Efficient solar disinfection (SODIS) using polypropylene based transparent jerrycans: An investigation into its effectiveness, viability, and water sample toxicity

Kris O'Dowd[1], Azahara Martínez-García[2], Isabel Oller[2], María Inmaculada Polo-López [2], Seila Couso-Pérez[3], Elvira Ares-Mazás[3], Hipólito Gómez-Couso[3,4], Ángela García-Gil[5], Javier Marugán[5], Ramesh Marasini[6], Kevin G McGuigan[7], Suresh C. Pillai[1*]

[1] *Nanotechnology and Bio-Engineering Research Group & Health and Bio-medical centre, Atlantic Technological University, ATU Sligo, Ash Lane, Sligo, Ireland*

[2]*Plataforma Solar de Almería-CIEMAT, Carretera de Senés Km 4, Tabernas (Almería), Spain.*

[3] *Laboratory of Parasitology, Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, A Coruña, Spain.*

[4]*Research Institute on Chemical and Biological Analysis, University of Santiago de Compostela, 15782 Santiago de Compostela, A Coruña, Spain.*

[5] *Department of Chemical and Environmental Technology (ESCET), Universidad Rey Juan Carlos, C/Tulipán s/n, 28933 Móstoles, Madrid, Spain*

[6] *Buckinghamshire New University, Queen Alexandra Road, High Wycombe, United Kingdom*

[7] *RCSI University of Medicine and Healthcare, Dublin D02 YN77, Ireland*

Abstract

Solar Disinfection (SODIS) has been identified as a suitable method for water disinfection using 2-L polyethylene terephthalate (PET) bottles. In this study we have examined the use of 10-L polypropylene (PP) transparent jerrycans (TJC) as an alternative plastic for SODIS. The TJC were analysed for their ability to inactivate a range of microorganisms, their structural properties after advanced weathering and their cytotoxicity after extended use. *Escherichia coli*, MS2 and *Cryptosporidium parvum* were used as microbial indicators in well water to ascertain the efficacy of the SODIS process. SODIS was capable of a 6 log reduction value (LRV) in *E. coli* after 105 minutes, a 3LRV in MS2 after 5 hours and 3 LRV in *C. parvum* after 2 days of treatment. This inactivation corresponds to a 2 star performance according to the Household Water Treatment and Safe Storage (HWTS) from the WHO) guidelines. Toxicity analysis using the MTT assay of water samples stored in the TJC over 9 months found that all samples had a cell viability of above 80%, according to ISO 10993-5, this indicates the samples are non-toxic to the cell line and are biocompatible. Advanced weathering of the PP found that after 5 weeks the plastic underwent photodegradation resulting in a decrease in UVA and UVB transmission that stabilised at 20% and 5% respectively. The photodegradation also impaired the mechanical properties of the plastic causing it to become brittle and more susceptible to breaks after two weeks. The PP was found to

be effective at inactivation of microorganisms, non-toxic to human cells but it was found to degrade after two weeks of accelerated ageing. Within the first two weeks of accelerated ageing the plastic retained its plasticity and had elongation of breaks of 570% and 550% for week 1 and 2, respectively, but after this the plastic became brittle and the elongation of break dropped to 10%. Future studies are directed toward developing appropriate UV stabiliser for PP to increase the structural lifetime.

Graphical Abstract: [Image removed]

Keywords:

Polypropylene; SODIS; Water disinfection; Toxicity; Structural integrity, life cycle

1. Introduction

Currently, over 40% of the world population has insufficient access to safe drinking water and this is expected to rise to over 50% by 2025 [1]. Unsafe water can contain multiple pathogens that can result in a variety of waterborne diseases such as diarrhoea, typhoid fever and cholera [2]. Solar Water Disinfection (SODIS) is a low-cost and effective method of disinfecting water that can be useful in low-income countries [3, 4] and has been recognized as such by the WHO [5]. The first research on the effect of light on bacteria was carried out in 1877 by Downes *et al.* that found that light had a germicidal effect on bacteria [6]. SODIS is a water disinfection process that first came about as a disinfection method in 1984 by Acra *et al.* [7] with its first guidelines being developed by Wegelin *et al.* in 1994 [8]. The process uses a transparent container that is filled with the water that is to be disinfected and is exposed to sunlight for times varying between 6 to 48 hours depending on whether there are normal irradiation conditions or cloudy weather [9] (see Figure 1). Typically a 2 L PET transparent plastic bottle is used for the inactivation of waterborne pathogens and to help reduce the incidence of diarrheal morbidity [10]. One of the key advantages of SODIS is the lack of need for a power source, where water treatment plants can require maintenance costs of between 30%-50% of the total cost of operation [11]. The main mechanism of SODIS inactivation comes from solar UVA and UVB, with UVA affecting the cell due to oxidative processes and UVB causing genetic mutations [9, 12]. Heat inactivation from the sunlight is possible but is only effective at temperatures above 45°C whilst light inactivation was possible at irradiances as low as 100W m^{-2} even in water with high turbidity [13].

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Figure 1. The SODIS technique: 1) Transparent bottle is filled with contaminated water, 2) Bottle is place in direct sunlight, 3) Bottle is left in the sun for a minimum of 6-8 hrs, 4) Water is now safe to drink.

The use of SODIS for the inactivation of microorganisms has seen a considerable amount of studies performed [14-17] with it being effective at different levels all year round [4, 18], seeing a minimum of a 4 log reduction in fungi, bacteria and protozoa [19, 20]. PET bottles as stated before, are generally used due to their availability and have been researched extensively [21-25]. PET bottles however, have displayed some negative traits for use in solar disinfection as the plastic is opaque to UVB light [26], with UVB being a key inactivation tool, this decreases the plastic's capacity for disinfection [27]. As part of the EU-India PANIWATER project collaboration [28] we have investigated the use of polypropylene (PP) transparent jerrycans as an alternative plastic SODIS container. In a comparison of four different plastics: polypropylene (PP), polymethylmethacrylate (PMMA), polyethylene terephthalate (PET) and polycarbonate (PC), PP was seen to provide a reasonable disinfection time whilst also capable of remaining stable with the addition of stabilisers, making them cheaper to produce and replace [29]. An examination of these four plastics with a thickness of 0.5 mm was found to have a solar UVA radiation transmission of 72.8%, 70%, 81% and 97.9% for PP, PET, PC and PMMA respectively and solar UVB radiation transmissions of 64.9%, 0%, 64.5% and 97% respectively. The 0% transmission of UVB for PET decreases its ability to inactivate microorganisms susceptible to UVB, such as viruses and protozoa, suggesting that the other plastics are better suited to SODIS. Polypropylene containers are proficient for use in disinfection as they are capable of eliminating a significant percentage of the highly resistant protozoan pathogen *Cryptosporidium parvum* oocysts after exposure to sunlight for 8-12 hours [30]. Using a solar calculator algorithm it was calculated that the exposure time required for the inactivation of bacteria was 0.97h for PP, 1.52h for PET, 0.90h for PC and 0.69h for PMMA [29]. SODIS containers typically will have to be robust due to their multifunctional role of not only being the site of disinfection but also the method by which the water is collected [31]. The durability of the plastic in both the physical transport to/from the water source and any potential weakness that may arise from prolonged exposure to solar irradiation during treatment will need to be examined to ensure the plastic is suitable for use. PMMA showed better suitability for SODIS than PP but as the plastic in PMMA is "relatively soft" it can be damaged easily during handling, so would be better suited to *in situ* SODIS only, and not for collection purposes. PP however is resistant to impact and is more elastic allowing for more robust handling of the TJC [32]. In this study, we have examined the use of PP as an alternative plastic for SODIS containers as it has a greater UVB transmission than PET and shows greater suitability as a container used for transporting and collecting water, than PMMA.

The use of plastic containers for SODIS has the potential for chemical compounds or leachates present in the plastic to have a cytotoxic effect on human cells [33]. These may arise from a multitude of sources during the production of the plastic such as phthalate plasticiser, unreacted monomers, organotin stabilisers, antioxidants, copolymers, etc [34]. Polypropylene has been studied extensively for use as packaging for food with any migration or degradation of compounds being seen to be non-toxic and not a threat to human health [35]. However, a cytotoxic analysis was undertaken after SODIS activity to ensure the process does not result in any compounds that would not have been present during standard food testing. The MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) assay is a colourimetric method of analysing cell densities [36] that was first described in the use of cell viability in 1983 by Mosmann [37]. The assay is based on the transformation of soluble yellow MTT into purple insoluble formazan that is caused by plasma membrane reductases of non-mitochondrial and mitochondria of viable cells. Any changes in metabolic activity can be observed as having a significant change in MTT allowing the detection of toxic compounds that can cause stress on the cell but do not result in “direct cell death” [38]. The MTT is performed in a 96-well plate that is analysed with a microplate reader and the absorbance obtained is relative to the number of cells present [39].

This study examines the use of 10L PP plastic TJC as SODIS containers that could provide greater UVB transmission than PET for increased inactivation of microorganisms whilst still being low cost, being structurally sound, having long term viability and are safe for use. The TJC containers were examined for their ability to inactivate the three microorganism species: *E. coli*; *C. parvum;* and MS2 as per WHO International Scheme to Evaluate Household Water Treatment Technologies. To ascertain their structural robustness the TJC were subjected to advanced weathering to record any photodegradation of the plastic, changes in UV transmission and changes in mechanical properties that may develop over time. Finally, to ensure the plastic was safe for use, a toxicity analysis was carried out over 9 months using the MTT assay.

2. Experiment analysis

2.1 Transparent Jerrycans (TJCs)

Polypropylene PP3321[was selected for use due to its suitability for blow moulding, impact resistance and food safety certification [40]. Polypropylene PP3321 has a melt flow index at 230°C/2.16 kg of 1.8 g/10 min analysed using ISO 1133. Its mechanical properties show an elongation at yield of <15% (ISO 572-2), a flexural modulus of 900 MPa (ISO 178), a Izod Impact Strength of 22 kJ/m^2 at 23°C and 3.6 kJ/m^2 at 0°C (ISO 180) and a hardness Rockwell of 82 kJ/m^2 (ISO 2039-2) [41]. The thermal properties at Vicat softening point at 50N-50°C and 10N-50°C per hour was 67°C and 130°C respectively (ISO 306). The plastic has a density of 902 kg/m^3 and a bulk density of 525 kg/m^3 (ISO 1183). The 10L TJC were produced using a blow moulding process by Gemini Prototyping Limited, UK and can be seen in Figure 2 (A). The transmittance of the TJC plastic material (Figure 2 B) was measured with an Evolution 220 UV-Vis spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) in the wavelength range of 200-800 nm.

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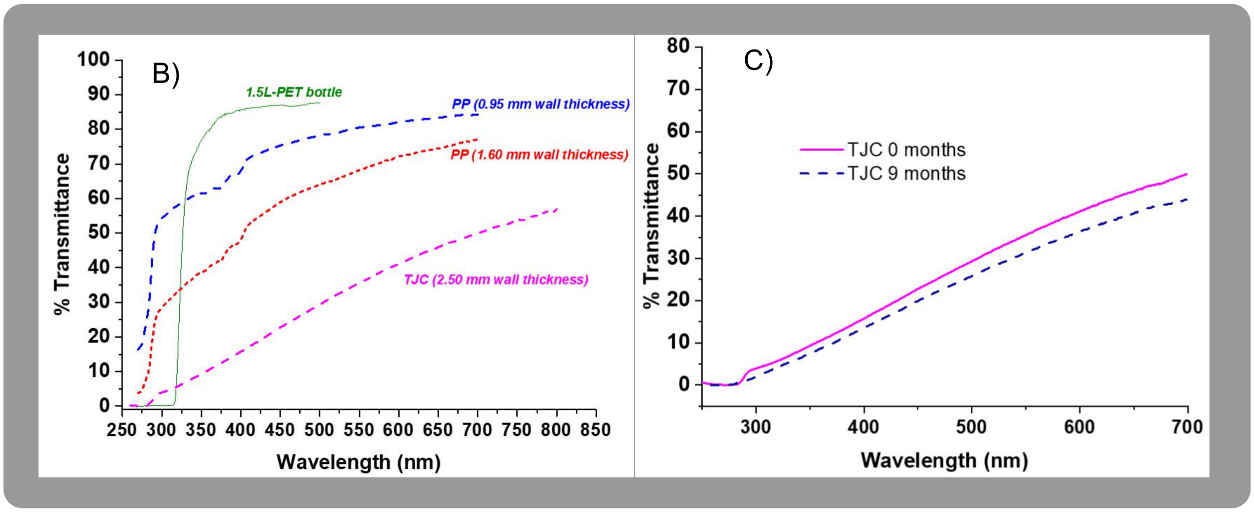

Figure 2. A) TJC filled with water and exposed to natural sunlight in vertical and horizontal positions during a solar disinfection test at PSA facilities in Spain. B) Comparison of transmittance of TJC-material with other transparent plastic containers (data from Polo-López et al., 2019). C) Transmittance of a TJC piece of wall before and after 9 months exposure to natural weather conditions at the solar research facility in PSA.

2.2 Solar water disinfection tests.

The solar water disinfection tests involving *E. coli* and MS2 as microbial targets were performed at Plataforma Solar de Almería (PSA) (Almería, Spain, latitude 37.0947°N, longitude 2.3584°W) under sunny conditions in 2020. The experiments were started between 10:30 and 11:00 am local time, over a period of 4-5 h to ensure similar radiation and temperature conditions between the results.

Water temperature (ºC) was measured during the treatment time with a portable thermometer (Checktemp, Hanna, Spain). UVA radiation was monitored using a UV pyranometer (in the range 295–385 nm, CUV4, Kipp and Zonen, Delftechpark, the Netherlands) with a sensitivity of 264 $\mu V/Wm^2$, located on site. The data recorded in W/m^2 were used to tabulate the UV energy dosage collected per unit area of illuminated surface according to equation 1, where t is equal to experimental time and UV(t) is the solar UV irradiance measured.

$Solar\ UVA\ Dose = \int UV\ (t)dt$ (equation 1)

The water used for the solar experiments was collected from a 200 m deep borehole well situated onsite at PSA. Physicochemical properties of water extracted from the well are shown in Table 1. The absence of naturally occurring fecal contamination in well water was confirmed by standard plate count technique using specific agar media (Chromocult® and Endo agar). A Total Organic Carbon (TOC) analyser (Shimadzu TOC-5050, Japan) was used to determine the total inorganic and organic carbon content. Turbidity, pH and conductivity were obtained with a turbidity instrument (Hach-2100N, Loveland, CO, USA), a pH-meter (GLP22, Crison), and a conductometer (GLP31, Crison Instruments, Barcelona, Spain) respectively.

Table 1. Well Water characterisation.

	Actual WW	Diluted WW	Diluted WW (after 9 months)
Conductivity (µS/cm)	2750	313	282.5
pH	7.14	7.75	6.89
Turbidity (NTU)	1.72	0.15	0.2
Total Organic Carbon(mg/L)	3	< DL	16.7
Total Inorganic Carbon (mg/L)	202.3	20.6	23.9
Cl^- (mg/L)	295.3	25.4	26.7
NO_3^- (mg/L)	13.36	0.8	0.1
SO_4^{2-} (mg/L)	198.5	14.9	16.2
Na^+ (mg/L)	466.9	45.2	46.8
K^+ (mg/L)	9.16	0.4	0.4
Ca^{2+} (mg/L)	86.9	7.5	7.4
Mg^{2+} (mg/L)	58.3	5.2	4.9

The experimental procedure for solar water disinfection tests was as follows: Two TJC were filled with freshly collected well water and individually spiked with previously prepared suspensions of each microorganism to guarantee an initial concentration of 10^6 Colony Forming Units (CFU)/ml for *E. coli*, 10^5 Particles Forming Units (PFU)/ml for MS2, and 5×10^5 purified oocysts/L of *C. parvum*. Initial samples were collected after ensuring a correct water homogenization and the TJCs were then immediately exposed to natural sunlight. During the treatment, samples were extracted at predetermined time intervals and analysed for microbial quantification as described below. The initial samples were stored in the dark at room temperature and assessed again at the end of each experiment as a microbial viability control and in all cases recorded stable microorganism concentrations throughout (data not shown). The inactivation profiles presented correspond to the average data of at least two replicate experiment for each microorganism.

Solar experiments involving *C. parvum* oocysts were carried out for 6 hours (from 11:30 to 17:30 h local time) on the roof of the Faculty of Pharmacy of the University of Santiago de Compostela (Galicia, Spain; latitude 42.8749 °N, longitude 8.5548 °W, altitude 260 m).

Each TJC was filled with 10 L of spiked well water containing 5×10^5 purified oocysts/L of *C. parvum*. For comparison, 1.5 L of spiked well water containing 5×10^5 purified oocysts/L of *C. parvum* was added to PET bottles. After agitation for homogenisation, all containers were exposed to sunlight. The temperature was monitored at the start and every hour with a Temp 3JKT thermometer equipped with a type J thermocouple probe ECTPGLPJ01M (Eutech Instruments Pte Ltd., Singapore). To measure the irradiance a radiometer PMA2100 fitted with a PMA2107 digital non-weighted UV- A + B Sensor (280–400 nm) (Solar Light® Company, Inc, Glenside, Pennsylvania, USA) was used. At 2 h intervals, varying volumes of sample (2-3.5 L) were removed and concentrated by filtration via nitrocellulose membrane (ø = 47 mm; pore size = 3 μm; Merck Millipore Ltd., Carrigtwohill, Ireland) using a vacuum pump FB70155 (Fisherbrand™, Fisher Scientific S.L, Madrid, Spain). The membranes were the washed three times with 4.5 ml of PBS (0.04 M pH 7.2) after being placed in polyethylene bags that can be re-sealed. Centrifuging the samples for 15 min at 2000×g to form a pellet and remove the supernatant, 1.5 ml of PBS (0.04 M pH 7.2) was then used to resuspend the pellet. All samples were incubated at 22 °C for 48 hours. Then, the total amount of oocyst structures in the sediment obtained was quantified using a modified Neubauer haemocytometer. Aliquots of each sample containing 1×10^5 oocyst structures were subjected to *hsp70* mRNA expression induction by incubation in a water bath at 45 °C for 20 min. Using liquid nitrogen to freeze and a water bath at 65 °C (6 cycles) to thaw the oocysts were disrupted and isolation of mRNA was performed by the oligo(dT)25 method using Dynabeads® mRNA DIRECT™ Kit (Invitrogen, Thermo Fisher Scientific, Vilnius, Lithuania) as per the manufacturer´s instructions. The mRNA obtained was analysed by RT-qPCR following the protocol described by Liang and Keeley (2012) using One Step PrimeScriptTM III RT-qPCR mix (Takara Bio Inc., Kusatsu, Japan) in a StepOne™ Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). A positive and a negative control was included in all RT-qPCR assays.

2.3 Accelerated ageing test

An Atlas Weather-Ometer Ci4000 was used to age the PP samples with ISO 4892-2 as basis for the procedure. The Ci4000 contains both inner and outer filters of type S borosilicate glass with a xenon lamp that simulates the visible and ultraviolet regions of sunlight. To analyse the PP samples they were exposed to weathering cycles. Each cycle included two segments with different conditions: in the first one, the samples were illuminated under wet conditions spraying deionised water for 18 minutes. In the second one, the samples were illuminated under dry conditions with a relative humidity of 50% for 102 minutes. The ageing took 8 weeks with a radiation intensity of 0.75 $W \cdot m^{-2}$ at 340 nm (3.6 $MJ \cdot m^{-2} \cdot nm$ in total).

2.4 Water toxicity tests

All materials used were of analytical grade. Acetone (C_3H_6O) and methanol (CH_3OH) were purchased from Analab. MCF7 cell line humane breast cells were purchased from Sigma. DMEM medium glucose (Dulbecco's Modified Eagle Medium)-High Glucose, Fetal Bovine Serum (FBS), catalase and Dulbecco's Phosphate Buffered Saline PBS were purchased from GE healthcare. Trypsin, 0.25% (1x), with 2.5g porcine in HBSS, Trypan blue and Thiazolyl blue tetrazolium bromide 98% (MTT) were purchased from fisher. Penicillin Streptomycin Sol 100 ml, L-Glutamine 200mm 100 ml and DMSO, LC-MC Grade were purchased from biosciences. T75 and T25Tissue Culture Treated Flasks (vented caps), 10/25/50 ml Serological Pipette (sterile), individually wrapped and 1/0.2/0.01 ml pipette tips were purchased from Starstedt. 96 well plates from MSC. Oasis HLB 5 cc Glass, 200 mg cartridges from waters. 4 ml screw neck vials were obtained from VWR. IST 20L VacMaster was obtained from the I.T. stores. A BMG Labteck FLUOstar Omega microplate reader was supplied by I.T. technical staff. Q-Sun Xenon test chamber supplied by Q-lab.

The samples for toxicity testing were obtained at weekly intervals during the exposure of filled TJCs to natural sunlight and weather conditions for an uninterrupted period of 9 months (from 21-09-2020 to 16-04-2021) at PSA. Three TJCs were filled with ten-fold diluted well water (to reduce the strong hardness of the actual well water). The diluted well water was physicochemically characterized at the beginning and the end of the solar exposure, and the results are shown in Table 1. Samples of 100 ml were taken out from each TJC regularly, stored in glass vials, refrigerated at 4 ºC and shipped to ATU Sligo for further analysis following the methodology described below.

3. Experimental Methods

3.1 Microbial enumeration and quantification

Escherichia coli

The culture-type strain *E. coli* K12 (CECT 4624) were provided by Spanish Type Culture Collection (CECT, Spain). Daily fresh bacterial stationary phase stock suspension of 10^9 CFU/ml was obtained by growing the bacteria in Luria-Bertani Broth (Merck KGaA®, Darmstadt, Germany). After incubation at 37 ºC under rotatory shaking for 20 h, the obtained suspensions made into bacterial pellets by centrifuging the sample for 15 min at 900×g, these were then re-suspended in PBS (Sigma-Aldrich®, Germany). The suspension stocks were then diluted in the water matrix to obtain a stock *E. coli* concentration of 10^6 CFU/ml. Enumeration of bacterial concentration was done according to the plate counting technique using ENDO Agar (Merck KGaA, Germany). 20 µl of sample was diluted ten-fold in PBS and this was then spread across the agar media. If lower concentration of microbes was required 500 µl of the sample was spread directly onto the media. After incubation for 24 h at 37 °C the colonies were counted. The detection limit (DL) of this procedure is 2 CFU/ml.

Bacteriophage MS2

The collection-type MS2 strain (ATCC 15597-B1) and its host *E. coli* C300 (ATCC 15597) were provided by the American Type Culture Collection (ATCC, USA). Stock suspension of MS2 was obtained after the growth of the virus with an active suspension of bacterial host previously cultivated in fresh liquid medium Tryptone Glucose Yeast Extract Broth (TYGB) in a rotatory agitation at 37 ºC for 6 hours. Afterwards, 100 µl of virus solution was added and left under the same conditions for another 20 hours. The culture medium was then filtered with a Milipore® nylon syringe filter of 0.2 nm (Merck KGaA, Darmstadt, Germany) to separate bacteria from virus and viral particle concentration determined by serial 10-fold dilutions using the double-layer agar method according to the manufacture instructions. To obtain a 10^5-10^6 PFU/ml concentration of the virus specific volumes from the stock were diluted using a water matrix. To accurately count infective MS2 particles in the samples the double layer agar method was used. 0.1–0.5 ml of sample (or 10-fold dilutions), 1 ml of *E. coli* C300 and 5 ml of melted semisolid TYG agar were mixed. After pouring this mix onto solid TYG agar plates they incubated for 24h at 37 ºC. The DL of the procedure is 2 PFU/ml.

Oocysts of *C. parvum*

A naturally infected neonatal Friesian–Holstein calf was used as the source for the *Cryptosporidium* oocysts. Concentration, purification, quantification and molecular characterisation of the isolate were carried out according to Gómez-Couso et al. Rectal sampling was used to collect obtain faeces that were kept at 5 °C in storage until their processing.
The faecal matter was added to 100-200 ml of phosphate-buffered saline (PBS, 0.04 M, pH 7.2) and homogenised, filtered via a 150 and 45 µm sieve, shaken vigorously with diethyl ether (2:1, v/v) and then centrifuged at 2000 × g, for 15 min, at 4 °C to concentrate. Discarding the supernatant and then washing the sediment with PBS (0.04 M, pH 7.2) the sample was centrifuged for 15 min at 4 °C using a centrifugal force of 2000 × g. To purify *Cryptosporidium* oocysts they were centrifuged for 30 min at 4 °C at 2000 x g on discontinuous caesium chloride gradients of 1.05, 1.10 and 1.40 g/ml. A modified Neubauer haemocytometer was used to count the oocysts using the colour stain 0.16% malachite green solution [43, 44]. PCR amplification and sequencing were used to identify the isolate *C. parvum* (subtype IIaA15G2R1) using the analysis of the fragments ~587bp of the small subunit rDNA gene (*SSU rDNA*) [45] and 850 bp of the 60 kDa glycoprotein (GP60) [46]. To ascertain the viability of the isolate, propidium iodide fluorogenic vital dye inclusion/exclusion was used along with a immunofluorescence antibody test modification to verify oocyst identification [47, 48] and by using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) against 70 kDa heat shock protein (*hsp70*) mRNA of *Cryptosporidium* [49].

3.2 Characterisation of aged PP

Differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and high-temperature gel permeation chromatography (HT-GPC) was used to study the evolution of the physicochemical properties of aged samples. For the DSC tests, 8-9 g of plastic samples were analysed under nitrogen from 20 °C to 220 °C at 10 °C/min rate in a DSC Mettler-822e.

To perform the HT-GPC tests the analysis was carried out at 150°C using three PLgel Olexis 3 columns and a GPC-IR6 from Polymer Char (CN1952C010) with a GMHH-R precolumn. Polystyrene standards were used to calibrate the columns, with a flow rate of 1 ml/min with 1,2-dichlorobenzene being used as the eluent and solvent. For the FTIR tests, the absorption spectra were collected in the 600-40000 cm^{-1} range (only shown from 1600-1850 cm^{-1}) with a resolution of 2 cm^{-1} for 32 scans in the attenuated reflection (ATR) method using a FTIR Varian Excalibur Series 3100 spectrometer. To calculate the carbonyl index the ratio of the carbonyl (C=O) peak area (1600-1800 cm^{-1}) relative to the C-C reference peak area (1316-1400 cm^{-1}) was analysed.

Tensile properties were measured to study the evolution of the mechanical properties of plastic samples with ageing. The tensile bars used were type 5A, and the test conditions followed the

ISO527 method in an MTS Alliance RT/5 tensile tester. The PP samples kept at 23 °C/50% RH prior to testing for 48 h to acclimatize. A speed of 20mm/min was used with a clamp length of 50 mm. all samples were run in triplicate and the average was found for them. The percentage elongation at break was selected as a representative tensile property.

The direct and diffuse transmittance were used to study the changes in the optical properties of plastic samples with ageing. A Vary Carian 500 spectrophotometer was used to record the spectra in the UVB (280-320 nm) and UVA (320-400) ranges in transmission mode with and without an integration sphere for diffuse and direct transmittance, respectively.

3.3 Toxicity analysis

3.3.1 Sample preparation

Oasis HLB 5 cc Glass, 200 mg cartridges were loaded onto an IST 20L VacMaster. 4 ml of acetone was loaded onto the cartridge and passed through and was repeated once. 4 ml of deionised water was loaded onto the cartridge and passed through and was repeated once. The 25 ml sample was loaded onto the cartridge in 5 ml aliquots and passed through. Waste was removed from the chamber and a 4 ml screw neck vial was placed below the cartridge. 4 ml of methanol was used to elute any contaminates on the cartridge. 100µl of DMSO was added to the vial and the methanol was removed by a gentle stream of nitrogen. Samples were stored at – 4 °C until needed for use.

3.3.2 MTT assay

MCF-7 cells were maintained in DMEM medium containing 20% fetal bovine serum and 1% pen/strep. Before analysis, a 96 well plate was seeded with a cell concentration of 3000 cell/well with a volume of 100 µl. After allowing the cells to attach for 24 hours the media was removed and 100 µl of controls were added; a blank (media), a negative control (media + 1%(v/v) DMSO) and a positive control (media + 10%(v/v) DMSO). Three concentrations of samples were added at 1.0, 1.5 and 2.5 concentrations. All were exposed for 24 hours after which the media was removed, and the wells were washed with 100 µl of PBS. 100 µl of fresh media and 10 µl of MTT reagent was added and the plates were incubated for at least 4 hours. After incubation, all contents were removed from the wells and 100 µl of DMSO was added. The absorbance was read at 580 nm on BMG Labteck FLUOstar Omega microplate reader with the shake plate feature on and analysed for 15 minutes. Comparing the absorbance of a control with treated cells can be used to calculate percentage viability of the treated cells. Viability of 40% or below is considered to be strongly cytotoxic, 40-60% moderately cytotoxic, 60-80% weakly cytotoxic and above 80% is seen to be nontoxic as per ISO 10993-5 [50].

4 Results

4.1 Solar water disinfection

The efficiency of the 10L TJC as potential containers for SODIS was evaluated in well water using *E. coli*, MS2 and *C. parvum* as a model of microbial indicators. To discard any acute toxic effect of the TJC material over the *E. coli* viability, a preliminary dark test was performed for 5 h at room temperature (25 ºC) in well water. The concentration of bacteria (10^6 CFU/ml) remained constant throughout the entire treatment time (data not shown).

Figure 3 shows the inactivation kinetics of *E. coli* after 5 h of SODIS treatment time under natural sunlight in two different exposure orientations, i.e., standing vertically erect on its base and lying horizontally on its broader side, to evaluate the influence of orientation on the disinfection performance, considering that both positions can be potential ways of use by end-users. Results showed that in both positions, greater than 6 Log Reduction Value (LRV) were obtained. No significant differences were detected between either positions; although a slightly enhanced inactivation was obtained for the horizontally positioned TJC where the detection limit (2 CFU/ml) was reached after being exposed to 220 kJ/m^2 (105 min of treatment time) in comparison with the vertical position (260 kJ/m^2 or 120 minutes of treatment time). Minimum and maximum solar UVA radiation values of 27.5 and 45.5 W/m^2, respectively, were recorded. Water temperature started at the same value of 25 ºC and rose to a maximum of 33 ºC with non-significant differences between both TJC exposure orientations.

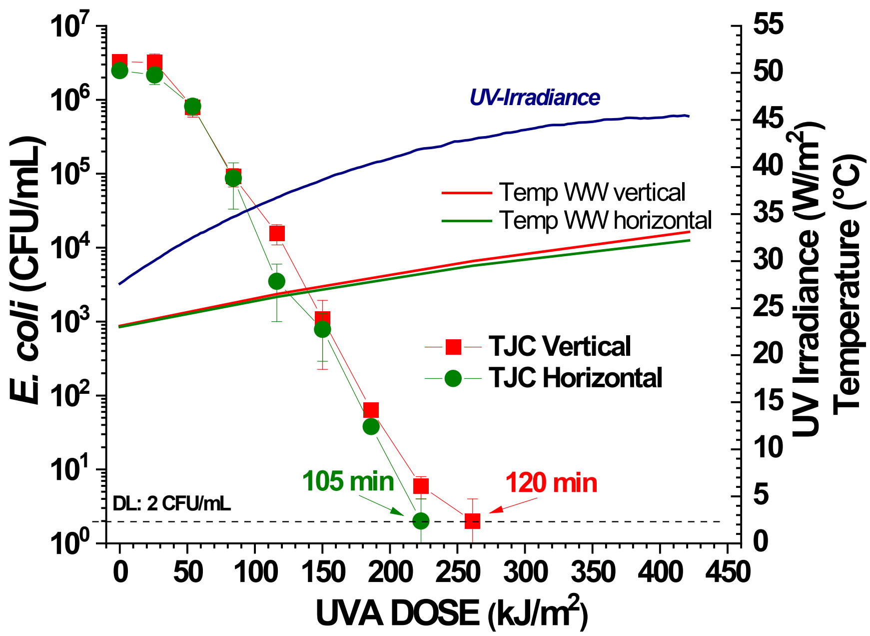

Figure 3. Solar inactivation kinetic of *E. coli* in 10L-TJC exposed to natural sunlight.

MS2 solar inactivation kinetics in well water within TJCs in vertical and horizontal exposure positions are shown in Figure 4. Results showed that in any case the DL (2 PFU/ml) was reached, being the highest LRV reached in horizontal position (3 LRV) compared with the vertical position (2.5 LRV) after ca. 800 kJ/m^2 of solar UVA dose or 5 h of solar exposure. In this test, water T was almost the same in both cases, reaching a maximum recorded value of 37 and 39.5 ºC for vertical and the horizontal position, respectively.

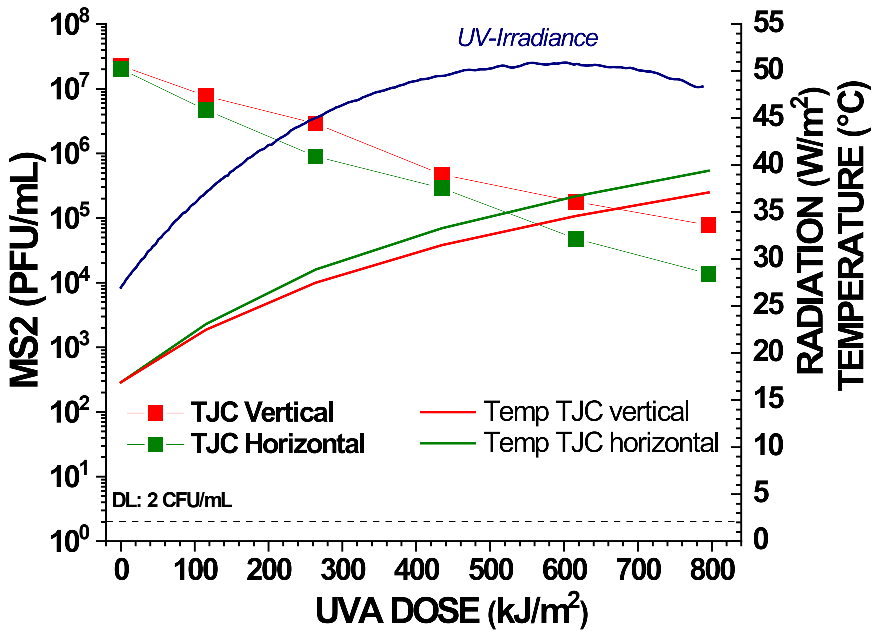

Figure 4. Solar inactivation kinetic of MS2 in 10L-TJC exposed to natural sunlight in well water.

Figure 5 shows the inactivation kinetics of *C. parvum* oocysts in 10 L TJC versus 1.5 L PET bottles containing well water and exposed to natural sunlight. Maximum temperatures of 34.6 °C for TJC and 37.0 °C in the case of PET bottles, were recorded. After 6 hours of exposure, the UV-A+B dose was 672.63±101.86 kJ/m^2. Under these conditions, a slight increase in the oocyst inactivation in TJC vs. PET bottles was observed. Thus, 1.14±0.10 LRV in the viability of *C. parvum* oocysts were determined in 10L TJC whereas in 1.5L PET bottles it was 0.69±0.16 LRV (*P*<0.0001).

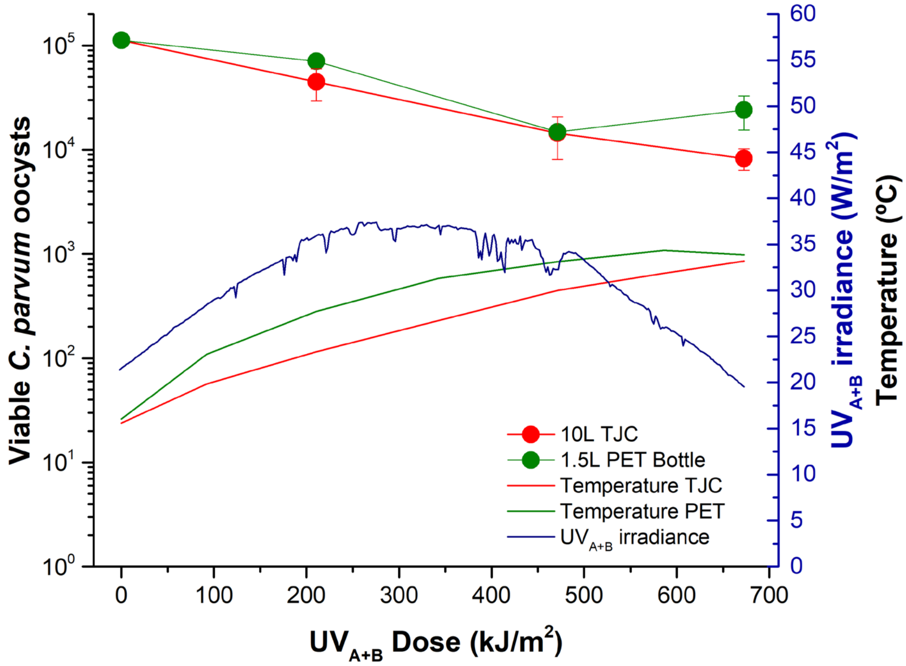

Figure 5. Inactivation kinetics of *C. parvum* oocysts in 10L TJC and 1.5L PET bottles containing well water and exposed to natural sunlight.

Considering that WHO recommends exposing the water for up to 48 hours in cloudy conditions, an experiment of exposure on two consecutive days (cloudy and sunny) was carried out. In these conditions and after a solar UV dose of 1316.7 KJ/m^2, 3.0 LRV in the oocyst viability was determined in 10L-TJC 1.0, statistically significantly higher than those observed in 1.5L-PET bottles (1.0 LRV) (*P*<0.0001) (Figure 6).

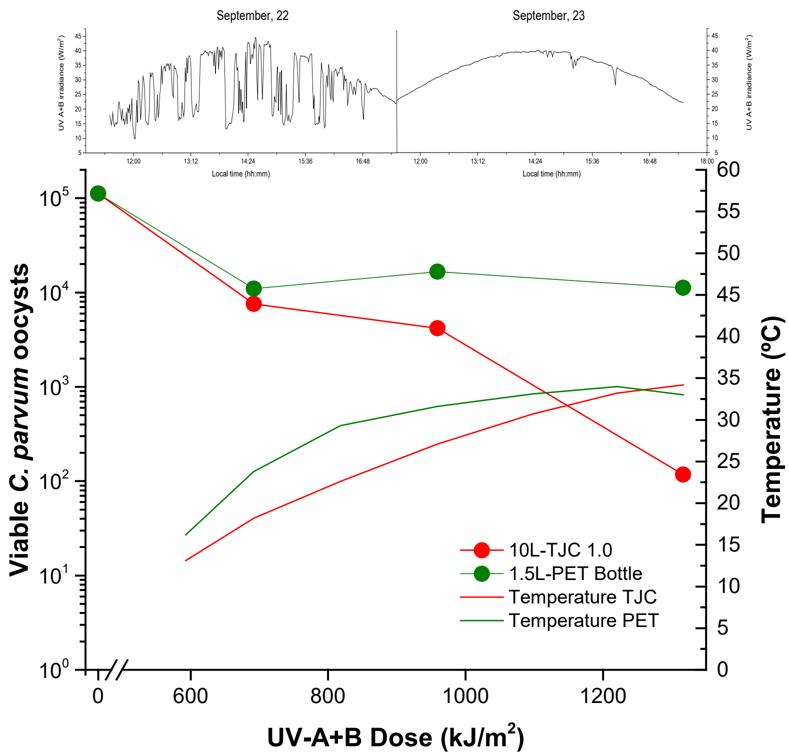

Figure 6. Inactivation kinetics of *C. parvum* oocysts in 10L TJC and 1.5L PET bottles containing well water and exposed to natural sunlight over two consecutive days.

4.2 Weathering

The physical properties of the plastic were seen to be affected after prolonged aging with the plastic becoming more translucent and less transparent over time. This can be seen in the physicochemical changes that occurred in the PP plastic that are listed in Table 2. A decrease in the first melting temperature (Tml) is observed from the second to the third week of weathering, dropping from 147.8 to 140.4 T_{m1} (°C) respectively. There was a decrease from 459770 M_w (g/mol) to 286700 M_w (g/mol) recorded in the plastic molecular weight from the initial 0 time to 2 weeks of ageing. A second decrease of 239700 M_w (g/mol) to 158900 M_w (g/mol) was observed between weeks 4 and 8, respectively. An increase in the polydispersity index (indicative of the width of the molecular weight distribution) confirming the breakage of the molecular chains of the polymer was observed from weeks 2-4 increasing over 50% from 9.24 to 15.5 with minimal change observed between weeks 0-2 and 4-8. The carbonyl index obtained via FTIR (indicative of the presence of oxygen groups in the polymer chain) increased confirming oxidative degradation of the materials through all the weeks, increasing from 0.43 to 0.83 for weeks 0 to 2, 0.83 to 1.38 for weeks 2 to 4 and 1.38 to 1.46 for weeks 4 to 8. This increase can also be seen in Figure 7 as the absorbance in the spectra 1850-1600 cm^{-1} increases as the accelerated ageing progresses.

Table 2. Physicochemical properties measured to study PP photodegradation by weathering

Technique	Measurement	Accelerated ageing week			
		0	**2**	**4**	**8**
DSC	T_{m1} (°C)	146.4	147.8	140.4	141.2
GPC	M_w (g/mol)	459770	286700	239700	158900
	PI	10.5	9.24	15.5	16.0
FTIR	Carbonyl Index	0.43	0.83	1.38	1.46

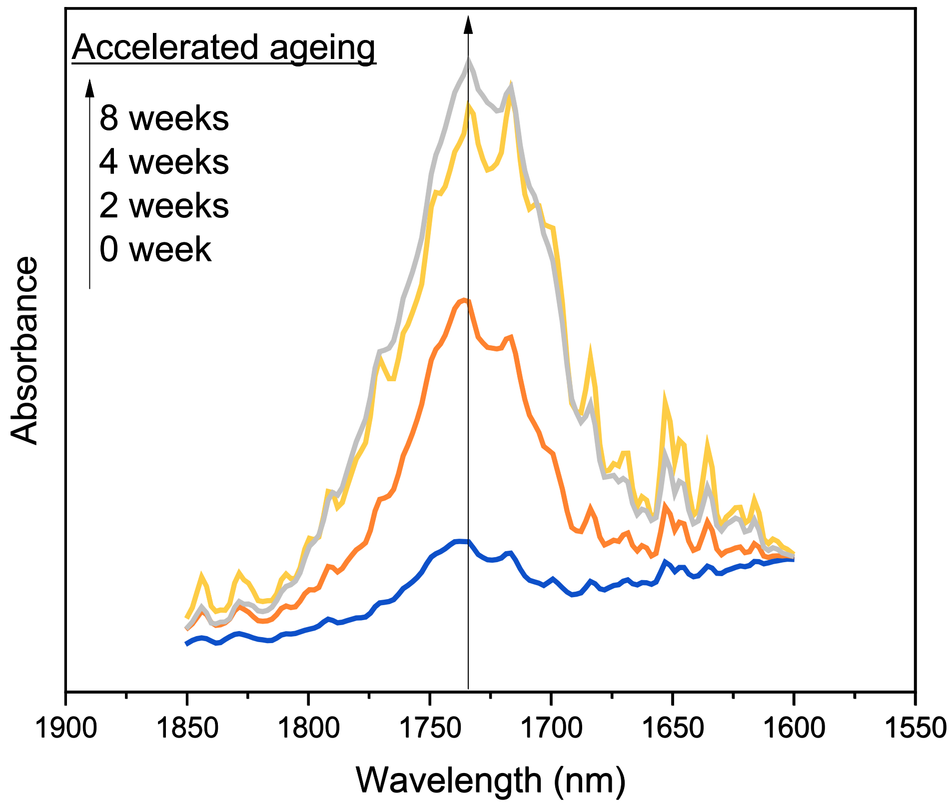

Figure 7. FTIR spectra in the 1850-1600 cm^{-1} region for the aged PP samples.

4.3 Viability for long term use

Figure 8 depicts the evolution of the transmission in the UVA and UVB regions of the PP samples with ageing time. For all samples, diffusely transmitted radiation was stronger than that directly transmitted, due to the translucency of this PP. Around the 5^{th} week of accelerated ageing, both transmittances in the UVA and UVB regions reduced by half, leading to longer exposure times being required to reach the same level of disinfection. However, the trend denotes stabilisation of the transmission at 20 % and 5% in the UVA and UVB regions, respectively.

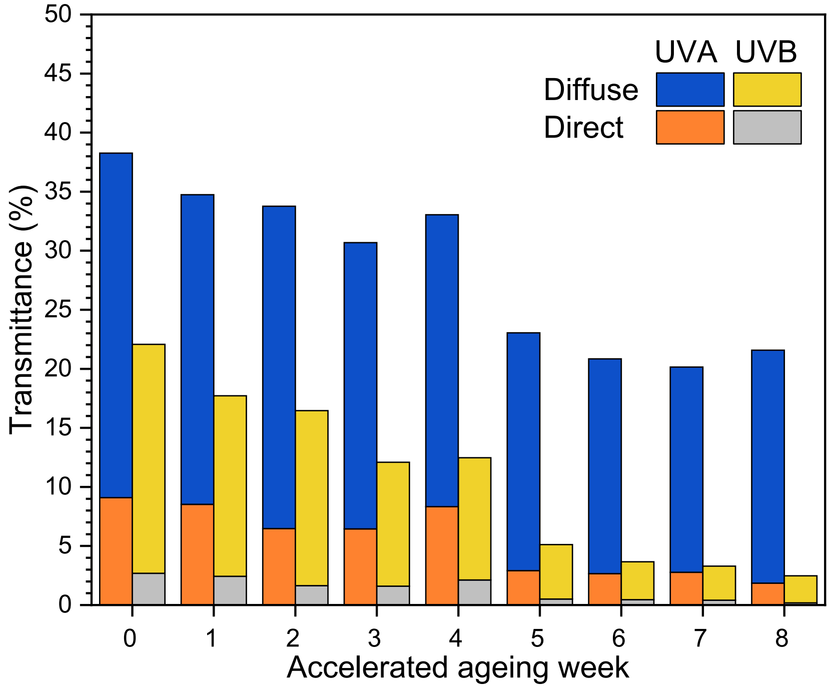

Figure 8. Evolution of UVA and UVB transmission of PP samples with ageing time. Total transmission is the sum of the direct and diffuse fraction. Percentage analysed as the average transmittance in the specific range (UVB: 280-320 nm and UVA:320-400 nm)

Evolution of the elongation at the break point is representative of the variation of mechanical properties of the plastic over time and is shown in Figure 9. In the first two weeks of accelerated ageing, the PP presented very high plasticity, but by the next ageing time-point, the PP had become very brittle. Weeks 1 and 2 had an elongation at break of above 570% and 550% respectively, which dropped to below 10% for the following weeks.

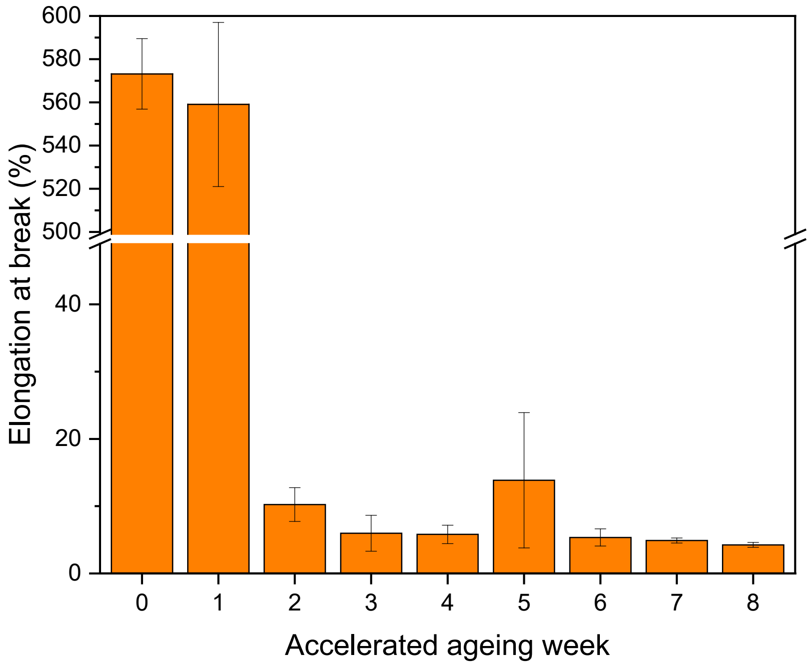

Figure 9. Evolution of the elongation at break of PP samples with ageing time.

4.4 Toxicity

Proliferation effects on cells can be seen in Figure 10 and supporting material. Cells were exposed to three concentrations of possible leachates at 2.5x, 1.5x and 1.0x concentrations at intervals of 0, 2, 3, 15, 30, 45, 60, 75, 90, 120, 180 days, 6, 7, 8 and 9 months. The cells were exposed for 24 hours before analysis. The effect on the cells was very limited across all the timeframes, with none of the timeframes or concentrations dropping below the 80% viability that would indicate toxicity to the cells. As can be seen in Figures 10 and supporting material the lowest viability was found in the 2.5x concentration after 60 days at 81.43% in TJC 1. As the concentration decreased in this time frame, the viability increased accordingly having a percentage viability of 95.4 and 99.3% for the 1.5x and 1.0 concentrations respectively. These results were consistent with TJC 2 and 3 not showing a drop in percentage viability below 81.43% and can be seen in Figures 1 and 2 of supporting material. Several time points showed proliferation activity with a viability above 100% with the highest of 107.8% at the 2.5x concentration after 7 months in TJC 3. Higher percentage viability of 117.7%, 112.7% and 113.9% was seen in TJC 2 at 60 days for 1.0x, 1.5x and 2.5x respectively this was not seen in TJC 1 and 3.

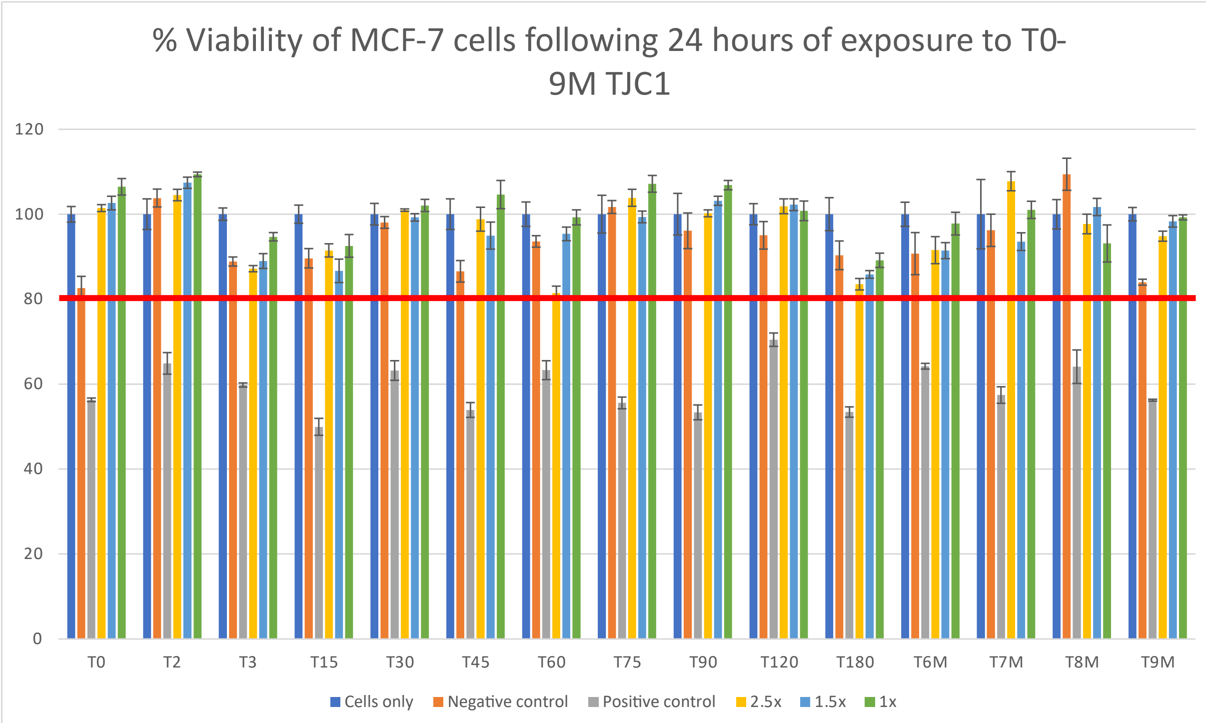

Figure 10. % viability of MCF-7 cells following 24 hours exposure T0 days to 9 Months TJC 1

5 Discussion

The microbial inactivation achieved by SODIS in 10L PP TJC in this study demonstrated good water disinfection efficiency and suggests that the PP transparent container is a promising drinking water treatment system for use at household level. According to the harmonized protocol of the WHO for Household Water Treatment and Safe Storage (HWTS), the inactivation reached (> 5 Log of *E. coli*, >2.5 Log of MS2 and from 1.2 to 3 Log of *C. parvum*) corresponds to the highest protection level for *E. coli*, and the protective (or limited protection) level for MS2 and *C. parvum* (only if two days of exposure are considered). Therefore, the 10L TJC performance is classified in the category of 2-star (minimum requirement of 2 Log_{10} reduction for bacteria and protozoa and 3 Log_{10} reduction for viruses). This 2-star category means that the tested product provides comprehensive protection against the three common classes of human pathogens responsible for diarrhoea diseases [51].

Microbial inactivation by SODIS is a well-known process attributed to the effects of solar photons (mainly UVA and UVB) and, sometimes, water temperature. It is recognized that UVB wavelengths (290-300 nm) affect DNA by direct photon absorption promoting the formation of pyrimidine dimers or single-strand breaks. UVA (320-400 nm) mainly causes damage by promoting the formation of internal Reactive Oxygen Species (ROS) including radical (hydroxyl, superoxide, hydroperoxyl, peroxyl, alkoxyl) and non-radical species (hydrogen peroxide, singlet oxygen, hypochlorous acid). These ROS are responsible for lipid peroxidation, protein oxidation and DNA damage leading finally to cell death. Nevertheless, there are several intrinsic differences among the three types of indicators tested that lead to different inactivation resistance (*E. coli* < MS2 coliphage < *C. parvum*). Bacteria are the most SODIS-susceptible indicator group, characterized by an active aerobic metabolism (basal ROS generation), rapid ROS accumulation when exposed to sunlight and low robustness of its protective cell wall (semi-permeable membranes) that is easily altered by oxidative attack causing ruptures and functional alterations.

Despite the biological simplicity of the viral indicator group, its resistance relies on the high robustness of the protective viral capsid structure and their low or absent metabolic activity. The inactivation of viruses (and MS2 coliphage) by SODIS has been attributed mainly to direct endogenous damage by UVB [52] and eventually increased by a strong synergy with water T above 40 ºC [53]. Therefore, the good efficiency observed in our results for MS2 inactivation can be explained and attributed mainly to the UVB transmittance of the PP material (Figure 7), removing water temperature as a key factor due to values consistently below 40 ºC being recorded during the SODIS treatment.

Water temperature is one of the most crucial elements in the case of *C. parvum* inactivation since it affects the durability of oocysts in the environment [54, 55]. Temperatures above 30-50°C cause the *Cryptosporidium's* viability to decrease because the oocyst wall contains fatty acids and hydrocarbons that have melting points near these temperatures and due to amylopectin metabolic activity increasing [55-58]. Spontaneous excystation [59] may also occur at high temperatures, whereby the sporozoites may excyst in small numbers as a result of the *C. parvum* oocysts being incubated at 37 °C without any other host stimulus [60]. As they are now outside of the host they are unable to survive. Also at temperatures above 45 °C solar thermal and optical effects are known to be strongly synergistic [8, 13].

Throughout the solar inactivation studies, the temperature ranged between 15.6 ± 3.0 °C and 33.2 ± 2.0 °C in the TJC and 16.0 ± 1.1 °C and 33.9 ± 4.5 °C in the PET bottles, reaching maximum values of 34.6 °C and 37.0 °C in TJC and PET bottles, respectively. Considering previous studies [59], and the thermal profile observed in the assays, with temperatures that did not exceed 37.0 °C, this abiotic factor can be ignored as the cause of *Cryptosporidium* inactivation. However, an increase is expected in oocyst inactivation when the water temperature reaches higher values [59].

Therefore, the solar inactivation of *C. parvum* oocysts observed during the experiments was caused predominantly by UV radiation, rather than temperature. Thus Liu *et al.* demonstrated that under solar full-spectrum oocyst inactivation is dominated by a mechanism of direct inactivation induced by UVB radiation [61, 62] The differences in the oocyst inactivation observed between 10L TJC and 1.5L PET bottles can be explained as a consequence of the different UVB transmission of the materials of the evaluated containers: 1% and 44%, in the global UVB range, and 59% and 60% in the UVA range, for PET and PP, respectively [63].

Regarding the two tested TJC with their exposure orientation facing both to the Sun, no significant differences were observed in this study using water with turbidity lower than 5 NTU. This indicates that under such conditions, the efficiency of the SODIS process is not strongly influenced by TJC orientation selected by the end-users. However it should be also mentioned that this conclusion may differ under other operational conditions, and therefore, the effect of turbidity, locations (latitude, altitude) and season of the year (related to the solar incidence angle) should be investigated in separate research, as the purpose of this study was to test the proof-of-principle capability of the TJC developed for SODIS purposes. In addition, the horizontal orientation, may have an additional drawback related to the possibility of leaks and loss of water if an unexpected rupture near the cap or stopper occurs during the solar exposure.

Our results are in good agreement with those previously reported by Polo-López et al. (2019) using 5L and 20L PP buckets for SODIS under natural sunlight [64]. In this research, no difference in the treatment performance were observed between both types of containers reaching ≥ 5.5 *E. coli* LRV in 250–300 kJ/m^2 of solar UVA dose or 3 h of solar exposure, 2.4-2.6 MS2 LRV in 500 kJ/m^2 or 5 h and from 93.21 ± 1.34% to 5.92 ± 3.73% and 8.23 ± 2.53% *C. parvum* oocysts viability in 850 kJ/m^2 or 6 h. In addition, field trials using the same 20L PP bucket container were tested at the household level in Malawi [65]. In comparison with the previous study under controlled conditions, lower efficiencies for naturally occurring bacteria inactivation were observed and attributed to the complexity of the natural water matrix used (unprotected water sources) and steady deterioration of the buckets following prolonged use.

For *C. parvum* oocysts, similar results were observed in previous studies in which the efficiency of *C. parvum* solar inactivation was evaluated in PP containers and PET bottles, despite slightly higher water temperatures and UV solar doses with respect to the present work [64]. Since similar temperature values were achieved in both types of SODIS reactors and the volume of the reactor has not influenced the microbial inactivation in clear water [66], the observed differences in oocyst inactivation between 10L TJC and 1.5L PET bottles may be the result of the different UV transmission of the evaluated materials: 1% and 44%, in the global UVB range, and 59% and 60% in the UVA range, for PET and PP, respectively [63].

Other transparent plastic materials and container configurations have been investigated as possible alternatives to PET bottles as containers for SODIS. Keogh et al., 2015 reported good SODIS efficacy for water containing *E. coli* within 19 L water cooler bottles made of transparent polycarbonate [67]. Several authors have also investigated the use of plastic bags and different materials, reaching similar conclusions in terms of food-grade polyethylene (PE) achieving good solar water disinfection in comparison with PET bottles, attributed to the UVB light transmittance of the material. In this regard, Lawrie et al., reported bacterial concentration below DL with a UV dose of 389 kJ/m^2 using plastic PE bags with a maximum capacity of 6 L [24]. Gutierrez-Alfaro *et al.* demonstrated 6 LRV of *E. coli* concentration in 60 min of normalized time under natural sunlight similar to using bags made of PE alone regardless of exposure orientation (vertical or horizontal)[16]. In the same study, the combination of PE with other chemical additives to improve the transmittance of the plastic material for SODIS in bags of 4 L was also investigated, reporting good results only using PE with ethylene vinyl acetate (EVA), with the advantage of a longer-life than PE bags alone (100% destroyed in 5 months).

The advanced weathering analysis of the PP showed definite degradation of the samples. The decrease in the first melting temperature seen in Table 2 may be a result of degradation due to crystallisation defects. The decrease in molecular weight observed in the plastics between weeks 0 to 2 and 4 to 8 and the increase in the polydispersity index between weeks 2 to 4 showed the polypropylene suffered high degradation from weathering that could possibly lead to chain scission and the formation of shorter chains that crystallize more easily. The increase observed in the absorption in the 1850-1600 cm^{-1} region also confirms photodegradation of the plastic due to the weathering. This increase is caused due to the formation of carbonyl species in the plastic as the photodegradation occurs. An increase in the carbonyl index occurs throughout the lifetime of the ageing of the polypropylene with it almost doubling after 2 weeks and doubling again after 4 weeks. This correlates with previous research by Lv et al., 2015, who observed increases in the carbonyl region and the carbonyl index when polypropylene was aged by natural and accelerated ageing. They reported an increase in absorbance in the carbonyl region and the carbonyl index after 9 days of accelerated ageing with a similar increase observed after 360 days of natural weathering [68]. They concluded it was a result of the formation of esters, carboxylic acids, lactones and ketones. These results obtained from the physico-chemical analysis denoted a critical time around the second week of accelerated ageing, which corresponds to 0.9 $MJ/m^2 \cdot nm$. The impact of the observed degradation to the detriment of both mechanical and optical properties, must be exhaustively studied to determine the lifetime of PP containers for further use.

As anticipated, the optical and mechanical properties also degraded because of photodegradation, however, the decrease was seen to stabilise after the 5th week of ageing with a transmission of 20% and 5% seen in the UVA and UVB respectively. This was consistent with previous studies whereby UV transmission dropped after 600 hours of accelerated ageing of 5 and 20-litre PP buckets stabilizing after this time period [64]. This does however show that a part of the UVB radiation was always transmitted despite the plastic degradation. As a result, the PP containers could be considered potential alternative materials to photo-inactivate UVB sensitive microorganisms, such as viruses and protozoa.

PP containers employed for solar disinfection must be suitably robust to withstand daily use and rough handling (falls, scratches, etc.). The result suggests from the variation of the break point that the brittleness of the PP containers should be checked after the time corresponding to a cumulative radiation dose of 0.9 $MJ/m^2 \cdot nm$, and new PP containers should be available for replacement in case of breakage. This effect of UV irradiation was reported in previous studies resulting in "important structural changes" of the PP, causing a decrease in the evolution of breaks as the exposure continued [69]. In any case, the addition of just 1% of UV-stabilizers during PP formation can extend the lifetime (and also the initial optical properties) of PP containers up to 9 months of solar exposure time (4 $MJ/m^2 \cdot nm$ at 340 nm)[29], which corresponds to 6 weeks of accelerated ageing under our conditions.

All samples and concentrations had a proliferation of 81.43% or higher. As per ISO 10993-5, this indicates the samples are non-toxic to cells and are biocompatible [50]. Samples exhibiting proliferation above 100%, with the highest being 117.74%, may indicate a proliferation effect that will require further genotoxicity testing such as E-screen. Diez *et al.* performed the E-screen assay on samples of PP 20L buckets used for SODIS and observed no mutagenicity over a 9 month period [22], no mutagenetic activity was observed when an Ames tests were performed also.

Figures 10 and Figures 1 and 2 in supporting material show the percentage of error bars of all wells, all are within acceptable parameters showing that the cell seeding was accurate and precise. In comparison to other research, MTT analysis of wastewater treatment plants have been used to analyse the toxicity of treated water. Zegura et. al. performed MTT analysis on chemical industry effluents, wastewater treatment plant effluents and river water samples [70]. Industrial effluents were seen to decrease cell viability as the concentration of the sample increased, chemical industry effluents displayed nontoxicity except in the higher concentrations and river water samples displayed toxicity through all the sample concentrations. The toxicity levels in the SODIS experiments showed lower toxicity when compared to the river water.

Analysis of three different water matrices with the MTT assay using Caco-2 cells found that when a 1:100 dilution of samples was used a decrease in cell viability of 18.0%. 24.5% and 33.8% was found for surface water, industrial wastewater and treated wastewater respectively. This decrease in cell viability in the industrial wastewater and treated wastewater was greater than the highest SODIS at 18.6%. Treated surface water was found to have a viability of between 71.2% and 91.2% [71] within the range of what was obtained from the SODIS. In the treatment of wastewater from a paper mill, the MTT assay found that the water was non-toxic to a max concentration of 30 v/v% sample [72]. The highest concentration used in SODIS was a 2.5 v/v% sample indicating a higher concentration may result in a decrease in cell viability

Toxicity analysis of photo-transformed microplastics with Caco-2 cells by Yu *et al.* showed a decrease in cell viability of 16.5% compared to ultra-pure water, this decrease was only 4.7% when the plastic were not photo-transformed [73]. In future experiments, a possible dark control could be used to ascertain if any leachates present are due to photolytic influences. In a comparison of the use of Caco-2 and MCF-7 cells it was concluded that there was "no significant difference" the analysis of toxicity with MCF-7 showing slightly less toxicity than that of the Caco-2 [74]. Future work will be carried out using Caco-2 cells to compare results to that of the MCF-7.

When polypropylene microplastics were analysed for toxicity the plastic was dissolved in DMSO and treated onto human dermal fibroblast cells. Polypropylene particles of a size of ~20 μm with concentrations of 500 μg/ml showed insignificant toxicity while a concentration of 1000 μg/ml showed a decrease of 20% in cell viability. When a polypropylene particle size of 25~200 μm was used, viability was only affected by 500 and 1000 μg/ml concentrations [75]. Further testing of dissolving the used plastics containers after completion of the 9 months could potentially ascertain if any additional compounds are present and are toxic, using HPLC. The examination of 5 leachates from polypropylene exhibited no toxicity on *Daphnia magna* [76]. Use of a similar toxicity analysis could be considered to further investigate if the containers are non-toxic.

6 Conclusions

This study found that 10 L polypropylene transparent jerrycans (TJC) are capable of an inactivation greater than 5 Log in *E. coli*, greater than 2.5 Log of MS2 and of between 1.2 to 3 Log for *C. parvum*. This inactivation is considered the highest protection in *E. coli* and protective with MS2 after 24 hours and *C. parvum* after 48 hours. According to the WHO guidelines for Household Water Treatment and Safe Storage (HWTS), this corresponds to an overall 2-star category treatment showing the TJC provides comprehensive protection against three common classes of waterborne pathogens. Regarding resistance to weathering, physicochemical tests confirmed that PP experienced photodegradation. Its impact on the optical properties was acceptable as the transmission ratios in the UVA and UVB ranges halved within 5 weeks of accelerated ageing, but subsequently, the ratios stabilised. This stabilisation shows that after 5 weeks the PP will have a standard transmission percentage of UVA and UVB allowing for treatment time to be altered accordingly. The effect of photodegradation on mechanical properties however, was significant after only the second week of accelerated ageing (solar exposure to 0.9 $MJ/m^2 \cdot nm$ at 340 nm), as PP became very brittle. New PP containers should be available for replacement in case of breakage. However, the lifetime of PP containers can be largely extended by adding small concentrations of UV-stabilizers to the PP formulation. Toxicity analysis using MTT found that all samples at all concentrations retained a cell viability above 80% with the lowest being 81.43% indicating the samples were biocompatible as per ISO 10993-5. The TJC proved to be capable of comprehensive protection from microorganisms and was non-toxic to humans but the plastic was not sufficiently robust for long-term use. Further studies will be carried out with the use of a UV stabiliser to increase the structural lifetime of the PP.

Acknowledgments

The authors acknowledge the financial support from the European Union's Horizon 2020 research and innovation programme for the PANIWATER project (GA 820718), funded jointly by European Commission and Department of Science and Technology, India.

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Supporting materials

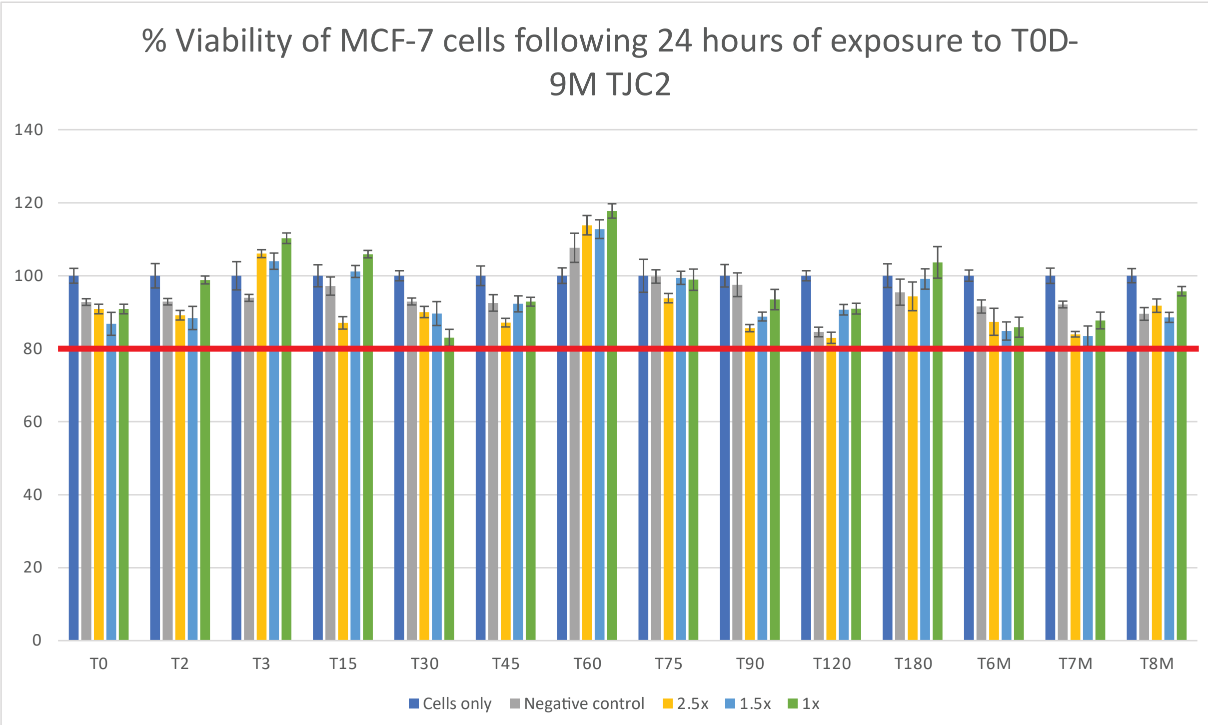

Figure 1. % viability of MCF-7 cells following 24 hours exposure T0 days-9 Months TJC 2

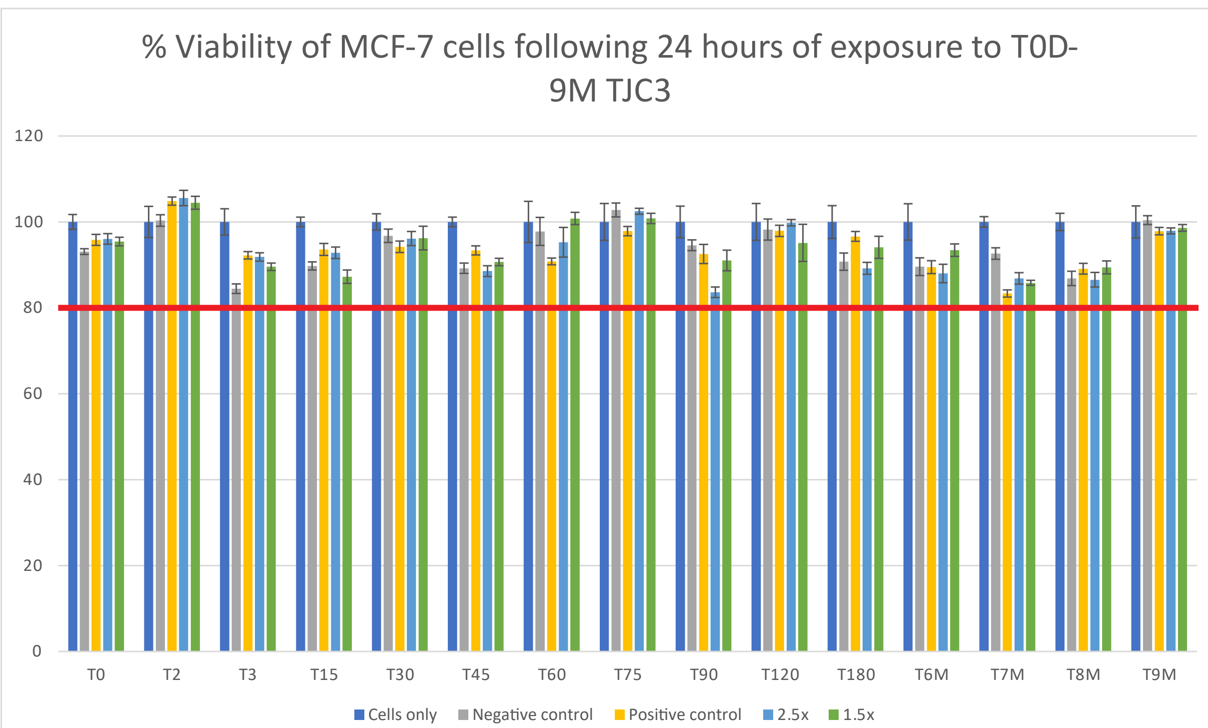

Figure 2. % viability of MCF-7 cells following 24 hours exposure T0 days-9 Months TJC 3