



Evaluation of peroxymonosulfate addition as a strategy to enhance *Cryptosporidium* inactivation in solar water disinfection

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ABSTRACT

Solar disinfection (SODIS) is a low-cost water treatment used in areas without access to safe water, with well-established efficacy against bacteria, but limited for virus and protozoa. *Cryptosporidium parvum* is a waterborne protozoan resistant to conventional water disinfection methods, representing a major challenge in public health. This study evaluates for the first time the enhancement of SODIS against *C. parvum* using peroxymonosulfate (PMS). Distilled and well water with different PMS concentrations, in the absence or presence of Fe^{2+} , were spiked with *C. parvum* oocysts and exposed to simulated solar radiation at 40 °C for 6 h, directly and under a polyethylene terephthalate (PET), polypropylene (PP) and polymethylmethacrylate (PMMA) covers. Oocyst viability was evaluated by quantifying 70 kDa heat shock protein mRNA using reverse transcription qPCR. Results showed that PMS concentrations ≥ 0.5 mM, in the absence and presence of Fe^{2+} , reached > 4 logarithmic reduction (LR) in the oocyst survival when water was exposed uncovered. Under all plastic covers, > 4 LR was achieved in the absence of Fe^{2+} in well water. Toxicity assays with *Daphnia magna* showed that, in the presence of Fe^{2+} , 0.5 mM PMS-treated well water under PP and PMMA covers caused only 10% mortality after 24 and 48 h. Overall, results demonstrated that the incorporation of PMS in SODIS procedures can effectively inactivate *C. parvum* oocysts and reduces exposure time.

1. Introduction

The World Health Organization (WHO) estimates that at least 2 billion people still consume water from sources contaminated with faecal matter [1]. This situation along with poor sanitation is highly related to the transmission of infectious diarrhoeal diseases [2], which remain a leading cause of child mortality and morbidity, mainly resulting from the consumption of contaminated food and water. This disease is responsible for almost 500 000 deaths in children every year and represents the second major cause of mortality in children under 9 years [3]. Moreover, the combination of poverty, inadequate drinking water and sanitation results in malnutrition, economic losses due to inability to work, lack of education and social development [4].

Solar disinfection (SODIS) has become a widespread household water treatment (HWT) in low- and middle-income countries (LMICs), where access to safe water is a challenging task. SODIS consists in filling transparent containers, mostly 1.5–2 L polyethylene terephthalate (PET)

plastic bottles, with microbiologically contaminated water and place them in direct sunlight for at least 6 h [5]. This method is an appropriate procedure to generate drinking water due to its effectiveness against a wide range of waterborne pathogens, low cost, ease of use and environmental sustainability [6,7]. Several studies have demonstrated the positive impact of this method on health in countries like Ethiopia, Kenya, Cameroon, Cambodia or India [8–13]. However, SODIS presents several limitations which influence the efficacy of the process: i) the low volume of the plastic bottles is not enough to satisfy daily family requirements; ii) the transmittance of the material which the containers are made. Specifically, PET is opaque to ultraviolet (UV)-B, which is responsible for DNA damage, resulting in decreased microbial inactivation, specially of viruses and protozoa; and iii) bacteria can also re-grow due to their capacity to repair genetic damage [6,14,15]. Furthermore, while the efficacy of SODIS procedures has been proven to be excellent for bacteria, this may be limited for viruses and protozoa [6,7,16–18].

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Advanced oxidation processes (AOPs) have been subject of studies given their capacity to enhance solar decontamination and disinfection through the generation of strongly reactive oxygen species (ROS), such as hydroxyl radicals (HO^\bullet), hydrogen peroxide (H_2O_2), among others, which are capable to inactivate microorganisms and degrade organic contaminants [19–21]. Recently, sulfate radicals (SO_4^\bullet) have attracted attention for their high redox potential (2.5–3.1 V), which is comparable to hydroxyl radicals (2.8 V vs. standard hydrogen electrode), reacting more selectively with organic compounds in a wide pH range of 2–8 [19]. Moreover, the half-life of sulfate radicals is longer, which facilitates better interaction with target compounds [22].

Peroxymonosulfate (PMS) is the active form of potassium hydrogen monopersulfate trisalt in aqueous solutions. PMS can be activated into hydroxyl and sulfate radicals through homolytic cleavage of the peroxide bond of HSO_5^- by UV radiation (Eq. (1)), heat or transition metals, being Fe^{2+} (Eq. (2)) one of the most commonly used [17,23].



Besides the main pathway of sulfate and hydroxyl radicals generation (Eq. (1)), there are propagation reactions that continue producing radicals. Sulfate radicals can react with PMS in the medium, resulting in sulfur pentoxide radicals (SO_5^\bullet) (Eq. (3)) [17].



PMS could be a suitable oxidant for water disinfection because it is easy to handle, stable, low cost and eco-friendly. Moreover, the enhancement of the SODIS procedures only requires micromolar concentrations, producing harmless residual compounds that are compatible with drinking water [20,24,25]. While several studies have proven that PMS is an effective inactivator of potential pathogenic microorganisms, such as viruses, bacteria and yeasts [23,26–31], there is a lack of research to determine its efficacy against the waterborne protozoa group.

Cryptosporidium is a genus of protozoan parasites that causes gastrointestinal disease in human and many vertebrate hosts [32]. The infective form (oocyst) is particularly resilient, as it is resistant to conventional disinfection methods, like chlorination, and represents a significant risk to public health [32–34]. Oocysts are transmitted by the faecal-oral route and their small size, the low infective dose, the high multiplication capacity and the absence of treatment facilitate their dissemination [34,35]. Thus, *Cryptosporidium* was responsible for 77.4% waterborne disease outbreaks reported between 2017 and 2022, being this parasite the most common protozoan agent [36]. It causes 2.9–4.7 million diarrhoeal episodes every year in children under 2 years, resulting in more than 200 000 deaths annually [37,38]. Although *Cryptosporidium* represents a major challenge in water treatment in both industrialised and developing countries, there is a significantly higher prevalence in LMICs due to socio-economic factors [39]. All these considerations lead to *Cryptosporidium* risk mitigation continuing to be a critical concern for water utilities globally [40]. The World Health Organization chose *C. parvum* as the reference pathogen for the waterborne protozoa group in the assessment of HWT due to its high environmental persistence, extreme resistance to chemical disinfection and difficulty to control in water treatments [41]. The WHO international scheme to evaluate HWT establishes that disinfection treatments must demonstrate at least 2-log or 4-log reduction (high protection) for protozoa. Using *C. parvum* as a benchmark ensures that technologies achieving 2 or 4 log reduction provide robust protection not only against *Cryptosporidium* but also against less resistant protozoa [41].

Over the past decades, several studies evaluated the effectiveness of SODIS against *Cryptosporidium*, demonstrating that temperature and UV radiation are key factors in the inactivation of *C. parvum* oocysts [42–45]. Thus, their synergistic effect and the strong influence of the

spectral distribution of the incident light were confirmed, with UV-B radiation being the main agent responsible for oocyst inactivation [44, 46–48]. Furthermore, effect of water turbidity was also assessed, emphasizing its role as a one of the major limiting factors for the efficiency of SODIS against *Cryptosporidium* [43,49].

In addition to these findings, research has focused on overcoming the limitations of the SODIS method against *Cryptosporidium*. Strategies to enhance light transmittance and increase the amount of treated water involved evaluating containers with different plastic materials and volumes [50–52]. Other approaches included the use of compound parabolic collector reactors and the integration of AOPs, such as TiO_2 and the photo-Fenton process [53–58]. Despite the variety of research in different SODIS approaches and strategies against *Cryptosporidium*, it is still scarce compared to studies conducted on other microorganisms, highlighting the need for further investigation.

SODIS method is a water treatment used in LMICs and its efficacy towards environmentally resistant pathogens, such as *C. parvum*, remains limited. Therefore, its enhancement is key to provide safer drinking water in resource-poor settings. To date, no studies have investigated the application of PMS in the SODIS method against *Cryptosporidium*. The present study evaluates the addition of PMS in the presence and absence of Fe^{2+} to determine whether these modifications can significantly increase oocyst inactivation compared with standard SODIS. Additionally, this work assesses the influence of three plastic materials used in SODIS containers with different water matrices and includes a toxicological approach. Its findings are expected to provide preliminary insights for improving SODIS efficacy against protozoa with potential applications for safer household drinking water in LMICs.

2. Material and methods

2.1. *C. parvum* oocysts

Cryptosporidium oocysts were obtained from naturally infected neonatal Friesian–Holstein calves in dairy farms in Galicia (northwest Spain). Concentration (0.04 M phosphate-buffered saline [PBS] pH 7.2 and diethyl ether), purification (discontinuous caesium chloride gradients), quantification (improved Neubauer counting chamber) and molecular characterization were performed as previously reported [54]. The isolate was identified as *C. parvum* subtype IIaA15G2R1 by PCR amplification and sequence analysis of ~587-bp fragment of the small subunit rDNA (*ssu-rDNA*) and 850-bp fragment of the 60 kDa glycoprotein (*gp60*) genes of *Cryptosporidium* [59,60]. The viability of *C. parvum* isolate was determined by inclusion/exclusion of the fluorogenic vital dye propidium iodide (PI) and induced excystation following the protocols described elsewhere [54].

2.2. Water matrices

To assess the influence of water composition on *Cryptosporidium* inactivation, experiments were conducted in distilled water (pH 6.9, conductivity < 10 $\mu\text{S}/\text{cm}$, total organic carbon < 0.5 mg/L) and natural well water collected in a private residence and stored at 8 °C until use. The main physicochemical and microbiological parameters of the well water are detailed in Table S1.

2.3. Experimental design

Assays were performed using PMS triple salt (Oxone™, Sigma-Aldrich, St. Louis, MO, USA) at different concentrations (0.1, 0.5, 2.5 and 5 mM) in the absence and presence of iron (18 μM Fe^{2+} , corresponding to 1 ppm, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, Panreac Química SLU, Barcelona, Spain).

A solar simulator (Suntest CPS+, ATLAS Material Testing Technology GmbH, Lisengericht, Germany) equipped with a 1100 W air-cooled xenon arc lamp and a combination of filters (Suprax, ATLAS, Material

Testing Technology GmbH) was used to simulate the outdoor solar radiation spectrum. Quartz tubes containing 3 mL of distilled water and the selected PMS concentration in the absence or presence of Fe^{2+} were contaminated with 2×10^6 oocysts/mL of *C. parvum* and placed in an open reaction-jacketed borosilicate beaker (135 × 75 mm) with 700 mL of distilled water. Samples were exposed to simulated solar radiation (40 W/m^2 , 280–400 nm) for 6 h. The temperature of the beaker during the experiments was kept at $40 \text{ }^\circ\text{C}$ by connecting the reactor to a refrigerated and heated bath circulator (Fisherbrand™ Isotemp™ 4100 R20, Thermo Fisher Scientific Inc., Waltham, MA, USA), and monitored by a thermometer (Eutech Instruments, Singapore) fitted with a type K probe (RS Components, Madrid, Spain). The temperature of $40 \text{ }^\circ\text{C}$ represents an average value reached under SODIS natural conditions, as reported in previous studies [42,43,45,61]. At 1, 2, 4 and 6 h of exposure, the tubes were mixed during 3 min and aliquots of 50 μL containing 10^5 oocysts were collected and stored at $4 \text{ }^\circ\text{C}$ overnight before processing.

Experiments were also performed under dark conditions at $40 \text{ }^\circ\text{C}$. For that, 1.5 mL RNase-free microtubes containing 300 μL of distilled water and the selected PMS concentration in the absence or presence of Fe^{2+} were contaminated with 2×10^6 oocysts/mL of *C. parvum* and placed in a refrigerated and heated bath circulator (Fisherbrand™ Isotemp™ 4100 R20,) at $40 \text{ }^\circ\text{C}$ for 6 h. Samples were collected and stored as previously described.

Moreover, the efficacy of PMS under different plastic materials was assessed. For this, quartz tubes containing 3 mL of distilled water or well water with the minimum concentration of PMS to inactivate *C. parvum* oocysts, in the absence or presence of Fe^{2+} (18 μM), were spiked with 2×10^6 oocysts/mL of *C. parvum*, placed in an open reaction-jacketed borosilicate beaker with 700 mL of distilled water and covered with three types of plastic sheets that show different transmission spectra: PET, polypropylene (PP) and polymethyl methacrylate (PMMA) (Fig. S1). Then, the samples were exposed to simulated solar radiation (40 W/m^2 , 280–400 nm) at $40 \text{ }^\circ\text{C}$ for 6 h. Samples were collected and stored as in previous simulated solar experiments.

The optical transmittance of the plastic sheets was measured by cutting them into 1.5 cm × 3 cm pieces and using a UV-Vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA). Fig. 1 shows the transmittance of the three different plastic covers employed in this study.

All the experiments were carried out in duplicate.

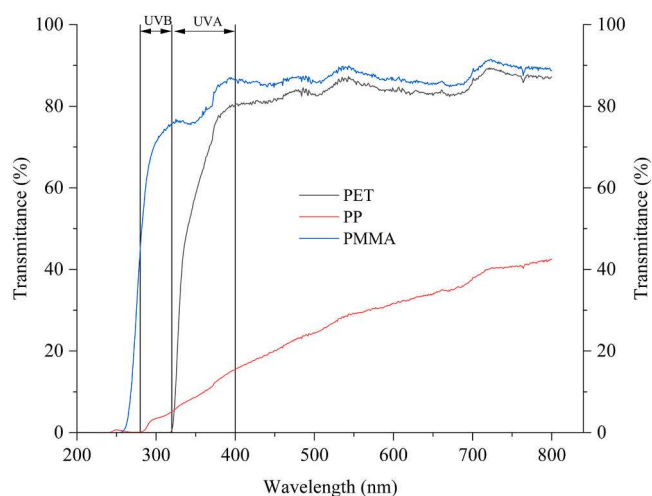


Fig. 1. Transmission spectra of polyethylene terephthalate (PET), polypropylene (PP) and polymethylmethacrylate (PMMA) plastic covers used in the present study.

2.4. Assessment of oocyst survival

The oocyst viability was determined by quantitative reverse transcription-polymerase chain reaction (RT-qPCR) targeting the messenger RNA (mRNA) of the 70 kDa heat shock protein (*hsp70*) gene of *Cryptosporidium* [62], with previous induction of the mRNA expression and extraction. Briefly, aliquots of each sample containing 1×10^5 oocyst structures were incubated in a water bath (Fisherbrand™ Isotemp™ 4100 R20, Thermo Fisher Scientific Inc.) at $42 \text{ }^\circ\text{C}$ for 20 min to induce the expression of the mRNA of *hsp70*. Then, the samples were subjected to 6 cycles of freezing in liquid nitrogen, thawing at $65 \text{ }^\circ\text{C}$ and short spinning at room temperature to break the oocyst wall. Finally, mRNA was isolated and purified by the oligo(dT)₂₅ method using Dynabeads® mRNA DIRECT™ Kit (Invitrogen, Thermo Fisher Scientific, Vilnius, Lithuania) in accordance with the manufacturer's instructions. The eluted mRNA was quickly placed on ice and used immediately or stored at $-80 \text{ }^\circ\text{C}$ until use.

The RT-qPCR reactions were performed in a StepOne™ Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) using 96 well cone-bottom plates (VWR International BV, Leuven, Belgium) covered with an optical adhesive film (Nerbe Plus GmbH, Winsen, Germany). Each reaction was 20 μL , containing 2 μL of mRNA, 10 μL of One Step PrimeScript™ III RT-qPCR Mix (Takara Bio Inc., Kusatsu, Japan), 0.6 μM of each primer (Cr_hsp70-1005-fp [5'-AACTTTAGCTCCAGTTGAGAAAGTACTC-3'] and Cr_hsp70-1095-rp [5'-CATGGCTCTTACCGTTAAAGAATTC-3']), 0.3 μM of the probe (Cr_hsp70-1062-probe [5'-FAM-AATACGTGTAGAACCACCAACCAATACAACATC-TAMRA-3']), 1X of carboxy-X-rhodamine (ROX) reference dye and molecular grade water. The thermal protocol consisted in a previous phase at $50 \text{ }^\circ\text{C}$ for 30 min to conduct reverse transcription, followed by $94 \text{ }^\circ\text{C}$ for 1 min and 45 cycles of $95 \text{ }^\circ\text{C}$ for 15 s and $60 \text{ }^\circ\text{C}$ for 20 s. To determine oocyst viability, a standard curve was built by using serial dilutions of viable oocyst suspensions containing 10^0 – 10^6 oocysts/50 μL . These oocyst suspensions were subjected to the same induction of the mRNA expression, extraction and purification, and RT-qPCR processes previously described. Based on the cycle threshold (Ct) values obtained from the exposed samples and the standard curve, the number of viable *C. parvum* oocysts was determined. The detection limit of the technique was established at 1 viable oocyst. Logarithmic reduction (LR) was calculated considering the viability of the *C. parvum* isolate used in each assay [41]. Negative controls, using molecular grade water, were included in all RT-qPCR runs.

2.5. Analytical determinations

PMS consumption, iron concentration and sulfate levels were determined in well water containing 0.5 and 1 mM PMS, in the presence and absence of Fe^{2+} , exposed to simulated solar radiation under three types of plastic covers (PET, PP and PMMA). PMS consumption was measured using the colorimetric *N,N*-diethyl-*p*-phenylenediamine (DPD) method, as described in Standard Methods for Examination of Water and Wastewater [63]. Iron concentrations were quantified by UV spectrophotometry according to ISO 6332. Both analyses were performed using a UV-Vis spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific, Wilmington, USA). Sulfate levels were determined by ionic chromatography (930 Compact IC Flex Metrohm AG, Herisau, Switzerland) in the RIAIDT service of the University of Santiago de Compostela.

2.6. Toxicological assays

Acute toxicity assays were conducted using *Daphnia magna* neonates following the standard operational procedure of the commercial Daphtoxkit F™ (Microbiotest, Gent, Belgium) in compliance with the ISO 6341 and OECD Guideline 202. *Ehippippa* were hatched under controlled conditions for 72 h. Neonates less than 24 h old were exposed to well

water samples treated with 0.5 and 1 mM PMS, in the presence and absence of Fe^{2+} , under simulated solar radiation covered with three types of plastic sheets (PET, PP and PMMA). Toxic effects were evaluated at 24 and 48 h.

2.7. Statistical analysis

Statistically significant effects on oocyst viability were identified by analysis of variance (ANOVA) and pairwise multiple comparison procedures (Tukey HSD test). Analyses were performed using Statgraphics Centurion 18 (©1982–2018 Statgraphics Technologies, Inc., The Plains, VA, USA). Differences were considered significant at $P < 0.05$. Normality and variance homogeneity were previously verified using Shapiro-Wilk test. The graphs presented were generated using Origin® software (©2012 OriginLab® Corporation, Northampton, MA, USA). The results shown are the average values of two replicate experiments, each of which was analysed in duplicate by RT-qPCR, and error bars represent the standard deviations.

3. Results

3.1. Inactivation of *C. parvum* oocysts by PMS

The viability of the *C. parvum* isolates determined by inclusion/exclusion of PI and induced excystation was $99.37 \pm 0.32\%$ and $97.63 \pm 0.72\%$, respectively. The incubation of the oocysts at 40°C in darkness during 6 h did not show significant effects in the oocyst survival, detecting reductions in the viability of 0.18 ± 0.39 log. The addition of PMS at concentrations of 0.1 and 0.5 mM reduced slightly the oocyst viability (0.30 ± 0.04 LR and 0.74 ± 0.12 LR, respectively). However, PMS concentrations of 2.5 and 5 mM caused a sharp decrease in the oocyst viability after 1 h of exposure (3.73 ± 0.08 LR and 3.52 ± 0.08 LR, respectively), reaching 5.08 ± 0.00 LR and 4.79 ± 0.35 LR, respectively, at the end of the assay (6 h). The incubation at 4°C in presence of 5 mM PMS did not show any significant effects on the viability of *C. parvum* oocysts (0.39 ± 0.55 LR) (Fig. 2a).

When samples were exposed to simulated solar radiation, higher oocyst inactivation was observed at all PMS concentrations (Fig. 2b), detecting statistically significant differences with the results obtained in dark conditions at PMS concentrations of 0.1, 0.5 and 2.5 mM ($P < 0.05$). At the lowest concentration of 0.1 mM, oocyst viability decreased progressively until 3.92 ± 0.17 LR at the end of the assay, very close to the oocyst inactivation observed without PMS (3.44 ± 0.22 LR) (Fig. 2b). At 0.5 mM, oocyst inactivation also proceeded gradually,

but exceeding 4 LR after 4 h of exposure and reaching 5.01 ± 0.09 LR at the end of the assay. In contrast, at concentrations of 2.5 and 5 mM PMS, a strong decrease in the survival of *C. parvum* was observed after only 1 h of exposure, leading to 5.07 ± 0.0 and 5.00 ± 0.09 LR, respectively, and causing total oocyst inactivation (Fig. 2b).

3.2. Inactivation of *C. parvum* oocysts by PMS in the presence of Fe^{2+}

The inactivation kinetics of *C. parvum* oocysts obtained in the presence of different concentrations of PMS and Fe^{2+} under dark conditions and simulated solar radiation at 40°C is shown in Fig. 3. Under dark conditions at 40°C (Fig. 3a), the presence of $18 \mu\text{M}$ of Fe^{2+} does not affect significantly to the viability of *C. parvum* oocysts (0.42 ± 0.10 LR vs. 0.18 ± 0.39 LR in the presence and absence of Fe^{2+} , respectively, after 6 h of exposure). The addition of PMS at a concentration of 0.1 mM also did not affect significantly to the oocyst survival (0.43 ± 0.03 LR). However, the use of PMS 0.5 mM/ Fe^{2+} led to a slight decrease in the oocyst viability (1.18 ± 0.01 LR), but higher than observed with only 0.5 mM PMS (0.74 ± 0.12 LR). At concentrations of 2.5 and 5 mM of PMS/ Fe^{2+} , a sharp decrease in the oocyst viability was observed, reaching 5.02 ± 0.06 LR after 1 h of exposure with 5 mM of PMS/ Fe^{2+} and 4.85 ± 0.12 LR after 2 h using 2.5 mM of PMS/ Fe^{2+} (Fig. 3a). At the end of the experiments, significant differences were not observed when comparing the results obtained in dark conditions with only PMS and PMS/ Fe^{2+} (Figs. 2a and 3a).

Similarly, under simulated solar radiation, the addition of Fe^{2+} did not improve the results of oocyst inactivation compared to experiments performed with PMS alone (Figs. 2b and 3b). While at concentrations of 2.5 and 5 mM of PMS/ Fe^{2+} , total oocyst inactivation was reached at 1 h of exposure, lower values of oocyst inactivation were determined at concentrations of 0.5 and 0.1 mM, although no significant differences were observed among them at the end of the assays (3.39 ± 0.32 , 4.63 ± 0.19 , 4.93 ± 0.26 and 5.01 ± 0.09 LR for $18 \mu\text{M}$ Fe^{2+} and concentrations of PMS of 0.1, 0.5, 2.5 and 5 mM, respectively, vs. 3.92 ± 0.17 , 5.01 ± 0.09 , 5.07 ± 0.0 and 4.97 ± 0.00 LR for PMS alone at concentrations of 0.1, 0.5, 2.5 and 5 mM, respectively). However, under simulated solar light, the presence of only $18 \mu\text{M}$ of Fe^{2+} gradually reduced the survival of *C. parvum* oocysts and statistically significant higher values of oocyst inactivation were observed compared to the samples exposed without PMS and Fe^{2+} after 6 h of exposure (4.28 ± 0.16 LR in the presence of Fe^{2+} vs. 3.44 ± 0.22 LR without PMS and Fe^{2+}) ($P < 0.05$) (Fig. 3b).

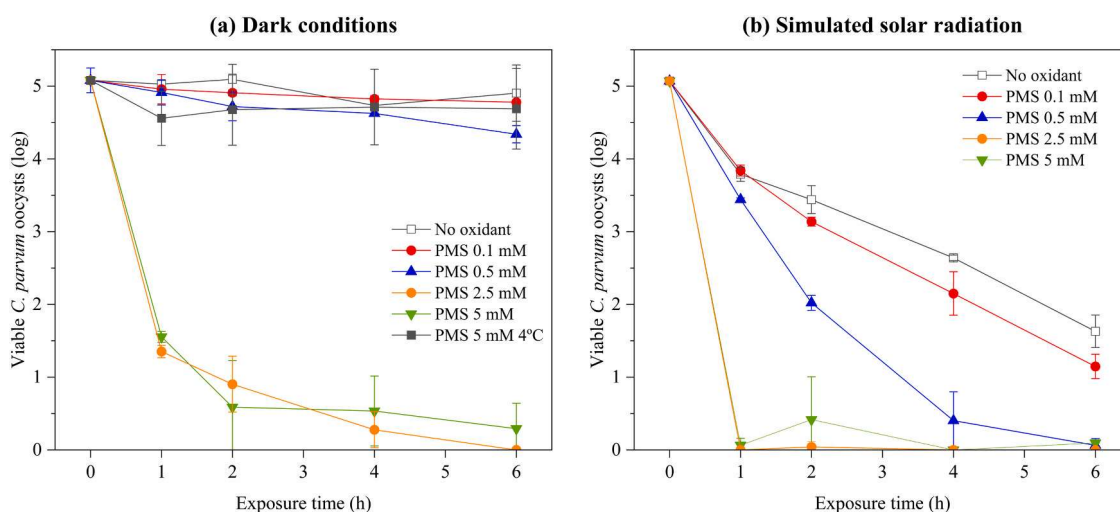


Fig. 2. Inactivation kinetics of *C. parvum* oocysts determined in distilled water at 40°C with different concentrations of peroxymonosulfate (PMS): (a) in dark conditions; (b) under simulated solar radiation (40 W/m^2 , 280–400 nm).

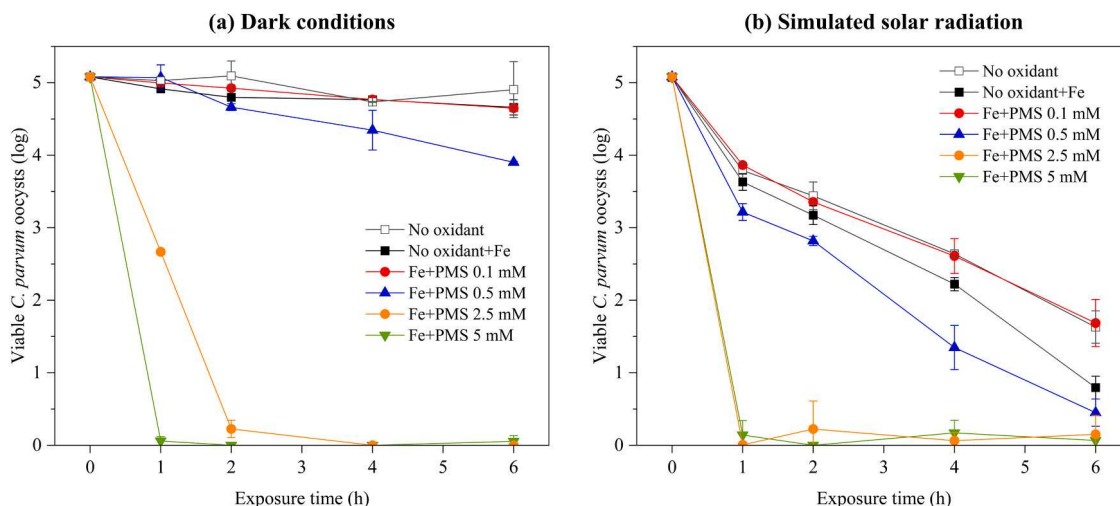


Fig. 3. Inactivation kinetics of *C. parvum* oocysts determined in distilled water at 40 °C with different concentrations of peroxymonosulfate (PMS) and Fe^{2+} (18 μM): (a) in dark conditions; (b) under simulated solar radiation (40 W/m^2 , 280–400 nm).

3.3. Efficacy of SODIS/PMS/ Fe^{2+} inactivation of *C. parvum* oocysts under different plastic materials

3.3.1. Distilled water

The inactivation of *C. parvum* oocysts with PMS in the presence and absence Fe^{2+} and under simulated solar conditions at 40 °C with PET, PP and PMMA covers is shown in Fig. 4. In the absence of PMS and Fe^{2+} , a statistically significant decrease in the oocyst inactivation was observed at the end of the experiments when PET and PP covers were employed ($P < 0.05$) compared to the results obtained with uncovered samples, whereas under PMMA cover, there was not statistically significant differences (0.60 ± 0.13 , 1.86 ± 0.05 , 3.20 ± 0.06 and 3.44 ± 0.22 LR for PET, PP, PMMA and without plastic cover, respectively) (Figs. 2b and 4). Similar behaviour was observed in the presence of only 18 μM of Fe^{2+} , obtaining significant lower oocyst inactivation with all the plastic covers in comparison to the experiments carried out without them (1.11 ± 0.52 , 2.49 ± 0.00 , 3.38 ± 0.07 and 4.28 ± 0.16 LR for PET, PP, PMMA and without plastic cover, respectively) (Figs. 3b and 4).

In the presence of 0.5 mM PMS, a reduction in the efficacy of oocyst inactivation was also observed when samples were exposed for 6 h under a PET cover compared to uncovered samples (3.65 ± 0.30 LR vs. 5.01 ± 0.09 LR, respectively) ($P < 0.05$) (Figs. 2b and 4a). However, very similar efficiencies were found when samples were exposed under PP and PMMA cover, reaching almost total oocyst inactivation at 4 h of exposure (4.94 ± 0.02 , 5.04 ± 0.00 , and 4.67 ± 0.40 LR, for PP, PMMA and uncovered samples, respectively) (Figs. 2b and 4b-c). The increase in PMS concentration to 1 mM in the samples exposed under PET cover led to higher oocyst inactivation compared to 0.5 mM, reaching > 4 LR after 4 h of exposure and the total oocyst inactivation at the end of the assay (Fig. 4a).

The combination of 18 μM Fe^{2+} and 0.5 mM PMS diminished the efficiency of the process in all types of plastic covers evaluated in comparison with the use of only 0.5 mM PMS, although under PP and PMMA, approximately 5 LR was determined after 6 h of exposure (Fig. 4). However, under PET cover, the addition of 18 μM Fe^{2+} improved the results only in the presence of 1 mM PMS, achieving 4.94 ± 0.20 LR after 4 h of exposure to solar radiation, while no improvement was observed with 0.5 mM (Fig. 4a).

3.3.2. Well water

Fig. 5 shows the inactivation of *C. parvum* oocysts with PMS in well water, in the presence and absence of Fe^{2+} , under simulated solar radiation at 40 °C with PET, PP and PMMA covers. Both in the absence of PMS and in the presence of 18 μM Fe^{2+} alone, similar inactivation rates

were observed in well water than in distilled water, except in experiments carried out under PMMA cover ($P < 0.05$) (Figs. 4 and 5).

In the presence of 0.5 mM PMS, after 6 h of exposure, oocyst inactivation reached 4.50 ± 0.02 , 4.45 ± 0.20 and 4.98 ± 0.08 LR for PET, PP and PMMA covers, respectively. When 0.5 mM PMS was combined with Fe^{2+} , oocyst inactivation was significantly reduced in all cases ($P < 0.05$), reaching 1.83 ± 0.19 , 2.35 ± 0.16 and 4.02 ± 0.23 LR for PET, PP and PMMA, respectively (Fig. 5). Increasing the PMS concentration to 1 mM in samples covered with PET resulted in almost 5 LR after 4 h of exposure (4.90 ± 0.04 LR), while in the presence of Fe^{2+} oocyst inactivation already reached 4.50 ± 0.13 LR at the same time.

3.4. Analytical determinations

As illustrated in Fig. 6, PMS consumption was monitored over time in well water exposed to simulated solar radiation at 40 °C under PET, PP and PMMA covers, in the absence and presence of Fe^{2+} . PMS consumption remained limited for all materials, in the absence of Fe^{2+} , with PMMA showing the highest PMS consumption (31.60%) at an initial concentration of 0.5 mM after 6 h, whereas PP and PET showed 14.51% and 15.60%, respectively. In contrast, the presence of Fe^{2+} resulted in complete PMS consumption after 4 h for PMMA and 6 h for PET and PP (Fig. 6).

Regarding iron concentration in well water (Table S2), in the absence of PMS, iron was predominantly present as Fe^{2+} at the beginning of the experiment. However, when PMS was added, iron was already fully oxidized. After 6 h of exposure to simulated solar radiation, iron was found exclusively as Fe^{3+} in all conditions, regardless the presence or absence of PMS.

Sulfate levels are reported in Table S3. After 6 h of exposure to simulated solar radiation at 40 °C, in the presence and absence of Fe^{2+} , sulfate concentrations were 101–117 mg/L for 0.5 mM PMS and for 1 mM PMS 230–238 mg/L.

3.5. Toxicological assays

Acute toxicity was evaluated in *D. magna* neonates exposed to well water samples treated with PMS in the absence and presence of Fe^{2+} under simulated solar radiation at 40 °C, using PET, PP and PMMA covers, with mortality assessed after 24 and 48 h of exposure. As shown Fig. 7, in the absence of iron, mortality was 100% in all conditions at both exposure times, regardless of the plastic material used or concentration tested. In contrast, in the presence of Fe^{2+} , for samples under PET, mortality at 24 h was 60% at 0.5 mM and 75% at 1 mM PMS, while

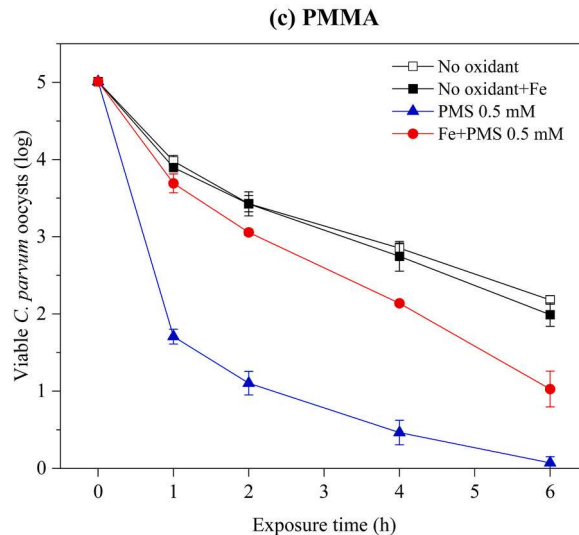
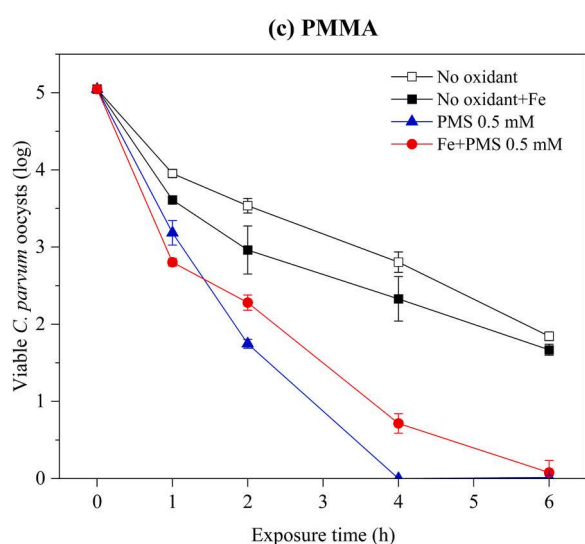
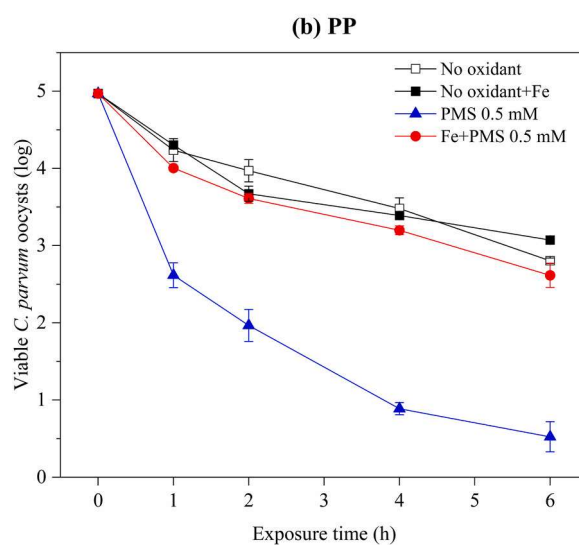
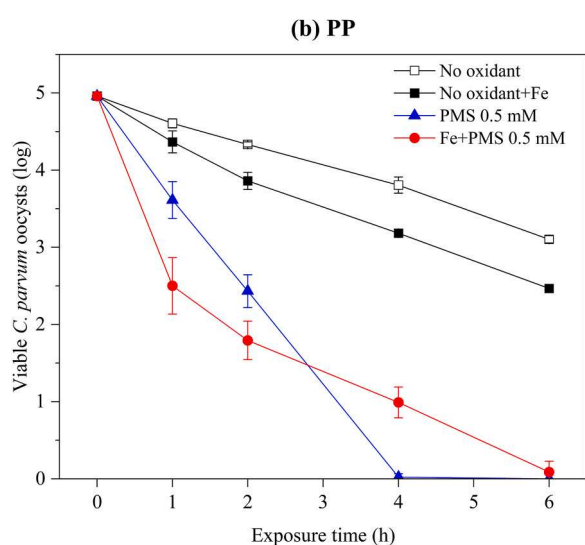
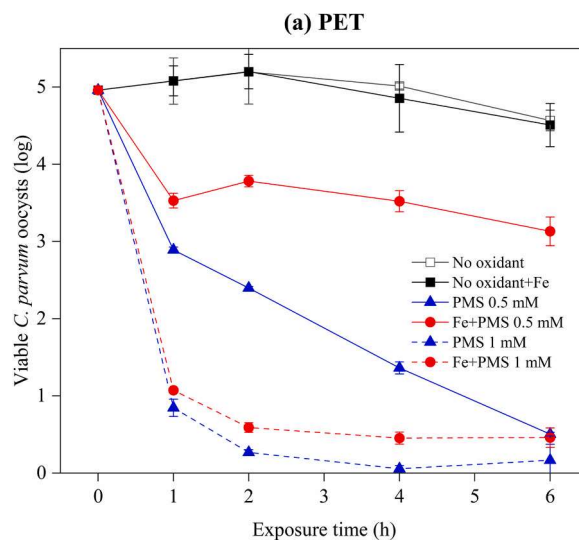
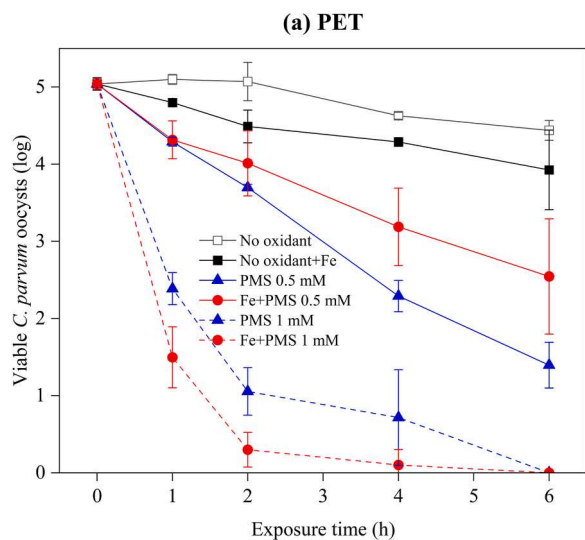


Fig. 4. Inactivation kinetics of *C. parvum* oocysts determined in distilled water under simulated solar radiation (40 W/m^2 , 280–400 nm) at 40°C with different concentrations of peroxymonosulfate (PMS) in the presence and absence of Fe^{2+} ($18 \mu\text{M}$) using different plastic covers: (a) polyethylene terephthalate (PET); (b) polypropylene (PP), and (c) polymethylmethacrylate (PMMA).

Fig. 5. Inactivation kinetics of *C. parvum* oocysts determined in well water under simulated solar radiation (40 W/m^2 , 280–400 nm) at 40°C with different concentrations of peroxymonosulfate (PMS) in the presence and absence of Fe^{2+} ($18 \mu\text{M}$) using different plastic covers: (a) polyethylene terephthalate (PET); (b) polypropylene (PP), and (c) polymethylmethacrylate (PMMA).

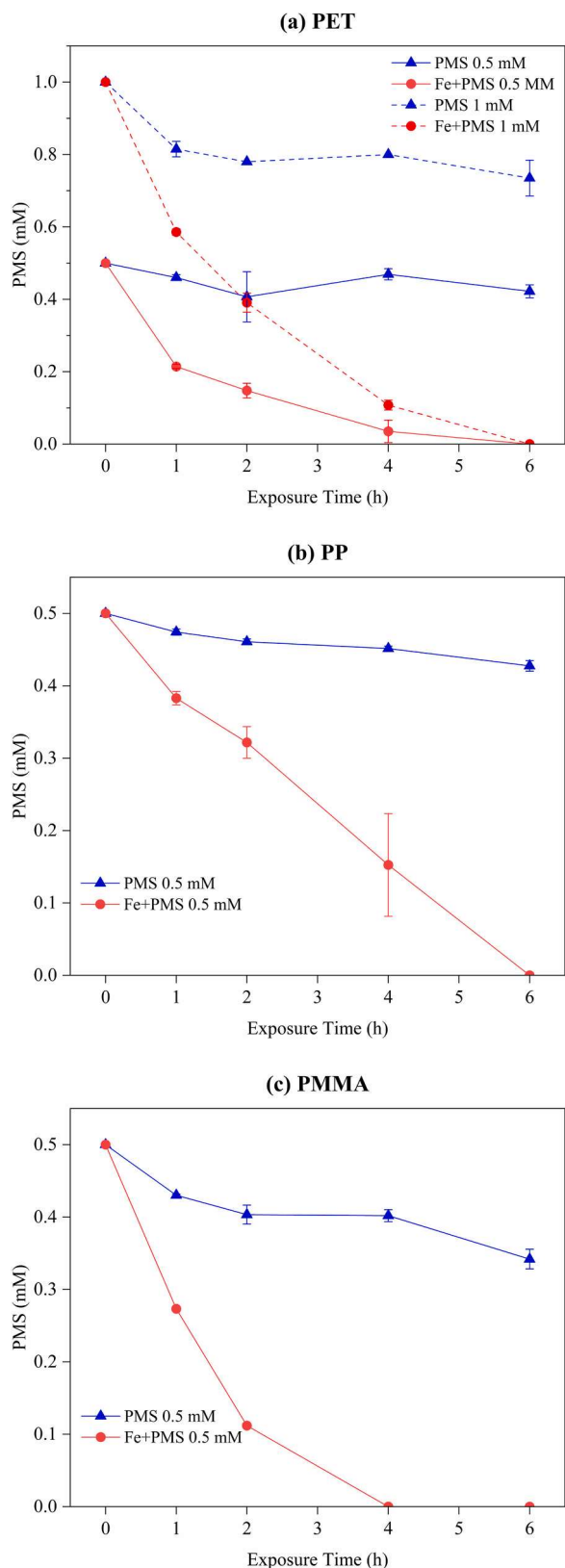


Fig. 6. Peroxymonosulfate (PMS) consumption determined in well water under simulated solar radiation (40 W/m^2 , 280–400 nm) at 40°C in the presence and absence of Fe^{2+} ($18 \mu\text{M}$) using different plastic covers: (a) polyethylene terephthalate (PET); (b) polypropylene (PP), and (c) polymethylmethacrylate (PMMA).

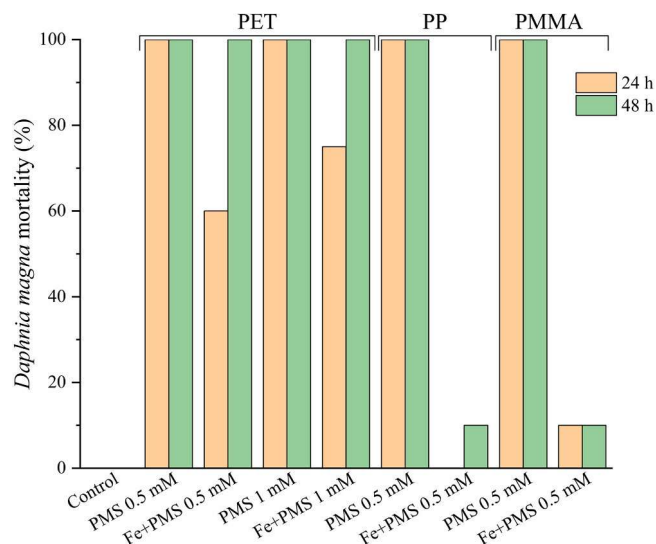


Fig. 7. Mortality of *Daphnia magna* neonates exposed to well water treated with Peroxymonosulfate (PMS; 0.5 and 1 mM) under simulated solar radiation (40 W/m^2 , 280–400 nm) at 40°C , in the presence and absence of Fe^{2+} ($18 \mu\text{M}$), using different plastic covers: polyethylene terephthalate (PET), polypropylene (PP) and polymethylmethacrylate (PMMA).

at 48 h mortality reached 100% for both concentrations. Under PP at 0.5 mM, no mortality was observed at 24 h (0%), whereas a mortality of 10% was recorded at 48 h. Similarly, samples under PMMA at 0.5 mM PMS showed a mortality of 10% at both 24 and 48 h.

4. Discussion

This is the first study evaluating the efficacy of PMS to inactivate *C. parvum* oocysts during SODIS procedures. Since no previous studies have investigated PMS application against *Cryptosporidium*, PMS concentrations (up to 5 mM) were selected on the basis of values reported in the scientific literature for other waterborne pathogens [17,23,26]. In this context, a PMS concentration of 0.5 mM led to 5.0 LR in the oocyst viability when water samples were exposed uncovered for 6 h to simulated solar radiation ($40 \text{ W/m}^2 - 40^\circ\text{C}$), while concentrations of 2.5 and 5 mM decreased oocyst survival 5 log just in the first hour of exposure (Fig. 2b). Previous studies showed that lower concentrations of PMS are effective to inactivate bacteria. Thus, water lake samples treated with 0.1 mM of PMS under a UV-A LED (23 W/m^2) achieved inactivation rates > 6 LR in *Escherichia coli* and *Bacillus mycoides* in 90 min and *Staphylococcus aureus* in 120 min [23]. In another work, a PMS concentration of 0.036 mM led to 5 LR in *E. coli* in distilled water stored in polycarbonate bottles that were exposed to natural sunlight during 60 min at maximum temperature of 23°C [26]. Additionally, it was demonstrated that, under natural sunlight, 0.005 mM of PMS inactivated *E. coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* in isotonic water to the detection limit (> 5 LR) in 20, 25 and 30 min, respectively, at temperatures ranging from 20 to 42°C [64]. However, a PMS concentration of 5 mM was required to achieve 5.61 LR of the fungus *Candida albicans* in 120 min under UVA-LED in lake water [23].

The disinfectant effect of PMS is significantly increased when the generation of sulfate and hydroxyl radicals is activated by heat, UV radiation or transition metals [65–68]. The present study was carried out under a controlled temperature of 40°C , as in SODIS under natural conditions, infra-red light increases water temperature, which already can cause lethal damage in microorganisms by denaturing proteins [42, 45,61,69]. The selected temperature in the present work (40°C) represents an average temperature achieved under natural sunlight to approximate the study to more realistic conditions and was applied in several previous studies [42–46,61]. This temperature value contributes

to the activation of PMS as previously reported by Rodríguez-Chueca et al. [17], who demonstrated that thermal activation of PMS at 40 °C led to complete inactivation of *E. coli* (6 LR) in less than 30 min with concentrations of 18–90 µM in MilliQ water. Under the same conditions (dark/40 °C), the results obtained in the present work support this finding since 2.5 and 5 mM of PMS reached total *C. parvum* oocyst inactivation after 6 h, whereas at 4 °C, 5 mM achieved only 0.39 ± 0.55 LR (Fig. 2a).

As previously mentioned, the activation of PMS can be also initiated by the addition of iron, leading to the production of sulfate radicals through the oxidation of Fe^{2+} to Fe^{3+} (Eq. (2)) [17]. According to the results obtained in the present study under dark conditions at 40 °C, when 18 µM of Fe^{2+} was added, *C. parvum* inactivation kinetics was slightly improved only with 2.5 and 5 mM of PMS in the first 2 h of the experiment (Fig. 3a). Similarly, the exposure to simulated solar radiation in the presence of Fe^{2+} did not lead to any enhancement in comparison with the inactivation results obtained with only PMS/sunlight (Figs. 2b and 3b). In the same way, Rodríguez-Chueca et al. [17] found that the activation of PMS only with Fe^{2+} resulted in similar bacterial inactivation than with the combination of sunlight/ Fe^{2+} /40 °C. In other studies, the addition of Fe^{2+} led to better disinfection results in some cases, but this improvement was not seen in every experiment [23,26]. This variability in the results may be attributed to the combination of sunlight and temperature, which can deprive Fe^{2+} available to activate PMS by promoting photo-oxidation and faster hydrolysis of Fe^{2+} to Fe^{3+} and, although Fe^{3+} may be regenerated to Fe^{2+} , this reaction occurs at a lower kinetic rate [17]. Moreover, Fe^{3+} can precipitate, causing a delay in the process by attenuation of the light transmission [17,26].

One of the major limitations of SODIS is the material of the water containers as certain plastics may block or reduce solar radiation [15]. Taking into consideration the previous results, a concentration of 0.5 mM was used to evaluate PMS efficacy in the presence of different plastic materials as it is the minimum PMS concentration capable of decreasing oocyst survival more than 4 log (Figs. 2b and 3b). Under a PET cover, oocyst viability was reduced 3.74 ± 0.30 log after 6 h of exposure to simulated solar radiation, whereas with PP and PMMA cover, oocyst inactivation was faster, reaching 4.94 ± 0.04 and 5.05 ± 0.00 LR in 4 h, respectively (Fig. 4). It is known that UV-B has a direct mutagenic effect in microorganisms, being more lethal than UV-A [6]. For *C. parvum* oocysts, solar inactivation is caused mainly by UV-B, showing a strong spectral dependence [46–48,70]. As it is shown in Fig. 1, PET is opaque to UV-B, justifying lower oocyst inactivation rates obtained compared to PP and PMMA. To compensate for the lack of UV-B radiation and enhance disinfection results under a PET cover, the concentration of PMS was increased to 1 mM, resulting in > 4 LR after 4 h of exposure. Furthermore, as reflected in our results, the presence of sunlight is key to both PMS activation and microorganism inactivation. Ozores Diez et al. [26] postulated that UV-A and visible light can effectively activate PMS. However, according to inactivation results obtained in the present study using a PET cover, when low concentrations of PMS are used, UV-A radiation alone is insufficient. Therefore, higher PMS concentrations or the presence of UV-B radiation is necessary to reach good values of *C. parvum* inactivation.

On the other hand, the transmission spectrum depends on the nature and thickness of the plastic material, which should balance adequate resistance with good optical properties [15]. Comparing results obtained after 6 h of exposure with PP and PMMA covers, which both allow UV-B transmission, the difference in the *C. parvum* inactivation values between them was 1.35 and 0.08 LR in the absence and presence of PMS, respectively. Although there is a significant difference in transmittance between PP and PMMA plastics (Fig. 1), in the presence of PMS (0.5 mM), similar oocyst inactivation was achieved under both plastics after 4 h (4.94 ± 0.04 vs. 5.05 ± 0.00 , respectively). This suggests that the use of more expensive materials with good optical properties like PMMA is unnecessary when PMS is added in SODIS. However, PP undergoes photodegradation and prolonged exposure can compromise its

physical properties. This leads to reduced UV transmission and increased brittleness, requiring the availability of new PP containers for replacement in case of breakage [50].

The chemical composition of water can influence also the PMS efficacy. Berruti et al. [64] studied the potential effect of chloride and carbonate ions with 0.01 mM of PMS under natural solar conditions for bacterial inactivation using different water matrices: distilled water, isotonic water (0.15 M of chloride ions), well water (1000 mg/L of carbonates) and diluted well water (100 mg/L of carbonates). They observed that water matrix already has an influence on bacterial inactivation in the absence of PMS and the addition of this oxidant enhanced disinfection in all water matrices. However, as water complexity increased, higher treatment times were needed, being the efficiency performing order: isotonic water > distilled water > diluted well water \approx well water [64]. In the present work, well water was used to assess the disinfection efficacy of PMS against *C. parvum* under PET, PP and PMMA covers (Fig. 5). A general decrease in the oocyst inactivation was observed compared to distilled water when PMS was combined with Fe^{2+} . However, inactivation rates > 4 LR were obtained by the end of the experiment using PMS in the absence of Fe^{2+} . Specifically, 0.5 mM PMS resulted in 4.45 ± 0.20 and 4.98 ± 0.08 LR under PP and PMMA covers, respectively, whereas a higher PMS concentration of 1 mM was required under PET to achieve a comparable inactivation level of 4.79 ± 0.21 LR. These results suggest that, despite the use of a more complex and realistic water matrix such as natural well water, the addition of PMS remains effective. However, future studies could include turbid waters to further explore its efficacy.

Although the application of PMS to SODIS procedures has been studied for bacteria, toxicity studies that ensure the complete safety of PMS-treated waters are scarce. Ozores Diez et al. [26] evaluated the toxicological safety of the plastic material (PET and polycarbonate) after the exposure to solar light in the presence of Fe^{2+} and peroxydisulfate (PDS). Even though estrogenic compounds were found, the process did not lead to the generation of dangerous amounts of leachables and no mutagenicity risk was detected in the average daily drinking water intake [26]. However, a much lower concentration of PDS (90 µM) was used, which is not comparable to the concentration of 0.5 and 1 mM of PMS that was employed in the present study. In this context, toxicity assays performed with *D. magna* in present work provide relevant information on the safety of water treated with PMS. Overall, toxicity was closely related to PMS consumption. In the absence of iron, PMS consumption remained limited in all conditions (Fig. 6), which was consistently associated with complete *D. magna* mortality at both 24 and 48 h (Fig. 7). In contrast, in the presence of iron, the kinetics of PMS consumption was influenced by the plastic material used as cover. Plastics with higher transmittance promoted faster PMS depletion. In particular, PMS was completely consumed under PMMA from the fourth hour of exposure, while under PP and PET longer exposure times were required (Fig. 6). PMS complete depletion resulted in lower toxicity, particularly at 24 h (Fig. 7). These results indicate that toxicity is strongly associated with the persistence of PMS in the treated water. In both PMMA and PP, mortality did not exceed 10%, remaining within safety levels. Notably, PET, which has the lowest transmittance among the plastic tested, showed higher toxicity even after PMS was fully consumed (Fig. 7). Although this study provides a preliminary toxicological assessment of PMS-treated water in SODIS, further studies are needed to fully confirm its safety for practical applications.

Moreover, it is important to consider laxative effects and noticeable taste changes that sulfates produce, as PMS can be a source of sulfate ions [17,64]. For that reason, PMS concentrations should be low enough to avoid exceeding the sulfate limit of 250 mg/L established by the WHO [71]. In this work, sulfate concentrations determined in well water containing 0.5 and 1 mM of PMS, in the presence and absence of Fe^{2+} , after 6 h exposed to simulated solar radiation were 101–117 and 230–238 mg/L, respectively, remaining under the limit of 250 mg/L (Table S3).

Regarding the possible microbial inactivation mechanisms, the global effect of PMS shows an integral and multilevel effect that explains its high efficacy. Tian et al. [72] described that during initial exposure of *E. coli* to this oxidant, external attacks on bacterial cell walls cause lipid peroxidation, which increases membrane permeability and leads to bacterial death. It has been proven that differences in the bacterial cell wall structure impact the efficacy of PMS, being gram-positive bacteria more resistant to oxidative stress than gram-negative bacteria due to a thicker peptidoglycan layer [73]. Furthermore, Tian et al. [72] proposed that there may be intracellular damage such as protein oxidation, although the primary targets of oxidation are lipids, along with potential DNA damage. This DNA damage may be mitigated by repair mechanisms that happen simultaneously or even faster than the damage itself. The limited research on *Cryptosporidium* makes challenging the identification of the mechanisms through which PMS attacks the oocyst. This parasitic form consists in a thick wall that encompasses four cells called sporozoites. Some studies have determined that the oocyst wall is a trilamellar structure, in which the inner layer is composed by disulfide-bridged wall proteins that provide mechanical rigidity, the central layer contains lipid-protein complex, while the outer one has been proven that the main component is a glucose-rich glycocalyx [74, 75]. Based on this knowledge and bacterial inactivation mechanisms described by Tian et al. [72], it could be expected that oxidation attacks may occur initially in the oocyst wall affecting the permeability, although the presence of glycoproteins may offer some resistance. This increase in the permeability can affect the oocyst survival, without excluding PMS diffusion through the oocyst wall. Once inside the oocyst, this reagent can target the sporozoite membrane or even diffuse through it, producing sulfate radicals inside and attacking lipids, proteins, RNA or even being capable of diffusing through the nuclear membrane to damage genomic DNA.

Finally, the application of PMS in SODIS method may represent a potentially useful approach in LMICs. Traditional SODIS typically employs 1.5 L PET bottles, which, although having poorer optical properties than alternatives like PP or PMMA, remain the most accessible and affordable option in these regions. Based on the results obtained in this preliminary study, a PMS concentration of 1 mM would likely be the most appropriate to achieve high oocyst inactivation rates using PET, with an estimated cost of 0.014€ per 1.5 L treated bottle. By contrast, plastics with better light transmittance, such as PP or PMMA, could allow the use of lower PMS concentrations (0.5 mM), which not only reduce costs but also enhance safety.

5. Conclusions

Access to safe water remains a significant challenge in many parts of the world. SODIS method is a HWT employed in LMICs, but it is insufficient to inactivate the most resistant waterborne pathogens. Therefore, improving its effectiveness is essential. The present work evaluates for the first time the enhancement of SODIS procedures using PMS against *C. parvum* oocysts, one of the most resistant waterborne pathogens. The results obtained in this first approach demonstrated that PMS, in presence of simulated solar radiation and temperature (40 W/m² – 40 °C), can effectively inactivate *C. parvum* oocysts and reduces exposure time compared to standard SODIS, meeting the WHO guidelines to provide high level of protection against waterborne protozoan diseases (4 LR). Toxicological assays using *D. magna* showed low mortality values when PMS was fully consumed in the presence of iron using PP and PMMA plastic covers.

Although further studies on PMS efficacy against *Cryptosporidium* under natural conditions in different water matrices, including turbid water, as well as additional toxicological assays should be conducted, the present study demonstrates that the use of PMS enhances the SODIS method, providing better protection against waterborne diarrhoeal diseases caused by protozoa. Furthermore, given the high resistance of *C. parvum* oocysts, water treatments capable of inactivating this

protozoan may also effectively eliminate other less resistant microorganisms.

CRedit authorship contribution statement

Sandra Martín-García: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Seila Couso-Pérez:** Writing – review & editing. **Hipólito Gómez-Couso:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jece.2026.122008](https://doi.org/10.1016/j.jece.2026.122008).

Data availability

Data will be made available on request.

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