier, (b) without optical clarifier and (c) 2 L PET bottles.



#### 2.4.2. MS2 bacteriophage

Tryptone Yeast Glucose (TYG) medium containing the following Sigma-Aldrich chemicals: Tryptone (10.0 g/L) Yeast Extract (1.0 g/L), NaCl (8.0 g/L), Glucose (10.0 g/L), CaCl<sub>2</sub> (2.94 g/L) and Thiamine (0.1 g/L) was used to enumerate and quantify MS2 (ATCC 15597B1) infective particle stocks and the enumeration procedure [12].  $\sim 10^6$  PFU/mL of MS2 bacteriophage was used to spike in the well water used for experiments. Prior to MS2 enumeration, the host *E. coli* C300 (ATCC 15597) was cultured for 6 h in fresh liquid medium (TYG) at 37 °C under rotatory agitation (90 rpm). A double-layer agar method was used to count infective MS2 particles. Briefly, 1 mL *E. coli* C300 is combined with 0.1 – 0.5 mL sample (or 10-fold dilutions) in PBS and 5 mL melted semi-solid TYG agar.

#### 2.4.3. *Cryptosporidium parvum*

*Cryptosporidium* oocysts were obtained from faeces collected directly from a naturally infected neonatal Friesian–Holstein calf by rectal sampling. Faecal sample was homogenised in PBS 0.04 M, pH 7.2, filtered through two sieves (mesh sizes 150 and 45  $\mu$ m), shaken vigorously with diethyl ether (2:1, v/v) and concentrated by centrifugation at 2000g, for 15 min, at 4 °C. The sediment was washed with PBS (0.04 M, pH 7.2) by centrifugation at 2000 g for 15 min at 4 °C and *Cryptosporidium* oocysts were purified on discontinuous caesium chloride gradients of 1.05, 1.10 and 1.40 g/mL by centrifugation at 2000 g for 30 min at 4 °C. The isolate was identified as *C. parvum* (subtype IIaA15G2R1) by PCR amplification and sequence analysis of fragments of  $\sim 587$  bp fragment of the small subunit rDNA gene (*SSU rDNA*) [20] and 850 bp of the 60 kDa glycoprotein (GP60) [21].

The initial concentration of *C. parvum* was  $5 \times 10^5$  oocysts per litre were spiked in GTW for SODIS experiments. Samples from TJC and PET bottles were taken every 2 h, 2 L volume of sample was withdrawn and vacuum filtered through nitrocellulose membranes with a pore size of 3  $\mu$ m (Merck Millipore Ltd., Carrigtwohill, Ireland). The membranes were removed from the vacuum filter and rinsed three times with 5 mL of PBS in re-sealable polyethylene bags. A modified Neubauer haemocytometer was used to count the number of oocyst structures in the sediment after centrifugation at 2000 g for 15 min. The oocyst viability was determined using aliquots containing  $10^5$  oocysts by quantitative reverse transcription-PCR (RT-qPCR) targeting the messenger RNA (mRNA) of the 70 kDa heat shock protein (hsp70) gene of *C. parvum* [22], prior induction at 45 °C for 20 min and mRNA extraction (Dyna-beads® mRNA DIRECT™ Kit, Invitrogen, Thermo Fisher Scientific, Vilnius, Lithuania). After each sampling, the volume of GTW contained in the 10 L TJC was kept at its full capacity by refilling the 10 L TJC with clean GTW.

#### 2.4.4. Ageing test of 9-month-old TJC

The TJC ageing study was carried out by exposing the 10 L PP TJC under uninterrupted, natural solar radiation for 9 months (October-2021 to June 2022) at CIEMAT-PSA, Almeria, Spain. TJC pieces of dimension 2 cm  $\times$  2 cm were cut out from the top and bottom of the exposed side wall of the TJC. Plastic degradation was measured by transmittance scanning from 270 – 600 nm using a UV-Vis Spectrophotometer (Evolution 220, Thermo Fisher Scientific).

The bactericidal efficacy of 9-month-old (weathered) TJC was also tested under natural sunlight conditions. TJC (WCL and W/out CL) were filled with well water spiked with  $\sim 10^6$  CFU/mL of *E. coli* K-12 from CIEMAT-PSA and exposed to sunlight for 5 – 6 h. Identical controls were kept in the dark. Samples were withdrawn at intervals of 30 min and the number of microorganisms was counted using the spread plate method as described in Section 2.4.1.

#### 2.4.5. Statistical analysis

The graphics and one-way ANOVA statistical analysis was accomplished using OriginPro (2023 ver. 10.0.0.154, Copyright © 1991–2022 OriginLab Corporation, Northampton, MA, USA). The microbial

inactivation results plotted in the graphs are the average of at least triplicates with the error bar representing the corresponding standard deviation. Significant differences between replicates were considered at  $p$  values < 0.05 (ANOVA), nevertheless, the results obtained showed no significant differences between both replicates and the different TJC investigated in this study.

### 3. Results

Fig. 3 depicts the inactivation profile of *E. coli* in both types of 10 L PP TJC under natural sunlight in comparison with 2 L PET bottles. *E. coli* LRVs of  $5.26 \pm 0.11$  and  $5.62 \pm 1.03$  were obtained for the W/out CL and WCL TJC, respectively, whereas the PET bottle showed  $6.54 \pm 0.07$  LRV on full sunny days after 6 h of exposure. While the water temperature in TJC (WCL and W/out CL) increased from 24 °C to 41 °C and 40 °C, respectively, in the PET bottle it rose from the same starting point to 45 °C. The initial ambient air temperature recorded at the start of the experiment was 29 °C (10:30–16:30 h, local time), and at the end of 6 h of exposure, the ambient temperature was 37 °C. No change in the concentration of *E. coli* was observed in control samples. The cumulative UV-A irradiance dose after six hours was 1100.16 kJ/m<sup>2</sup>.

In all experiments carried out with *E. coli*, the inactivation efficacy of SODIS achieved using TJC in comparison with PET bottles showed LRV > 4.0 which is a drinking water pre-requisite value set by WHO in its Harmonised Testing methodology as part of the international plan to assess household water treatment [3]. In every instance, the detection limit (DL = 2 CFU/mL) was reached, indicating LRV > 5 in the case of *E. coli* after 6 h of solar exposure.

Fig. 4a shows the results of MS2 virus inactivation studies. LRVs of  $2.34 \pm 0.20$  and  $2.42 \pm 0.04$  were obtained for the W/out CL and WCL TJC, respectively. The differences observed between LRVs obtained using the WCL & W/out CL TJC models were negligible demonstrating that the presence of the clarifier did not impart any additional improvement in the SODIS performance of these containers. The LRV achieved using 2 L PET bottles was  $1.74 \pm 0.26$  after 5 h of solar exposure on full sunny days (763 kJ/m<sup>2</sup>). Water temperature was in all cases 25 °C at the beginning of the experiment, a similar maximum value was attained in both TJC (40.5 °C) while a slightly higher value for PET bottles (43.8 °C) was observed after 5 h of solar exposure.

In relation to *C. parvum*, the oocyst LRVs obtained after 6 h of solar exposure in 10 L TJC (WCL and W/out CL) and 2 L PET bottles were 0.73, 0.88 and 0.18, respectively. The days during which the assays were carried out were sunny with some cloud cover. The mean value for

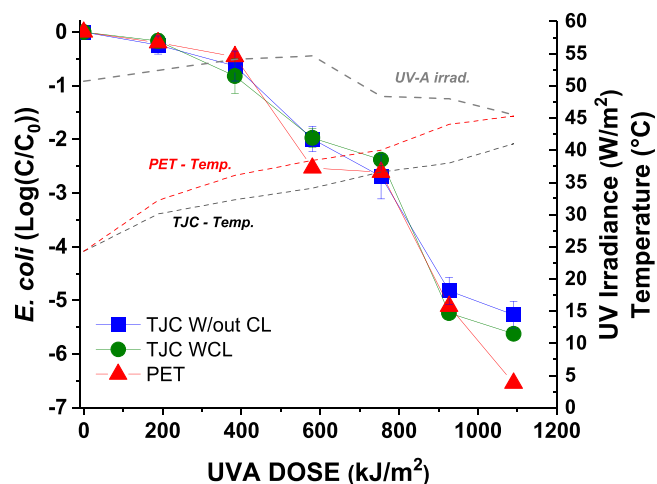


Fig. 3. Solar inactivation profiles of *E. coli* by SODIS in 10 L polypropylene transparent jerry cans with (WCL) and without optical clarifier (W/out CL) in comparison with 2 L polyethylene terephthalate bottles in well water at CSIR-NEERI, Nagpur, India.

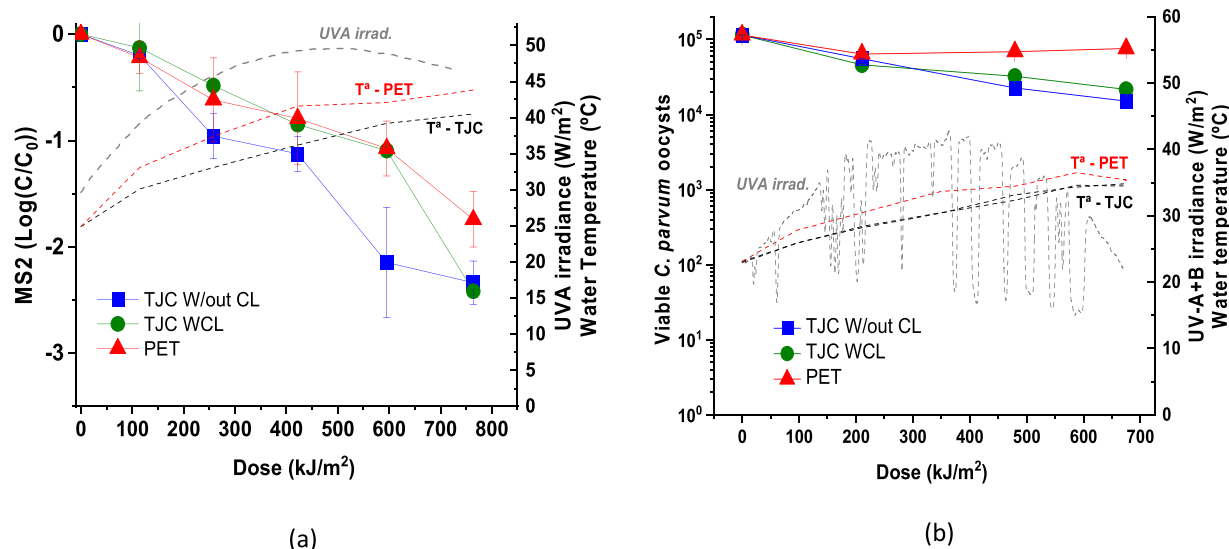


Fig. 4. Solar inactivation profiles in 10 L polypropylene transparent jerry cans (TJCs) with (WCL) and without optical clarifier (W/out CL) in comparison with 2 L polyethylene terephthalate bottles for: (a) MS2 virus in well water at CIEMAT-PSA, Almeria, Spain; (b) *C. parvum* in general test water (GTW) at University of Santiago de Compostela, Spain.

accumulated UV irradiance was 674.9 kJ/m<sup>2</sup> and the maximum value for UV radiation was 41.4 W/m<sup>2</sup>. The maximum temperatures reached in water samples contained in 10 L PP TJC WCL and W/out CL and 2 L PET bottles were 34.6 ± 0.1 °C, 34.9 ± 0.1 °C and 36.5 ± 0.3 °C, respectively (water temperature at the beginning of approximately 23 °C). The results are presented in Fig. 4b. This disparity between LRVs of TJC and PET is presumably due to their different UV transmission properties, especially in the UV-B range (1% and 44% in the global UV-B range, and 59% and 60% in the UV-A range for PET and PP, respectively) [12,23].

One of the most critical components for successful SODIS treatment is the container material. In addition to the optical properties of the SODIS container, the mechanical qualities that improve the durability of SODIS device material are also important. However, weathering resulting from prolonged exposure can cause plastic containers to deteriorate both mechanically and optically leading to shorter lifetimes and less effective disinfection rates [24]. This was observed for the TJCs over 9 months of exposure to natural weather conditions. The container material became brittle and prolonged exposure of TJCs for 9 months under natural sunlight altered the container material, causing it to become fragile (Fig. 5). The changes in texture of container material and brittleness had also been observed visually.



Fig. 5. A sample 10 L polypropylene (PP) transparent jerry can (TJC) damaged due to weathering caused by 9 months of continuous exposure to the elements at CIEMAT- PSA, Almeria, Southern Spain.

The UV transmittance spectra of new and weathered TJCs for 9 months of exposure were measured and are shown in Fig. 1. The SODIS efficacies of TJCs (new / un-weathered and weathered for 9 months) for bactericidal inactivation were also compared (Fig. 6). LRVs of 5.61 ± 01 and 4.42 ± 03 were obtained for new and 9 months-weathered TJCs WCL, respectively. Similarly, bacterial LRVs for new and 9 months-weathered TJCs W/out CL were 5.63 ± 0.3 and 4.15 ± 1.19, respectively. It is worth noticing the increase in the time required to achieve 4 LRV using 9 months-old weathered TJCs was five hours, whereas only three hours of exposure was sufficient to achieve 5 LRV using new TJCs under similar conditions. The control studies with *E. coli* performed using new and weathered TJCs in the dark did not show any effect on bacterial viability.

#### 4. Discussion

Our results show that the inactivation kinetics of *E. coli*, MS2-phage

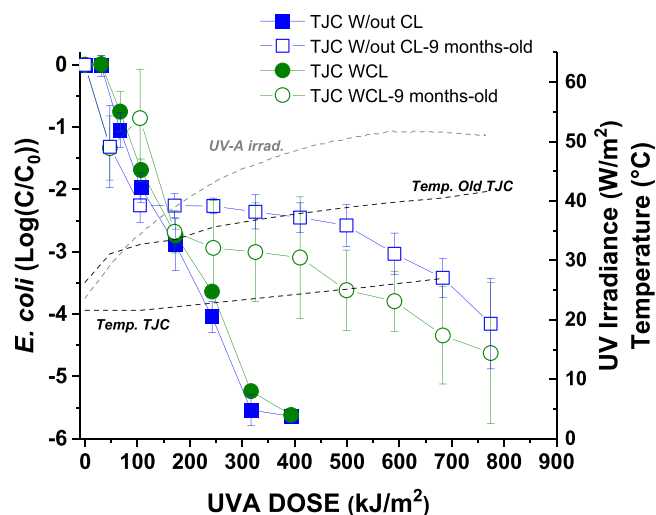


Fig. 6. Inactivation profiles of *E. coli* in well water contained in 10 L polypropylene (PP) transparent jerry cans (TJCs) with (WCL) and without optical clarifier (W/out CL), before and after 9 months of exposure to natural sunlight at CIEMAT-PSA.

and *C. parvum* were found to be broadly similar under natural sunlight for both 10 L PP TJC, i.e., WCL and W/out CL. Despite the poorer transparency of the TJC pathogen inactivation efficacies achieved using 10 L PP TJC (WCL and W/out CL) and 2 L PET bottle which is the standard container most frequently used for SODIS, were found to be comparable (Figs. 3, 4a and 4b).

It has been observed that the increased UV-B transmittance of PP compared to PET contributes to a notable improvement in the inactivation of viruses and protozoa. Although the 10 L PP TJC prototype had less transparency than standard 2 L PET bottles (Table 2) (UV-B 0.001%, UV-A 4.29%, and visible 92% for W/out CL TJC, and UV-B 1.36%, UV-A 8.01%, and visible 90.01% for WCL TJC, and for PET (UV-B 0.72%, UV-A 10–85%, and visible 80–90%), > 5 LRV, 2 and 0.8 LRVs were obtained in just six hours for *E. coli*, MS2 virus and *C. parvum*, respectively.

SODIS inactivate pathogens through a combination of solar UV induced, oxidative activity arising from dissolved oxygen and other endogenous components in the cells, and heat conditions during solar exposure [12]. UV-A mostly causes sublethal effects, such as oxidative stress, protein damage, delayed growth, and impaired energy consumption, on cells but does not cause them to die [13].

The majority of cell death caused by solar radiation results from the UV-B middle ultraviolet (UV-B, 290–320 nm) spectrum, as it is the most energetic portion of terrestrial solar UV radiation, wherein DNA can absorb the photons resulting in the damage [13]. As a result, it is frequently regarded as the primary factor in cell death when exposed to sunlight. Season (more UV-B in summer), time of day (more at solar noon), altitude (more at high altitudes), latitude (more at low latitudes), and atmospheric ozone (more UV-B in areas with less O<sub>3</sub> above) all have a significant impact on the amount of solar UV-B that is received at the Earth's surface [25]. In the present study, the amount of UV-B radiation received for each container is 0.001%, 1.36%, and 0.72% for TJC W/out CL, TJC WCL, and PET respectively.

According to previous studies, PET bottles showed effective inactivation of pathogens but need to replace after six months as the container material degrades due to prolonged exposure and handling [26]. SODIS has been conducted using 19 L PC WDC, and showed good inactivation performance against pathogens. It was noticed that PET material transmits substantially more UV radiation than the PC WDC over the range of 290–400 nm [17]. Previously Polo-López et al. showed that, the transmission properties of 20 L PP buckets remain steady, allowing for usage for up to 6 months under direct sunshine. However, transmission properties of smaller volume 5 L buckets made of the same PP material and exposed under the same circumstances were seriously impacted, indicating that the smaller containers would require replacement every three months [12].

The mechanical and optical qualities of plastic containers change with age as a result of exposure to solar radiation over an extended period of time and weathering. Fig. 5 illustrates the visible textural changes to the TJC as the ageing process continued, as well as increased brittleness; however, SODIS efficacy was not compromised as LRV > 5 were still obtained from these containers for the inactivation of bacteria. These results can be explained due to the transmittance of the material. Fig. 1 demonstrates the variation in transmittance spectra that has been observed as a result of weathering. The TJC W/CL showed a 10–15% loss

in transmittance qualities after 9 months of natural weathering, while TJC W/out CL showed a corresponding 15–20% decrease.

When the SODIS efficacies of fresh, unweathered TJC and TJC that had been weathered for nine months were also examined for bacterial inactivation, > 5 and > 4 LRVs were found for the new and nine months-weathered TJC WCL, respectively. Corresponding to this, the bacterial LRVs for unweathered TJC W/out CL that had been weathered for 9 months were > 5 and > 4 respectively. The difference in exposure time needed to achieve 4 LRV using 9-month-old weathered TJC was five hours, compared to the three hours needed to achieve 5 LRV using new TJC under the same circumstances. TJC's ability to inactivate bacteria did not change, despite physical changes and changes in the mechanical properties of the PP TJC prototypes being noted.

The experiments described in this article were conducted under conditions of uninterrupted, strong sunlight in Southern Spain and the sub-continent of India. It is clear from the experimental process that the water matrix, bacterial strain, geographic factors, and meteorological conditions varied between the two sites. Despite the variations in the factors mentioned, the SODIS efficiency was unaffected by changes in the local climate or the water matrix. Inactivation of the bacteria produced LRVs > 5 for both TJC with and without CL in Spain and India.

According to these findings, effective SODIS does not require that the containers have good optical transparency. This study also suggests that 10 L PP TJC could be a practical substitute for 2 L PET bottles for the implementation of efficient and scaled-up SODIS. Compared to the usual 1.5–2 L volume of the PET bottles, the TJC have the added benefit of treating a larger batch volume of 10 L.

The design and development of the TJC studied in this work, formed part of a wider body of research to examine possible configurations for large volume (5–10 L) SODIS containers. Transparent PP was examined as a candidate material primarily because of its frequent use in food-safe containers. However, initial prototypes all exhibited very poor transparency. Much time and effort were allocated to improving the transparency of the prototypes through the inclusion of clarifying agents in the manufacturing process. Consequently, it is surprising to observe that the benefits associated with improved transmissibility and transparency do not translate into any significant advantage in SODIS microbicidal efficacy. Indeed, as we can see in Fig. 5, even as the PP transparency deteriorates as a result of corresponding weathering, the bacterial inactivation achieved, although poorer than observed in the pristine samples, is still sufficient to produce a LRV > 4. The realisation that optical transparency is no longer a pre-requisite broadens the range of possible candidate containers and materials that can be used for SODIS. Clearly, the authors are not advocating that opaque containers can be considered, however translucent containers that may have been previously rejected on the grounds of insufficient optical clarity, may in fact be used if their optical transmission properties facilitate the passing of UV and photons.

## 5. Conclusions

Our studies suggest that for effective solar water disinfection, UV transmission properties of the container material are important but the optical transparency of the SODIS container material is not as critical as previously thought. The translucent nature of the PP SODIS container material does not hinder its ability to inactivate pathogens. Despite poor transparency, the PP material of the SODIS TJC facilitates diffuse transmission which is sufficient to inactivate *E. coli*, MS2-phage and *C. parvum*. Thus, it can be concluded that optical transparency (i.e. direct transmission) is not a critical parameter determining the efficacy of SODIS treatment, since diffuse transmission due to scattering in translucent materials provides sufficient available radiation within the container to drive the subsequent inactivation. Practically speaking, containers that appear to be translucent but not visibly transparent to the naked eye, may still be used for SODIS if their optical properties allow transmission of sufficient UV energy.

**Table 2**

Comparison of transmission properties of the 10 L polypropylene (PP) transparent jerry cans (TJC) with (WCL) and without optical clarifier (W/out CL) and 2 L polyethylene terephthalate (PET) bottle.

Container	UV-B Transmission (%)	UV-A Transmission (%)	Visible Transmission (%)
10 L PP TJC W/ out CL	0.001	4.29	92
10 L PP TJC WCL	8.01	1.36	90.01
2 L PET Bottle	0.72	10–85	80–90



Ageing and weathering studies also demonstrated that prolonged exposure to sunlight adversely affects the durability of the PP TJC with the polymer becoming progressively more fragile. Surprisingly, however, despite the deterioration in mechanical durability, this did not severely affect the SODIS efficacy of the container with a bacterial LRV of  $> 4$  being consistently achieved. The addition of an optical clarifying agent during fabrication did not result in any improvement in SODIS treatment of water, aside from increasing the visual/optical transparency of the container.

Access to transparent containers and the workload associated with filling and managing smaller volume bottles were both previously identified [1] as obstacles to uptake in adopting the SODIS technique. The results reported herein indicate that much of the time, resources and efforts that have been invested in recent years in developing large volume plastic containers with good optical transparency to facilitate solar water disinfection [1,12,17] were unnecessary. Ultraviolet translucency (rather than transparency) is more than sufficient for the purposes of SODIS. From the perspective of microbial inactivation, it is of little importance whether the UV-photons that are incident upon the microbial pathogen arrived there via diffuse or direct transmission. The resulting cellular damage remains the same. From a user perspective, SODIS uptake can be improved by removing the need for highly transparent containers in order to practice solar water disinfection. This work provides reliable evidence that SODIS could be more widely accepted/practiced by communities in resource poor settings through the use of high-volume translucent PP TJC.

The current study and others [27] have demonstrated that even though polypropylene (PP) facilitates good solar disinfection characteristics, it is not of sufficient durability for long-term use in the field as a transparent SODIS jerry can. The search for more suitable materials for larger volume SODIS containers will have to examine other plastic formulations. One possible candidate for future study might be polymethyl methacrylate (PMMA) [28]. Field evaluations of a PMMA based V-profile reflector SODIS reactor using 45 L transparent PMMA tubes to treat harvested rainwater in Uganda, has shown this material to be both UV transparent and durable [29]. PMMA was not initially considered in the current study primarily for reasons of cost.

Our observations over the past 25 years of work in Sub-Saharan Africa indicate that opaque containers such as buckets and jerry cans, seem to be most frequently used for transporting water from water sources to the household. The maximum volume used tends to be in the range of 10–20 L. Larger volume SODIS containers or reactors are possible but clearly cannot be portable without some form of mechanised transport. Otherwise, the larger volume reactors would have to be located on-site near the household. Volumes of 140 L and 90 L of rain-harvested treated drinking water have been SODIS treated using V-trough solar reactors manufactured of PMMA [29]. Consequently, SODIS can be applied using large volume containers and might be advantageous for end users in settings with limited resources.

#### Credit author statement

**B. Sawant:** Experimental procedure and design in India and Spain, writing, editing. **M. J. Abeledo-Lameiro, Á. García Gil, S. Couso-Pérez:** Experimental design and procedure in Spain, review and editing. **S. Sharma, U. Sethia:** Data collection. **R. Marasini, L. Buck:** Study design and development, review and editing. **M. I. Polo-López:** study design, review and editing. **I. Oller Alberola:** Supervision, review and editing. **J. Marugán, H. Gómez-Couso:** review and editing. **E. Ares-Mazás:** Supervision review and editing. **K. Vijaya Lakshmi, S. Pal, R. Dhodapkar:** Supervision, review and editing. **K.G. McGuigan:** Study design, experimental procedure, supervision, review & editing, conceptualization, funding acquisitions.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data Availability

Data will be made available on request.

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