Elsevier Editorial System(tm) for Water Research
Manuscript Draft

Manuscript Number:

Title: Solar disinfection of wastewater to reduce contamination of lettuce crops by E. coli in reclaimed water irrigation

Article Type: Research Paper

Keywords: E. coli; hydrogen peroxide; irrigation; lettuce; solar disinfection; wastewater reuse

Corresponding Author: Ms. Françoise Bichai, Ph.D.

Corresponding Author's Institution: Harvard University

First Author: Françoise Bichai, Ph.D.

Order of Authors: Françoise Bichai, Ph.D.; M. Inmaculada Polo Lopez; Pilar Fernández Ibañez

Abstract: Low-cost disinfection methods to allow safe use of recycled wastewater for irrigation can have important beneficial implications in the developing world. This study aims to assess the efficiency of solar disinfection to reduce microbial contamination of lettuce crops when solar-treated wastewater effluents are used for irrigation. The irrigation study was designed as a complete experimental loop, including (i) the production of irrigation water through solar disinfection of real municipal wastewater treatment plant effluents (WWTPE), (ii) the watering of cultivated lettuce crops at the end of solar treatment, and (iii) the detection of microbial contamination on the irrigated crops 24h after irrigation. Solar disinfection was performed using two types of reactors: (i) 20-L batch borosilicate glass reactors equipped with CPC to optimize solar irradiation, and (ii) 1.5-L PET bottles, i.e. the traditional SODIS recipients commonly used for disinfection of drinking water in developing communities. Both solar and H2O2-aided solar disinfection processes were tested during <5h exposure of WWTPE, and E. coli inactivation was analysed. A presence/absence detection method was developed to analyse lettuce leaves sampled 24 hours after watering for the detection of E. coli. Results of inactivation assays show that solar disinfection processes can bring down bacterial concentrations of >103-104 E. coli CFU mL-1 in real WWTPE to <2 CFU mL-1 (detection limit). The absence of E. coli on most lettuce samples after irrigation with solar-disinfected effluents (26 negative samples/28) confirmed an improved safety of irrigation practices due to solar treatment, while crops irrigated with raw WWTPE showed contamination.

Suggested Reviewers: Erick Bandala
Univ. Americas de Puebla, Mexico
erick.bandala@udlap.mx
EXPERTISE: SOLAR ENERGY FOR WATER DECONTAMINATION

Kevin McGuigan
Royal College of Surgeons in Ireland (RCSI), Ireland
kmcguigan@rcsi.ie
EXPERT ON SODIS AND ITS IMPLICATIONS IN DEVELOPING COUNTRIES

Myrai Beckbölet

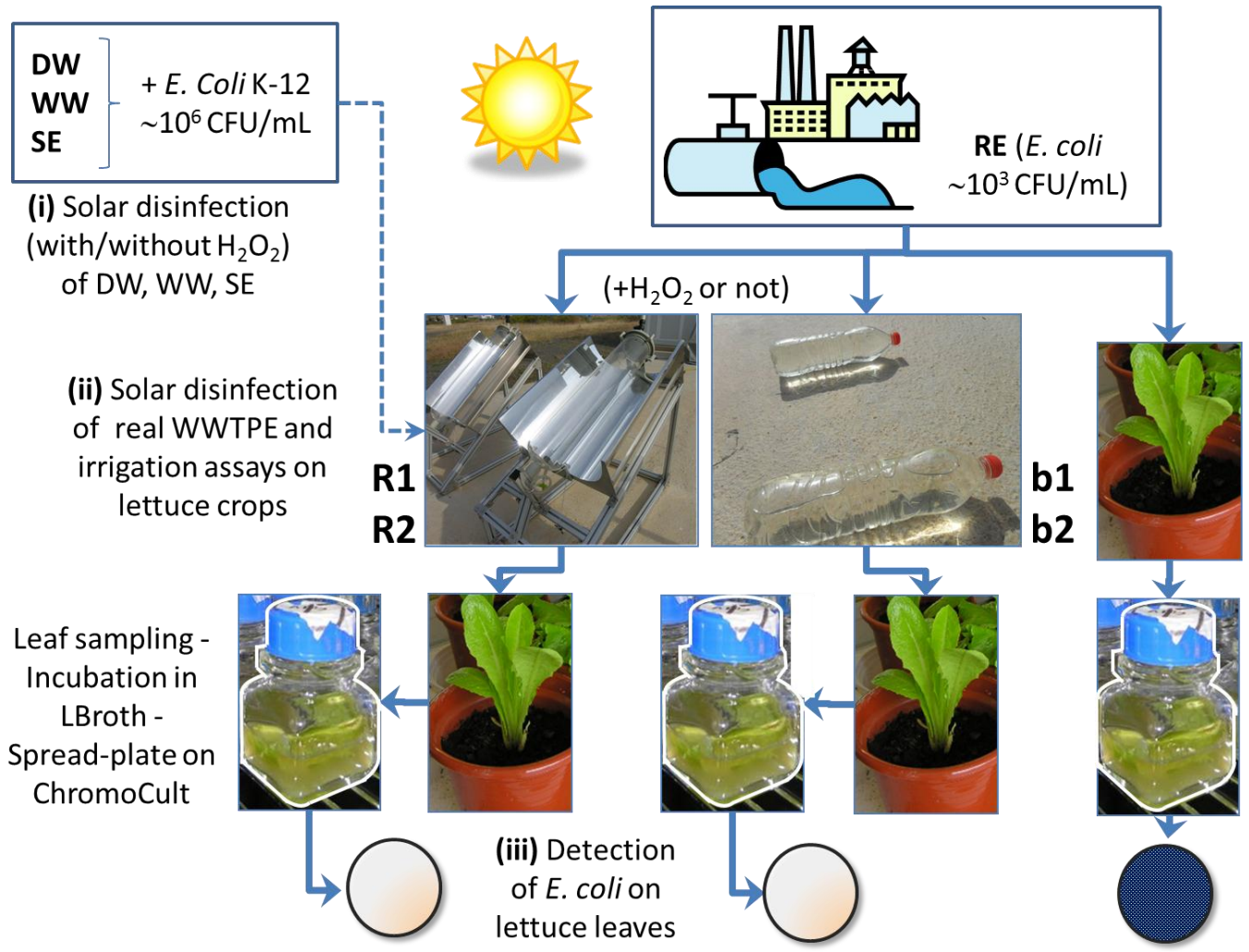
Bogazici University, Institute of Environmental Sciences
bekbolet@boun.edu.tr
EXPERTISE: WASTEWATER TREATMENT TECHNOLOGIES AND WATER REUSE

Eunice Ubomba-Jaswa
Council for Scientific and Industrial Research (CSIR), South Africa
eubombajaswa@rcsi.ie
SOLAR DISINFECTION AND HEALTH IMPACT ASSESSMENT

Highlights

- Real wastewater effluents were solar-disinfected with and without H_2O_2.
- Inactivation kinetics in real effluents was compared with standard water matrices.
- Lettuce crops were irrigated with solar-treated or non-treated effluents.
- Lettuce leaves were analysed 24h after irrigation for detection of *E. coli*.
- Solar treatments effectively reduced *E. coli* contamination on irrigated crops.

Graphical Abstract



Solar disinfection of wastewater to reduce contamination of lettuce crops by *E. coli* in reclaimed water irrigation

***Françoise Bichai (corresponding author)**
Plataforma Solar de Almería-CIEMAT, Carretera Senés km 4, 04200 Tabernas (Almería), Spain
Phone: 1-617-496-5961
Fax: 1-617-496-1457
Cell: 1-857-277-8116
E-mail: **fbichai@seas.harvard.edu**

M. Inmaculada Polo Lopez
Plataforma Solar de Almería-CIEMAT, Carretera Senés km 4, 04200 Tabernas (Almería), Spain
E-mail: **mpolo@psa.es**

Pilar Fernández Ibañez
Plataforma Solar de Almería-CIEMAT, Carretera Senés km 4, 04200 Tabernas (Almería), Spain
E-mail: **pilar.fernandez@psa.es**

Abstract

Low-cost disinfection methods to allow safe use of recycled wastewater for irrigation can have important beneficial implications in the developing world. This study aims to assess the efficiency of solar disinfection to reduce microbial contamination of lettuce crops when solar-treated wastewater effluents are used for irrigation. The irrigation study was designed as a complete experimental loop, including (i) the production of irrigation water through solar disinfection of real municipal wastewater treatment plant effluents (WWTPE), (ii) the watering of cultivated lettuce crops at the end of solar treatment, and (iii) the detection of microbial contamination on the irrigated crops 24h after irrigation. Solar disinfection was performed using two types of reactors: (i) 20-L batch borosilicate glass reactors equipped with CPC to optimize solar irradiation, and (ii) 1.5-L PET bottles, i.e. the traditional SODIS recipients commonly used for disinfection of drinking water in developing communities. Both solar and H_2O_2-aided solar disinfection processes were tested during ≤5h exposure of WWTPE, and *E. coli* inactivation was analysed. A presence/absence detection method was developed to analyse lettuce leaves sampled 24 hours after watering for the detection of *E. coli*. Results of inactivation assays show that solar disinfection processes can bring down bacterial concentrations of $>10^3$-10^4 *E. coli* CFU mL^{-1} in real WWTPE to <2 CFU/mL (detection limit). The absence of *E. coli* on most lettuce samples after irrigation with solar-disinfected effluents (26 negative samples/28) confirmed an improved safety of irrigation practices due to solar treatment, while crops irrigated with raw WWTPE showed contamination.

Keywords: *E. coli*; hydrogen peroxide; irrigation; lettuce; solar disinfection; wastewater reuse

1. Introduction

The world's population living in water-stressed areas is projected to reach 44% by 2050 (Scheierling et al. 2011). As the world faces increasing freshwater scarcity, wastewater use is gaining attention as an option for augmenting available water supplies. Agriculture irrigation is by far the most established application of wastewater reuse in the world (Scheierling et al. 2011). Yet, agriculture still accounts for over 70 percent of the world's total freshwater withdrawal (FAO 2012). Recycled domestic wastewater has beneficial properties for agriculture use, such as nutrients which have a natural fertilizer value for crops, leading to higher crop yields while reducing demand for chemical fertilizers (Drechsel et al. 2010). It also provides a climate-independent source of water allowing year-round crop production. Wastewater recycling for agriculture irrigation is particularly cost-effective in low-income arid and semi-arid countries (Drechsel et al. 2010). Moreover, land application of wastewater can be viewed as a low-cost wastewater treatment reducing pollution to water bodies.

Unplanned use of wastewater in agriculture, involving direct or indirect use of untreated wastewater, is by an order of magnitude more commonly found than planned use globally (Scheierling et al. 2011).The use of inadequately treated domestic wastewater effluents for irrigation raises public health concerns arising from the presence of pathogens in the wastewater. Such risk is especially critical in the case of vegetables eaten raw, such as leafy greens (Beuchat 2002). Secondary treatment is standard practice as an environmental management strategy for a majority of domestic wastewater systems worldwide. The typical quality of such wastewater effluents is estimated to 10^5 total coliforms/100 mL (Moulin et al. 2010, Levantesi et al. 2010). The WHO guidelines for the use of wastewater forunrestricted irrigation (including irrigation of salad crops and vegetables eaten uncooked) require a water quality of <1000 faecal coliforms per 100 mL (WHO 2006).

Many arid and semi-arid countries facing water scarcity are well positioned to receive sufficient UV radiation from natural sunlight yearly, enhancing the potential for solar disinfection applications (UNDP 2006). While solar and solar photo-catalytic disinfection processes have been proven to efficiently inactivate various pathogens in drinking water (Boyle et al. 2008, Sichel et al. 2009; Gomez-Couso et al., 2009) or in simulated water effluent (Polo-López et al. 2010), the use of solar radiation had never been assessed for enhancing the microbial quality of real wastewater effluents for crop irrigation. The addition of low amounts of hydrogen peroxide in water was previously demonstrated to increase the inactivation rate of microorganisms exposed to sunlight at a low cost (Polo-López et al. 2010). Hydrogen peroxide decomposes into water and oxygen in the disinfection process. Therefore the reagent is consumed without producing toxic by-products nor requiring pH correction, as it is the case in other advanced photo-oxidation processes (using titanium dioxide or photo-Fenton catalysis) which require a post-treatment (Polo-López et al. 2010). While wastewater effluents are spontaneously used in developing countries when other sources for irrigation are scarce (Drechsel et al. 2010), low-cost solar disinfection processes could help reducing health risk for consumers of wastewater-irrigated crops.

The objective of this study is to assess the performance of solar disinfection processes, with or without the addition of a low hydrogen peroxide dose (5 and 10 mg L^{-1}), as a low cost wastewater treatment to enhance microbial safety of secondary-treated wastewater effluents used for irrigation. This study aims at (i) measuring *E. coli* inactivation in wastewater effluents freshly collected from a municipal wastewater treatment plant with a standard secondary treatment, during exposure to natural sunlight with or without hydrogen peroxide addition, and (ii) comparing *E. coli* contamination (presence/absence) on lettuce crops irrigated with the fresh wastewater effluent vs. the solar-disinfected effluent on the day after irrigation was practiced.

Indigenous *E. coli* was chosen as a target microorganism in this study because it is naturally present in municipal secondary-treated wastewater effluents in sufficient concentrations to likely allow measurements of (i) a significant reduction during disinfection assays, and of (ii) a detectable contamination on lettuce leaves watered with the untreated wastewater effluent. *E. coli* is also the most commonly used bacterial indicator of faecal contamination in water.

This study is, to our knowledge, the first to present a direct assessment of microbial contamination on crops irrigated with solar-treated vs. untreated real wastewater effluents, through an innovative complete experimental loop design.

2. Material and Methods

2.1 Experimental design

To emulate the transport of *E. coli* in a cycle of wastewater reuse for irrigation of edible crops, the following experimental study was designed (**Figure 1)**: (i) The efficiency of solar disinfection processes was evaluated in standard water matrixes (distilled water, natural well water, and simulated wastewater effluent) inoculated with *E. coli* K-12, and in real municipal wastewater treatment plant effluents (WWTPE) contaminated with naturally occurring *E. coli*. Two types of solar static batch reactors, i.e. 20-L CPC reactors and 1.5-L PET bottles, were used. The disinfection effects of natural sunlight with and without the addition of a low dose of H_2O_2 were evaluated. (ii) Cultivated lettuce crops were watered with solar-treated real WWTPE and (iii) *E. coli* was detected on lettuce leaves 24h after irrigation. For this purpose, a presence/absence detection method of *E. coli* on lettuce leaves was developed.

2.2 E. coli detection and enumeration

136 *E. coli* K-12 (ATCC 23631) was used in solar disinfection processes for characterizing
137 inactivation kinetics of the solar reactors. This strain was also used to contaminate the
138 synthetic irrigation water as a positive control in lettuce irrigation tests. Cultures of
139 *E. coli* K-12 were generated from frozen stocks by streaking onto Luria Bertani (LB)
140 (Sigma-Aldrich, USA) agar and were incubated at 37 **º**C for 18-24 h. A single colony
141 from the plate was inoculated into 14 mL sterile LB-broth (Sigma-Aldrich, USA) and
142 incubated at 37 **º**C for 18 h on a rotary shaker to obtain a stationary phase culture.
143 Cells were harvested by centrifugation at 800 x *g* for 10 min and the pellet was re-
144 suspended in 14 mL Phosphate Buffer Solution (PBS, Oxoid), yielding a final
145 concentration of ~10^9 CFU mL^{-1}. Appropriate volumes were diluted to reach a starting
146 concentration of ~10^6 CFU mL^{-1} in the reactors.
147
148 The samples collected during solar disinfection experiment were enumerated using the
149 standard plated counting method through serial 10-fold dilutions in PBS, and volumes
150 of 20 µL were plated in triplicate on Endo Agar (Sigma-Aldrich, USA) plates. Colonies
151 were counted after incubation of 24 h at 37 ºC. When colony counts were very low,
152 500µL-samples were spread over a plate to decrease the detection limit down to 2 CFU
153 mL^{-1} (DL). Data obtained in the studies were analysed using the one-way ANOVA
154 analysis tool (Origin v7.0300, OriginLab Corp., Northampton, USA).
155
156 Naturally occurring *E. coli* in real municipal WWTPE was evaluated by spreading
157 appropriate sample volumes (25-500 µL depending on the bacterial load) on
158 ChromoCult® Coliform Agar (Merck KGaA, Darmstadt, Germany) plates in duplicate
159 (DL of 2 CFU mL^{-1}). ChromoCult® allows the selective detection of coliforms and
160 distinguishes *E. coli* in a heterogeneous bacterial community, as its colonies appear in
161 a characteristic dark-blue to violet color (Merck, 2004).
162

Bacterial re-growth in treated WWTPE was evaluated after all solar treatments keeping the final samples at room temperature for 48h in the dark. Then they were plated on ChromoCult® Agar as described above for *E. coli* enumeration.

2.3 Water types

2.3.1 Standard water matrixes

For the inactivation kinetics characterization assays using laboratory-cultivated *E. coli* K-12 bacteria, (i) distilled water (DW), (ii) natural well water (WW) and (iii) simulated municipal WWTP effluent (SE) were used:

(i) Distilled water (DW, conductivity <10 μS cm^{-1}, organic carbon <0.5 mg L^{-1}) was used as a reference for observation and comparison of inactivation kinetics in standard laboratory study conditions, excluding the contribution or interference of any other compound.

(ii) Well water (WW) was used to represent clean natural water that could be used for irrigation in agriculture. The water was collected from a well located on the PSA site at a depth of approximately 200 m. A single batch of WW (~100 L) was withdrawn to ensure that the same stock of water was used for all the experiments. To preserve the chemical integrity of the WW, it was not autoclaved. Naturally occurring organisms in WW were determined by standard plate counting technique using Endo Agar before adding *E. coli* K-12. They were found to be lower than the DL. WW had an average turbidity of 0.3 NTU and a pH of 7.5.

(iii) Simulated municipal wastewater effluent (SE) was used as a synthetic model of wastewater effluent with 25 mg L^{-1} of dissolved organic carbon (DOC). The same model effluent was described elsewhere (Klamerth et al. 2010, Polo-Lopez et al., 2012). The solution composition is: $NaHCO_3$ (96 mg L^{-1}), NaCl (7 mg L^{-1}), $CaSO_4 \cdot 2H_2O$ (60 mg L^{-1}), urea (6 mg L^{-1}), $MgSO_4$ (60 mg L^{-1}), KCl (4 mg L^{-1}), $CaCl_2 \cdot 2H_2O$ (4 mg L^{-1}), peptone (32 mg L^{-1}), $MgSO_4 \cdot 7\ H_2O$ (2 mg L^{-1}), meat extract (22 mg L^{-1}).

191

192 *2.3.2 Real wastewater effluents (WWTPE)*

193 For irrigation assays, freshly collected 'real effluent' water from the municipal
194 wastewater treatment plant of Almería, El Bobar (Spain), was used (WWTPE). This is
195 the effluent of a secondary treatment, i.e. a standard biological treatment (activated
196 sludge) followed by sedimentation in ponds. The real effluent was used for irrigation
197 assays either raw or following solar disinfection treatment. WWTPE was freshly
198 collected from the treatment plant on the morning of each disinfection assay.

199

200 WWTPE had DOC values ranging from 15.9-17.1 mg L^{-1}, DIC 66.4-77.8 mg L^{-1},
201 turbidity 7.11-8.93 NTU, pH 7.4–7.8, and conductivity between 1739 and 1819 µS cm^{-1}.
202 The DOC concentration was not significantly reduced at the end of each solar
203 treatment.

204

205 The concentrations of ions present in water were evaluated using ion chromatography
206 (IC) with a Dionex DX-600 (Dionex Corporation, Sunnyvale, California, USA) system
207 for anions and with a Dionex DX-120 system for cations. Dissolved organic carbon
208 (DOC) and total carbon (TC) were analyzed using a Shimadzu TOC-5050 (Shimadzu
209 Corporation, Kyoto, Japan), and turbidity was measured using a turbidity meter (Model
210 2100 N Hach, Hach Company, Laveland, Colorado, USA).

211

212 ***2.4 Hydrogen peroxide (H_2O_2) analysis***

213 Two H_2O_2 concentrations were used in all experiments: 5 and 10 mg L^{-1}. These
214 concentrations were chosen based on those used in previous disinfection assays
215 (Sichel et al. 2009; Polo-López et al. 2011; García-Fernández et al. 2012), which
216 demonstrated a significant increase in microbial inactivation rates compared with solar
217 disinfection without H_2O_2. It was intended to use low doses of H_2O_2 in order for the
218 process to remain as low-cost as possible while avoiding the presence of high

concentrations of chemical reagents in irrigation water, which could contaminate the soil and the crops and increase the costs. Concentrations of hydrogen peroxide below 50 mg L^{-1} have been shown non-toxic for crops (Sichel et al., 2009).

H_2O_2 (Riedel-de Haën, Germany) at 30 wt% was used as received and diluted into the reactor filled with water. H_2O_2 concentration was determined by a colorimetric method based on the use of Titanium(IV) oxysulfate (Riedel-de Haën, Germany), which forms a stable yellow complex with H_2O_2 detected by absorbance measurements at 410 nm. Absorbance was measured using a spectrophotometer (PG Instruments Ltd T-60-U). This method is used for drinking water analysis and has a DL of 0.1 mg L^{-1}. The signal was read with reference to a H_2O_2 standard in distilled water. Absorbance measurement was linearly correlated with H_2O_2 concentration in the range 0.1–100 mg L^{-1}.

Dark control experiments were previously conducted using the chosen H_2O_2 concentrations (5 and 10 mg L^{-1}) for 5 h observing that the viability of both types of *E. coli* (indigenous in WWTPE and *E. coli* K-12) remained unaffected. Catalase was added to water samples to eliminate residual hydrogen peroxide: 1-mL samples were mixed with 100 mL of 2300 U mg^{-1} bovine liver catalase at 0.1 g L^{-1} (Sigma–Aldrich, USA). Dark control tests with *E. coli*, H_2O_2 and catalase under the same conditions as the solar experiments showed no effect (positive or negative) of catalase on bacterial count results (García–Fernandez et al. 2012).

2.5 Solar reactors

2.5.1 20L-CPC solar reactors

Two 20-L CPC reactors (R1 and R2, **Figure 2**) were used for solar treatments. These twin reactors were used to duplicate the solar tests under simultaneous solar irradiation conditions. They are cylindrical prototypes made of borosilicate glass of 5-mm

247 thickness (hardness type 3.3., Shott-Duran, Germany), which allows a 92%
248 transmission of UVA in the natural solar spectrum. Although the total volume of the
249 reactors is 30 L (irradiated length: 92.5 mm, irradiated width: 62.5 mm, external
250 diameter: 200 mm), they were filled with 20 L for the experiments in this study. Both
251 reactors are inclined at 37º with respect to the horizontal to maximize collection of solar
252 radiation. They are equipped with Compound Parabolic Concentrators (CPC). CPCs
253 are static solar collectors with a shape and reflective surface designed to optimize the
254 collection of solar UVA radiation at all daylight times. CPCs used for this work have
255 been described elsewhere (Ubomba-Jaswa, 2010) and have a concentration factor
256 equal to 1. The total irradiated volume for each reactor is 20 L and the total irradiated
257 area is 0.58 m^2. All experiments were carried out in duplicate during 3-5 h of solar
258 exposure on clear sunny days at the Plataforma Solar de Almería (PSA, South of
259 Spain).
260
261 *2.5.2 PET 1.5L-bottles*
262 Two 1.5-L poly(ethylene) terephthalate (PET) bottles (b1, b2; **Figure 2**) were also used
263 in this study as they are the traditional SODIS recipients used for disinfection of
264 drinking water in developing communities (Byrne et al. 2011). These bottles were used
265 as solar reactors only for real WWTPE disinfection tests. Solar assays with PET bottles
266 were conducted simultaneously to CPC reactor experiments, using the same batch of
267 WWTPE collected that day. For each experiment, two PET bottles of 1.5 L were used
268 and laid on the ground aside from the 20-L CPC reactors and exposed to natural
269 sunlight. The total irradiated area of each bottle was 0.028 m^2.
270
271 ***2.6 Solar experiments***
272 For disinfection assays using standard water matrices described above (section 2.3),
273 solar reactors were filled with 20 L of water (DW, WW or SE); *E. coli* K-12 was added
274 to an initial concentration of ~10^6 CFU mL^{-1} and the suspension was homogenized

while reactors were protected from sunlight using an opaque cover. When real WWTPE was used, there was no addition of bacteria to the water. In disinfection assays using sunlight + H_2O_2, the hydrogen peroxide was added and agitated once the bacterial suspension was homogeneous and while protected from sunlight. The cover was then removed and disinfection experiments started. Experiments were performed from April 2011 to June 2011, starting at 10.30 am local time, for ≤5 h.

Water temperature was measured hourly in each reactor using a thermometer (Checktemp, Hanna instruments, Spain). pH, *E. coli* and H_2O_2 concentrations were measured for all collected water samples. For each disinfection test, a water sample was taken and kept in the dark at laboratory temperature as a control which was plated at the end of the experiment.

2.7 Measurement of solar UVA radiation

A global UV-A radiometer (295–385 nm, Model CUV3, Kipp & Zonen, Netherlands) inclined at 37° was used to measure UV irradiance (in W m^{-2}). Another equal radiometer placed horizontally was used for the monitoring and evaluation of UV-A irradiance in the horizontal plane for the experiments performed in PET bottles. To compare results under different solar radiation conditions and in reactors with different shapes (R1 and R2 *vs*. b1 and b2), the inactivation kinetic was presented as a function of the cumulative solar UV dose (J·m^{-2}):

$$Dose_{UV} = \int_{t_1}^{t_2} I_{UV} \cdot dt \qquad \text{(Eq. 1)}$$

where I_{UV} is the incident solar UV radiation on the irradiated area (W m^{-2}) at time t (s).

2.8 Irrigation assays

2.8.1 Selection of a test crop

Lettuce was chosen as a target crop for irrigation assays. It has the practical advantage of growing relatively fast. In lettuce, the edible part (leaves) are directly exposed to irrigation water when irrigation spray drift or watering cans are used, the latter being a common practice among small farmers in developing communities (Dreschel et al. 2010). Lettuce, as other leafy greens eaten raw, has been the object of frequent reports of microbial contamination and food-associated enteric illnesses and outbreaks (Beuchat 2002), including reports of contamination by *E. coli* (Solomon et al. 2002, Berger et al. 2010, Habteselassie et al. 2010, Luo et al. 2010, Niemira et al. 2010).

2.8.2 Crop preparation and maintenance

Lettuce was grown in pots kept outside of the PSA under the natural environmental conditions in Tabernas (April to June 2011, average ambient temperature ranging from ~12 ºC-29 ºC). Growing lettuce crops (>10-cm height) were obtained from a local provider, transplanted into individual pots and watered daily with 50 mL of chlorine-free mineral water (CO_3H^-: 57 mgL^{-1}, SO_4^{-2}: 7 mg L^{-1}, Cl^-: 6 mg L^{-1}, Ca^{2+}: 11 mg L^{-1}, Mg^{2+}: 9 mg L^{-1}, Na^+: 5 mg L^{-1}, SiO_2: 4.3 mg L^{-1}, K^+ <1 mg L^{-1}). The mineral water quality was monitored daily (on Endo Agar plates) to ensure no bacterial contamination.

2.8.3 Crop irrigation experiments

Two lettuce crops were evaluated for each experimental condition using real WWTPE only, either untreated or solar treated. All the crops used for irrigation tests were ~10-15 cm in height and were irrigated with ~50 mL of each test-water using a Pasteur pipet to carefully cover the entire surface of every lettuce leaf. For each irrigation test the following protocol was used: (i) At the end of each solar experiment using either of the two solar processes evaluated in this study, the treated water collected from reactors R1, R2, b1 and b2 was immediately used for watering the lettuce crops. (ii) One lettuce

crop was watered with untreated real WWTPE, which had been kept in the dark at 4^{o}C to preserve its characteristics. (iii) One lettuce crop was irrigated with 50 mL mineral (potable) water to provide a negative control. (iv) One additional crop was irrigated with 50 mL of mineral water spiked with *E. coli* K-12 (10^6 CFU mL^{-1}) as a positive control.

2.8.4 Detection of microbial contamination on lettuce

A presence/absence method was developed to detect *E. coli* on lettuce leaves. Several pieces (0.1 g) of lettuce leaves were collected randomly from each test-crop using sterilized scissors. Combined samples were made of 3 pieces of leaves from one same crop for analysis. Each 0.3-g sample was incubated in LB-broth (15mL) at 37 ºC for 24h to guarantee the growth of even one single *E. coli* colony in the sample through the broth enrichment, and to avoid false negatives. Then suspensions were 10-fold diluted in sterile PBS and plated (50 µL) on ChromoCult agar plates. After incubation, plates showing dark blue to violet-stained *E. coli* colonies were counted as positive samples and others were counted as negative ones (Finney et al. 2003, Prats et al. 2007).

3. Results and Discussion

3.1 Inactivation assays

The potential of solar disinfection with and without added H_2O_2 to reduce bacterial contamination in real WWTPE was assessed. Additionally, the performance of solar treatments in real effluents was compared with performances in standard water matrices.

Figures 3 and **4** show, respectively, results of solar and solar/H_2O_2 inactivation of *E. coli* in DW, WW, SE, and real WWTPE in 20-L CPC reactors against solar UV dose. A good reproducibility of results for all water types was observed. No significant reduction

was observed in control samples (data not shown). **Table 1** shows inactivation rates (*k*) based on a linear regression of the logarithm of *E. coli* concentrations vs. UV dose (kJ m^{-2}) for all disinfection assays.

Time and average accumulated UV doses to reach ~6-log reduction without H_2O_2 addition varied from <1.5h and 140(± 40) kJ m^{-2} in DW (**Figure 3**) to <2h (254 (±13) kJ m^{-2}) in WW, and <3h (442 (±12) kJ m^{-2}) in SE. In comparision, with the addition of either 5 or 10 mg L^{-1} of H_2O_2 (**Figure 4**), ~6-log reduction was achieved in ≤0.5h (60 (±20) kJ m^{-2}), <1h (130 (±10) kJ m^{-2}) and <1.5h (190 (±10) kJ m-2) in DW, WW and SE, respectively. Initial *E. coli* concentrations in WWTPE collected for solar disinfection assays were of $2.4x10^3$ CFU mL^{-1} and $1.3x10^4$ CFU mL^{-1}. UV doses and treatment times required to reach the DL were higher than for standard waters, i.e. 512 (±9) kJ m^{-2} and 605 (±5) kJ m^{-2} (<4 and 5h), respectively. For the H_2O_2/solar treatment of real WWTPE (initial *E. coli* concentration ~$3.5x10^3$ CFU mL^{-1}),average UV doses of 590 (±5) kJ m^{-2} and 470 (±10) kJ m^{-2} were required to lower *E. coli* concentrations below the DL for 5 and 10 mg L^{-1} H_2O_2 doses, respectively (<4 and 3h, respectively).

Inactivation rates in different water matrices were ordered as expected for both solar treatments, i.e. DW > WW > SE > WWTPE (**Table 1**). The low doses of H_2O_2 allowed an increased activation rate in all water matrices . The H_2O_2 dose of 10 mg L^{-1} did not significantly enhance disinfection rates as compared with the 5 mg L^{-1} dose during disinfection of DW, WW, and SE; however in WWTPE a significant enhancement was observed at the 10 mg L^{-1} dose (k = 0.0105 ±0.0005 vs. k = -0.0068 ±0.0004 at 5 mg L^{-1}).

In solar disinfection of real WWTPE in 1.5 L-PET bottles (**Figure 5**), the addition of H_2O_2 was also observed to enhance *E. coli* inactivation rates. Maximal efficiency was observed at the 10 mg L^{-1} H_2O_2 dose, with ~3.6 Log reduction measured in <3h. Inactivation rates as compared with CPC reactor experiments were similar although slighly lower in PET-bottle reactors.

Concentration of hydrogen peroxide decreased through all disinfection experiments. In DW, WW, and SE, a low consumption of H_2O_2 was observed consistently to the findings of Polo-López et al. (2010) using similar water matrices. H_2O_2 consumption was the lowest in DW (DW < WW < SE). At the end of solar experiments in SE, H_2O_2 concentrations were lowered to 4.3 and 8.8 mg L^{-1}, for initial doses of 5 and 10 mg L^{-1}, respectively (H_2O_2 demand of 0.7 and 1.2 mg L^{-1}). The highest H_2O_2 consumption was observed in real WWTPE, reaching a final concentration of ~40 % of its initial dose at the end of exposure (2.0-2.4 mg L^{-1} and 3.9-4.2 mg L^{-1} for 5 and 10 mg L^{-1} initial doses, respectively).

The bacterial inactivation under UVA light lies in the accumulation of DNA mutations provoked by UVA photons, and the oxidative stress induced by reactive oxygen species (ROS) generated in the water under UVA (Boyle et al., 2008). Another source of cells injures is the internal photo-Fenton reaction assisted by the iron inherently existing in microorganisms (Spuhler et al. 2010), which reaction is enhanced, among other mechanisms, by the addition of H_2O_2. The enhancement of microbial inactivation in water by the addition of low doses of H_2O_2 under natural solar radiation has been previously reported on *E. coli* cells (Garcia-Fernandez et al. 2012), *Salmonella* sp (Sciacca et al., 2010) and fungi spores (Polo-Lopez et al., 2011). Mechanisms involved in the photo-inactivation of bacterial cells in the presence of H_2O_2 have been extensively explained by Polo-Lopez et al. (2011). In this study, the best improvement in solar treatment efficacy due to the addition of H_2O_2 was found in DW (**Table 1**), with

411 an ~120% increase in inactivation rate (*k*). The decomposition of H_2O_2 is influenced by
412 the presence of organic matter in the water. In the presence of natural
413 photosensitizers like porphyrins and analogues, natural organic matter may benefit the
414 disinfection process (DeRosa et al. 2002).The simulated wastewater (SE) used in this
415 study does not contain such compounds; hence, a positive effect from the presence of
416 organic matter on inactivation kinetics in SE was not expected. This study is the first
417 report of H_2O_2-aided solar disinfection tested on real WWTPE. The WWTPE contains,
418 among others, carbonates/bicarbonates, sulfates, nitrates, chlorides and phosphates
419 which partially scavenge H_2O_2, as well as other ROS. This may explain the different
420 H_2O_2 consumption found amongst different water types tested, and the lower efficiency
421 of H_2O_2 in real WWTPE as compared with the SE results (i.e. ~80% reduction of the
422 inactivation rate with 5 mg L^{-1} H_2O_2 in real WWTPE vs. SE in CPC reactors).
423
424 Water temperature was monitored during all experiments. Temperatures higher than
425 45^{o}C were never reached in any of the reactors during the first 3h of solar exposure,
426 when most of the inactivation was achieved. Above this temperature, a strong
427 synergistic effect of UVA photons and solar heating is reported to induce enhanced
428 bacterial inactivation rates (McGuigan et al. 1998). Nevertheless, the positive effect of
429 thermal increase, including mild heat, has been reported in most of solar disinfection
430 experimental studies within the range of 25-55°C, using PET bottles or other solar
431 reactors (Berney et al. 2006; Ubomba-Jaswa et al. 2009). In all real WWTPE in CPC-
432 equipped reactors, initial temperatures were within 27-33°C reaching maximal values of
433 39-50°C usually during the last hour of exposure. Temperature in PET-bottles was
434 consistently lower than in CPC reactors (maximal temperature of 40-43°C), which was
435 expected due to the optimized design of the CPC to concentrate solar radiation.
436
437 ***3.2 Irrigation assays***

Table 2 summarizes the results of all irrigation assays. All negative control samples (from crops irrigated with mineral water) were detected as negative, whereas all positive control samples (irrigated with mineral water spiked with 10^6 CFU mL^{-1} *E. coli* K-12) were detected as positive. This confirms adequate handling of the samples throughout the steps of the detection protocol developed in this study. Positive control samples were identified as plates covered with a blue-stained colony coating, whereas negative control sample plates could be observed as clean empty agar plates or showing a few colourless or rosy-tinted colonies, which can indicate the presence of non-*E. coli*- total coliforms or other Gram-negative bacteria (Finney et al. 2003).

Through all irrigation assays, out of a total of 16 combined samples (~0.3 g each) from crops irrigated with raw wastewater effluent (not exposed to solar disinfection processes), 14 were detected as positive for the presence of *E. coli*. In comparison, out of a total of 28 combined samples (~0.3g each) analysed from crops irrigated with solar- or solar+H_2O_2-disinfected real WWTPE, 26 samples were negative for the presence of *E. coli* (2 positives). These results are the first experimental observations of the potential for low-cost solar disinfection processes to enhance the microbial safety of wastewater reuse in agriculture irrigation practices.

The first positive sample from a lettuce sample irrigated after a solar treatment can be tracked back to a highly contaminated WWTPE (initial *E. coli* concentration $1.3x10^4$ CFU mL^{-1} vs. $2.4\text{-}3.8x10^3$ CFU mL^{-1} in all other WWTPE assays), and regrowth was observed after storing the disinfected water at room temperature for 48h (1.0 and 3.4 CFU mL^{-1} in water from reactors R1 and R2). This suggests that a low concentration had possibly remained in the disinfected water, leading to subsequent bacterial regrowth. The second positive sample was associated with a lettuce sample irrigated with WWTPE treated with H_2O_2/solar disinfection (5 mg L^{-1} dose) in PET bottle b2. After 4h of exposure, no *E. coli* was detected in the disinfected water (<2 CFU mL^{-1}), but

466 regrowth was observed after 48-h dark storage ($3.6x10^2$ CFU mL^{-1}). In previous
467 studies, injured bacterial cells irradiated with solar or UVA light were not reported to be
468 able to repair damage or regrow during post-disinfection storage (Bosshard et al.
469 2009), which is one main advantage of solar disinfection compared with
470 monochromatic UVC disinfection. However, previous assays had not been performed
471 in real WWTPE matrices. Our preliminary observations suggest that solar inactivation
472 of *E. coli* was possibly incomplete in the 2 samples identified above, and that the real
473 WWTPE matrix possibly allowed favorable conditions for bacterial survival/replication
474 during dark storage. To ensure safe practice of solar disinfection for wastewater reuse,
475 exposure time should be maximized and, according to our preliminary observations in
476 real WWTPE, storage time of the disinfected water before irrigation should be limited to
477 prevent possible bacterial regrowth in case inactivation was incomplete.
478
479 The findings presented in this study are innovative, since they include the first
480 demonstration of solar disinfection processes efficiency (with and without H_2O_2) using
481 real WWTPE. They are also the first attempt to include direct detection of bacterial
482 contamination on locally-cultivated lettuce crops irrigated with the solar-treated vs. non-
483 treated real wastewater. This study also presents the first original idea to use the
484 traditional PET-bottle SODIS reactor, widely disseminated for drinking water
485 disinfection at household level by the NGO-sector and recommended by the WHO, to
486 improve microbial safety of common wastewater irrigation practices in developing
487 countries. Developments on the use of a simple, low-cost solar disinfection process
488 suggested in this study can have significant positive implications for rural health and
489 rural livelihoods in the developing world. Results presented in this study should yet be
490 viewed as preliminary and deserving further investigation. Ambient climatic conditions
491 and condition of the lettuce crop could influence the attachment of bacteria on the
492 leaves surface following irrigation, and impact die-off on lettuce leaves.
493

494 ***3.3 Interpretation and Preliminary Recommendations for Application in***
495 ***Developing Countries***
496 The WHO guidelines recommend ≤1000 CFU faecal coliforms per 100 mL (i.e.10 FC
497 mL^{-1}) in water used for unrestricted irrigation (WHO 2006). Our results suggest that
498 solar disinfection processes could bring down unacceptable bacterial concentration
499 levels (>10^3-10^4 CFU mL^{-1} of *E. coli*) in real WWTPE to concentrations <2 CFU mL^{-1}.
500 This demonstrates a removal capacity of >3 Log for *E. coli*, which can likely predict a
501 similar performance for the inactivation of other fecal coliforms. Although fecal
502 coliforms were not measured in this study, it is likely that the solar-disinfected irrigation
503 water produced from WWTPE in our experiments would satisfy the <10 FC mL^{-1} WHO
504 criteria for unrestricted irrigation, as *E. coli* typically accounts for the majority of the
505 fecal coliform group (Gannon and Busse 1989).
506
507 Previous studies estimating the health risk associated with the consumption of crops
508 irrigated with recycled wastewater presented calculations based on an estimated
509 volume of the contaminated water retained on a crop surface (Mara et al. 2007,
510 O'Toole et al. 2010, Drechsel et al. 2010). For instance, the Australian Guidelines for
511 Water Recycling (EPHC-NRMMC-AHMC 2006) provide the indicative value of 5 mL of
512 water retained on 40 g of lettuce leaves (considered one serving) after irrigation. Based
513 on these values and theoretical calculations, each lettuce sample (0.3 g) analysed for
514 *E. coli* contamination in this study would have retained 0.0375 mL of water, and roughly
515 90-490 *E. coli* cells when irrigated with the untreated WWTPE, as compared with
516 <$7.5x10^{-2}$ cells in samples from crops watered with the disinfected WWTPE (<2 CFU
517 mL^{-1}). Our presence/absence detection method would likely allow detecting a single
518 colony if present on a lettuce sample, as this colony would be amplified by several
519 orders of magnitude during the incubation in LB-broth. The present study aimed at
520 providing new, additional information about the safety of the disinfected irrigation water
521 and practice. Analysing crops a day after irrigation, when the irrigation water retained

on the leaves would not be captured in the sample (as would be the case immediately after irrigation), was meant to allow assessing additional information on 'surviving' or established contamination on the 'dry' lettuce leaves. Although a ~0.5–2 Log reduction per day can be expected on crops surface in the field after the last irrigation depending on climate, crop type, pathogen, etc. (Drechsel et al. 2010), *E. coli* was still detected 24h after irrigation in most cases (14 positive samples/16) when crops were watered with untreated wastewater in this study. In contrast, when crops were irrigated with the solar-treated wastewater, *E. coli* could not be detected in most cases (26 negative samples/28), indicating a potentially increased health safety.

In this study, sunlight exposure was limited to ≤5 h. In field application collected effluent in PET bottles could be exposed to sunlight all day for maximal disinfection. Safe practice of the SODIS technique for drinking water disinfection as recommended by the WHO (Clasen and Haller, 2008) includes an exposure time under direct sunlight of ≥6 hours. The use of a reflective surface can increase the efficacy of SODIS for drinking water, for instance when laying SODIS bottles on the metallic roofs of houses in developing communities, which practice could be applied for wastewater disinfection as well. Further experiments should be conducted to determine optimal practices for this newly described wastewater disinfection method for agricultural use.

In this study, *E. coli* was used as an indicator of faecal contamination to estimate the risk to humans consuming the irrigated crops. Further inactivation assays should be performed to assess the efficacy of the disinfection process (with or without H_2O_2) using more resistant microorganisms with public health significance. Previous studies have shown that solar and H_2O_2/solar disinfection processes could significantly reduce *Fusarium* spp spore concentrations in simulated wastewater effluents (Polo-Lopez et al. 2010). *Fusarium* spp spores can be used as a surrogate for highly resistant

549 organisms to sunlight disinfection, and they are known to be a phytopathogen agent,
550 which can cause significant reduction in crop yields. Reducing plant pathogens through
551 the application of solar disinfection process for enhanced crop yields and reduced
552 economic losses can be an additional benefit to protecting public health in wastewater
553 irrigation.
554
555
556 **Conclusions**
557
558 The results of this study provide preliminary indications that solar and H_2O_2/solar
559 disinfection processes can be thought of as a potential means for enhancing the
560 microbial quality of wastewater effluents used for irrigation of edible crops. The
561 presented inactivation assays using solar disinfection with or without H_2O_2 addition are
562 the first indication of their potential efficacy to treat real WWTPE. Additionally, the
563 demonstration that traditional SODIS reactors (PET bottles) can potentially produce
564 satisfying irrigation water quality when exposing real wastewater effluents to natural
565 sunlight, with or without the addition of H_2O_2, opens the doors to a novel use and
566 dissemination of the SODIS process in some developing communities where local
567 farmers rely on poor quality wastewater effluents for the irrigation of their crops. In the
568 water and sanitation sector, public health protection through drinking water treatment at
569 household level tends to be compartmented from work in the agriculture sector where
570 irrigation is recognized as a central issue. Improved water supplies for irrigation
571 including safer microbial quality of reused wastewater can contribute to both water and
572 food security.
573
574
575 **Acknowledgements**
576

577 The authors thank the NSERC (National science and engineering research council of
578 Canada) for funding the first author's post-doctoral fellowship. We also acknowledge
579 the contribution of Agustín Carrion Muñoz, Irene García-Fernández, and Isabel
580 Fernández Calderero in the experimental work. We also thank Dr. Pierre Servais
581 (Université Libre de Bruxelles, Belgium) for his advice on the protocol.
582
583
584 **References**
585
586 Beuchat, L.R. 2002. Ecological factors influencing survival and growth of human
587 pathogens on raw fruits and vegetables. Microbes and Infection 4, 413-423.
588
589 Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand P., Frankel G.
590 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens.
591 Environmental Microbiology 12(9), 2385–2397
592
593 Berney, M., Weilenmann,, H.-U., Simonetti, A., Egli, T. 2006. Efficacy of solar
594 disinfection of Escherichia coli, Shigella flexneri, Salmonella Typhimurium and Vibrio
595 cholera. Journal of Applied Microbiology 101, 828–836.
596
597 Bosshard F., Berney, M., Scheifele,M., Weilenmann, H.U., Egli, T. 2009. Solar
598 disinfection (SODIS) and subsequent dark storage of Salmonella typhimurium and
599 Shigella flexneri monitored by flow cytometry. Microbiology (155), 1310–1317.
600
601 Boyle, M., Sichel, C., Fernández-Ibáñes, P., Arias-Quiros, G.B., Iriarte-Puña, M.,
602 Mercado, A., Ubomba-Jaswa, E., McGuigan, K.G. 2008. Bactericidal effect of solar
603 water disinfection under real sunlight conditions. Applied and Environmental
604 Microbiology 74 (10), 2997–3001.

605

606 Byrne, J.A., Fernández-Ibáñez, P., Dunlop, P.S.M., Alrousan, D.M.A., Hamilton. J.W. J.
607 2011. Photocatalytic Enhancement for Solar Disinfection of Water: A Review.
608 International Journal of Photoenergy 2011 (Art.ID 798051), 1-12.
609
610 Clasen, T.F., Haller, L. 2008. Water Quality Interventions to Prevent Diarrhoea: Cost
611 and Cost-Effectiveness. WHO.
612
613 DeRosa M.C., Crutchley R.J. 2002. Photosensitized singlet oxygen and its
614 applications. Coordination Chemistry Reviews 233-234, 351-371.
615
616 Dreschel, P., Scott, C.A., Raschid-Sally, L., Redwood, M., and Bahri, A.
617 2010.Wastewater Irrigation and Health -Assessing and Mitigating Risk in Low-Income
618 Countries. London: IDRC/IWMI.
619
620 EPHC-NRMMC-AHMC. 2006. NWQMS Australian Guidelines for Water Recycling:
621 Managing Health and Environmental Risks. Environment Protection and Heritage
622 Council, the Natural Resource Management Ministerial Council and the Australian
623 Health Ministers' Conference, Canberra.
624
625 FAO (Food and Agriculture Organization of the United Nations), 2012. Water NEWS:
626 Climate Change & Water. http://www.fao.org/nr/water/news/clim-change.html
627
628 Finney, F., Smullen, J., Foster, H.A., Brokx, S., Storey, D.M. 2003. Evaluation of
629 Chromocult coliform agar for the detection and enumeration of Enterobacteriaceae
630 from faecal samples from healthy subjects. Journal of Microbiological Methods 54,
631 353– 358.
632

633 Gannon, J.J., Busse, M.K. 1989. Escherichia-coli and enterococci levels in urban
634 stormwater, river water and chlorinated treatment-plant effluent. Water Research 23
635 (9),1167-1176.
636
637 García-Fernández, I., Polo-López, M.I., Oller, I., Fernández-Ibáñez, P. 2012. Bacteria
638 and fungi inactivation using Fe^{3+}/sunlight, H_2O_2/sunlight and near neutral photo-Fenton:
639 A comparative study. App. Cat. B: Environ. 121-122, 20-29.
640
641 Gómez-Couso, H., Fontán-Saínz, M., Sichel, C., Fernández-Ibáñez, P., Ares-Mazás, E.
642 2009. Efficacy of the solar water disinfection method in turbid waters experimentally
643 contaminated with Cryptosporidium parvum oocysts under real field conditions.
644 Tropical Medicine and International Health 14 (6), 620-627.
645
646 Habteselassie, U.Y., Bischoff, M., Applegate, B., Reuhs, B., TURCO R.F.
647 2010.Understanding the Role of Agricultural Practices in the Potential Colonization and
648 Contamination by Escherichia coli in the Rhizospheres of Fresh Produce. Journal of
649 Food Protection 73 (11), 2001–2009.
650
651 Klamerth, N., Rizzo, L., Malato, S., Maldonado, M. I., Agüera, A., Fernández-Alba, A.R.
652 2010. Degradation of fifteen emerging contaminants at ugL-1 initial concentraions by
653 mild solar photo-Fenton in MWTP effluents. Water Research 44, 545-554.
654
655 Levatensi, C., La Mantia, R., Masciopinto, C., Böckelmann, U., Ayuso-Gabella, M.,
656 Salgot, M., Tandoi, V., Van Houtte, E., Wintgens, T, Grohmann, E. 2010. Quantification
657 of pathogenic microorganisms and microbial indicators in three wastewater reclamation
658 and managed aquifer recharge facilities in Europe. Sci. Total. Environ. 408, 4923-4930.
659

660 Luo, Y., He, Q., McEvoy J.L. 2010, Effect of Storage Temperature and Duration on the
661 Behavior of Escherichia coli O157:H7 on Packaged Fresh-Cut Salad Containing
662 Romaine and Iceberg Lettuce. Journal of Food Science 75 (7), M390-M397.
663
664 Mara, D. D., Sleigh, P.A., Blumenthal U.J., Carr, R. M. 2007 Health risks in wastewater
665 irrigation: Comparing estimates from quantitative microbial risk analyses and
666 epidemiological studies. Journal of Water and Health 5 (1), 39-50.
667
668 McGuigan, K. G., Joyce, T. M. Conroy, R. M. Gillespie, J. B., and Elmore-Meegan, M.
669 1998. Solar disinfection of drinking water contained in transparent plastic bottles:
670 characterizing the bacterial inactivation process, Journal of Applied Microbiology 84,
671 1138–48.
672
673 Merck 2004. ChromoCult® Coliform Agar For detection of coliforms in drinking water
674 and processed food samples. Merck KGaA, Darmstadt, Germany.
675
676 Moulin, L., Richard, F., Stefania, S., Goulet, M., Gosselin, S., Goncalves, A., Rocher,
677 V., Paffoni, C., Dumètre, A. 2010. Contribution of treated wastewater to the
678 microbiological quality of Seine river in Paris. Water. Research 44, 5222-5231.
679
680 Niemira, B.A., Cooke, P.H. 2010. Escherichia coli O157:H7 Biofilm
681 Formation on Romaine Lettuce and Spinach Leaf Surfaces Reduces Efficacy of
682 Irradiation and Sodium Hypochlorite Washes. Journal of Food Science 75.(5), M270-
683 M277.
684
685 Polo-López, M.I., I. García-Fernández , I. Oller and P. Fernández-Ibáñez, 2011. Solar
686 disinfection of fungal spores in water aided by low concentrations of hydrogen
687 peroxide. Photochemical & Photobiological Sciences 10, 381-388.

688

689 Polo-López, M. I., Fernández-Ibáñez, P., García-Fernández, I., Oller, I., Salgado-
690 Tránsito, I., Sichel, C. 2010. Resistance of Fusarium spores to solar TiO2
691 photocatalysis: influence of spore type and water (scaling-up results). J. Chem.
692 Technol. Biotechnol. 85, 1038–1048.
693
694 Polo-López, M.I., García-Fernández, P., Velegraki, T., Katsoni, A., Oller, I.,
695 Mantzavinos, D., Fernández-Ibáñez, P. 2012. Mild solar photo-Fenton: an effective tool
696 for the removal of Fusarium from simulated municipal effluents. Applied Catalysis B
697 111-112, 545-554.
698
699 Prats, J., Garcia-Armisen, T., Larrea, J., Servais, P. Comparison of culture-based
700 methods to enumeraten Escherichia coli in tropical and temperate freshwaters. Letters
701 in Applied Microbiology 46, 243–248.
702
703 O'Toole, J., Sinclair, M., Leder, K. 2010. Quantitative microbial risk assessment and
704 Australian Guidelines for Water Recycling: two case studies. Food Australia 62 (9),
705 408-412.
706
707 Sichel, C., Fernández-Ibáñez, P., Tello, J., de Cara, M. 2009. Lethal synergy of solar
708 UV-radiation and H_2O_2 on wild Fusarium solani spores in distilled and natural well
709 water. Water Research 43, 1841-1850.
710
711 Scheierling, S.M., Bartone, C.R., Mara, D.D., and Drechsel, P. 2011. Towards an
712 agenda for improving wastewater use in agriculture. Water International 36 (4), 420–
713 440.
714

715 Sciacca F, Rengifo-Herrera JA, Wéthé J, Pulgarin. 2010. Dramatic enhancement of
716 solar disinfection (SODIS) of wild Salmonella sp. in PET bottles by H2O2 addition on
717 natural water of Burkina Faso containing dissolved iron. Chemosphere 78 (9),1186-
718 1191.
719
720 Solomon, E.B., Yaron, S., Matthews, K.R. 2002. Transmission of Escherichia coli
721 O157:H7 from Contaminated Manure and Irrigation Water to Lettuce Plant Tissue and
722 Its Subsequent Internalization. Applied and Environmental Microbiology 68 (1), 397–
723 400.
724
725 Spuhler, D., Rengifo-Herrera, J.A., Pulgarín, C. 2010. The effect of Fe2+, Fe3+, H2O2
726 and the photo-Fenton reagent at near neutral pH on the solar disinfection (SODIS) at
727 low temperatures of water containing Escherichia coli K12. Applied Catalysis B:
728 Environmental 96, 126-141.
729
730 Ubomba-Jaswa, E., Fernández-Ibáñez, P., Navntoft, C., Polo-López, M.I. McGuigan,
731 K.G. 2010. Investigating the microbial inactivation efficiency of a 25 L batch solar
732 disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for
733 household use. J. Chem. Technol. Biotechnol. 85, 1028–1037.
734
735 Ubomba-Jaswa, E., Navntoft, C., Polo-López, I., Fernández-Ibáñez, P., McGuigan,
736 K.G. 2009. Solar disinfection of drinking water (SODIS): An investigation of the effect of
737 UVA dose on inactivation efficiency. Photochem. Photobiol. Sciences 8 (5), 587-595.
738
739 UNDP (United Nations Development Programme). 2006. Human Development report
740 Beyond Scarcity. Power, Poverty and the Global Water Crisis. New York: Palgrave
741 Macmillan. http://hdr.undp.org/en/reports/global/hdr2006/
742

743 World Health Organisation (WHO). 2006. Guidelines for the safe use of wastewater,
744 excreta and greywater. Wastewater use in agriculture, Volume 2. Geneva: World
745 Health Organisation.
746

747 **Captions**

748

749 **Table 1.** *E. coli* inactivation rates (*k*) during solar disinfection of DW, WW, SE and real
750 WWTPE with and without H_2O_2.

751

752 **Table 2.** *E. coli* presence/absence on lettuce leaves for irrigation assays with real
753 WWTPE prior- and post- solar treatments with positive and negative controls of mineral
754 water.

755

756 **Figure 1.** Schematic view of experimental design: (i) Solar disinfection (with/without
757 H_2O_2) of DW, WW, SE in 20-L CPC equipped solar reactors; (ii) Solar disinfection
758 (with/without H_2O_2) of real WWTPE in 20-L CPC-equipped solar reactors and 1.5-L
759 PET-bottle reactors, followed by irrigation assays on lettuce crops; (iii) Detection of *E.*
760 *coli* on lettuce leaves.

761

762 **Figure 2**. Solar disinfection batch reactors used at Plataforma Solar de Almería
763 (Spain): (a) R1 and R2, 20-L borosilicate glass reactors equipped with CPC; and (b) b1
764 and b2, 1.5-L PET SODIS bottle reactors.

765

766 **Figure 3.** Solar inactivation of *E. coli* in distilled water (– ■ –), well water (– ▲ –), SE
767 (– ◆ –), and real WWTPE (– ● –) using the 20-L CPC solar reactors against solar UV
768 dose and time. The detection limit (DL) was 2 CFU mL^{-1}.

769

770 **Figure 4.** H_2O_2-assisted solar inactivation of *E. coli* in distilled water (– □ – and – ■ –),
771 well water (– △ – and – ▲ –), SE (– ◇ – and – ◆ –), and real WWTPE (– ○ – and – ● –)
772 using the 20-L CPC reactors. Open symbols: 5 mg L^{-1} H_2O_2 and solid symbols: 10 mg
773 L^{-1} H_2O_2.

774

775 **Figure 5.** Solar (– ■–) and H_2O_2/solar inactivation of *E. coli* with 5 mg L^{-1} (– ▲–) and

776 10 mg L^{-1} (– ●–) of H_2O_2 in real WWTPE against solar UV dose in 1.5L PET-bottles.

777

778

779

780

781

782

Table 1

Click here to download Table: Table 1.pdf

Table 1

Water	Solar treatment		*k*	R^2	Exposure time to DL	Log (initial conc. *E. coli*)
	Reactor	H_2O_2 dose (mg L^{-1})	Log (CFU mL^{-1})/(kJ m^{-2})		h	Log (mg L^{-1})
DW	R1-R2	-	-5,2E-02	0,97	<1,5	5,9
	R1-R2	-	-4,8E-02	0,95	<1,5	6,2
	R1-R2	5	-1,1E-01	0,99	<0,5	6,9
	R1-R2	5	-1,0E-01	0,98	<0,5	6,1
	R1-R2	10	-1,1E-01	0,93	<0,5	6,9
	R1-R2	10	-1,1E-01	0,64	<0,5	6,0
WW	R1-R2	-	-2,7E-02	0,98	<2	6,3
	R1-R2	-	-2,8E-02	0,98	<2	6,4
	R1-R2	5	-4,8E-02	0,99	<1	6,1
	R1-R2	10	-5,4E-02	0,98	<1	6,1
SE	R1-R2	-	-2,3E-02	0,94	<3	6,6
	R1-R2	-	-2,1E-02	0,97	<3	6,7
	R1-R2	5	-3,5E-02	0,96	<1,5	6,0
	R1-R2	10	-3,6E-02	0,97	<1,5	6,3
WWTPE	R1-R2	-	-7,3E-03	0,99	<4	3,4
	R1-R2	-	-6,4E-03	0,96	<5	4,2
	b1-b2	-	-5,2E-03	0,99	>5	4,1
	R1-R2	5	-6,8E-03	0,98	<4	3,5
	b1-b2	5	-6,5E-03	0,98	<4	3,5
	R1-R2	10	-1,1E-02	0,99	<3	3,6
	b1-b2	10	-9,4E-03	0,99	<3	3,6

k =*E. coli* inactivation rate (linear regression of Log (concentration) vs. UV dose) with R^2 = regression coefficient. Exposure time to DL (h) shows the time at which the first negative sample was collected (<2 CFU mL^{-1}) in every disinfection assay. All subsequent samples were below DL.

Table 2

Click here to download Table: Table 2.pdf

Table 2

Irrigation water	**Presence (+) / Absence (-) of *E. coli* on lettuce leaves after irrigation**							
Without solar treatment								
Initial concentration of *E. coli* in real WWTPE (CFU mL^{-1})	2.4×10^3		1.3×10^4		3.8×10^3		3.1×10^3	
Untreated real WWTPE	+	+	+	+	+	+	+	+
	-	+	+	+	+	-	+	+
After solar treatment								
	Solar disinfection				H_2O_2/solar disinfection			
	(1^{st} assay)		(2^{nd} assay)		10 mg L^{-1}		5 mg L^{-1}	
20-L CPC-equipped solar reactors (R1)	-	-	+	-	-	-	-	-
20-L CPC-equipped solar reactors (R2)	-	-	-	-	-	-	-	-
PET-bottle (b1)	N/A	N/A	-	-	-	-	-	-
PET-bottle (b2)	N/A	N/A	-	-	-	-	-	+
Controls								
Negative control: Mineral (potable) water	-	-	-	-	-	-	-	-
Positive control: Mineral water + *E. coli* K-12 (10^6 CFU mL^{-1})	+	+	+	+	+	+	+	+

+/- indicate the presence/absence of *E. coli* in a ~0.3 g (3 x 0.1 g) lettuce leaves combined-sample from a single lettuce crop. For each disinfection assay,2 lettuce crops were irrigated with the water from each of the solar reactors (R1, R2, b1, b2) and 2 combined samples (one from each crop) were analyzed for the presence of *E. coli*. Note: No results (N/A) are presented for the 1^{st} solar disinfection assay in PET-bottles (b1, b2) since these reactors were not used during that first experiment.

Figure 1

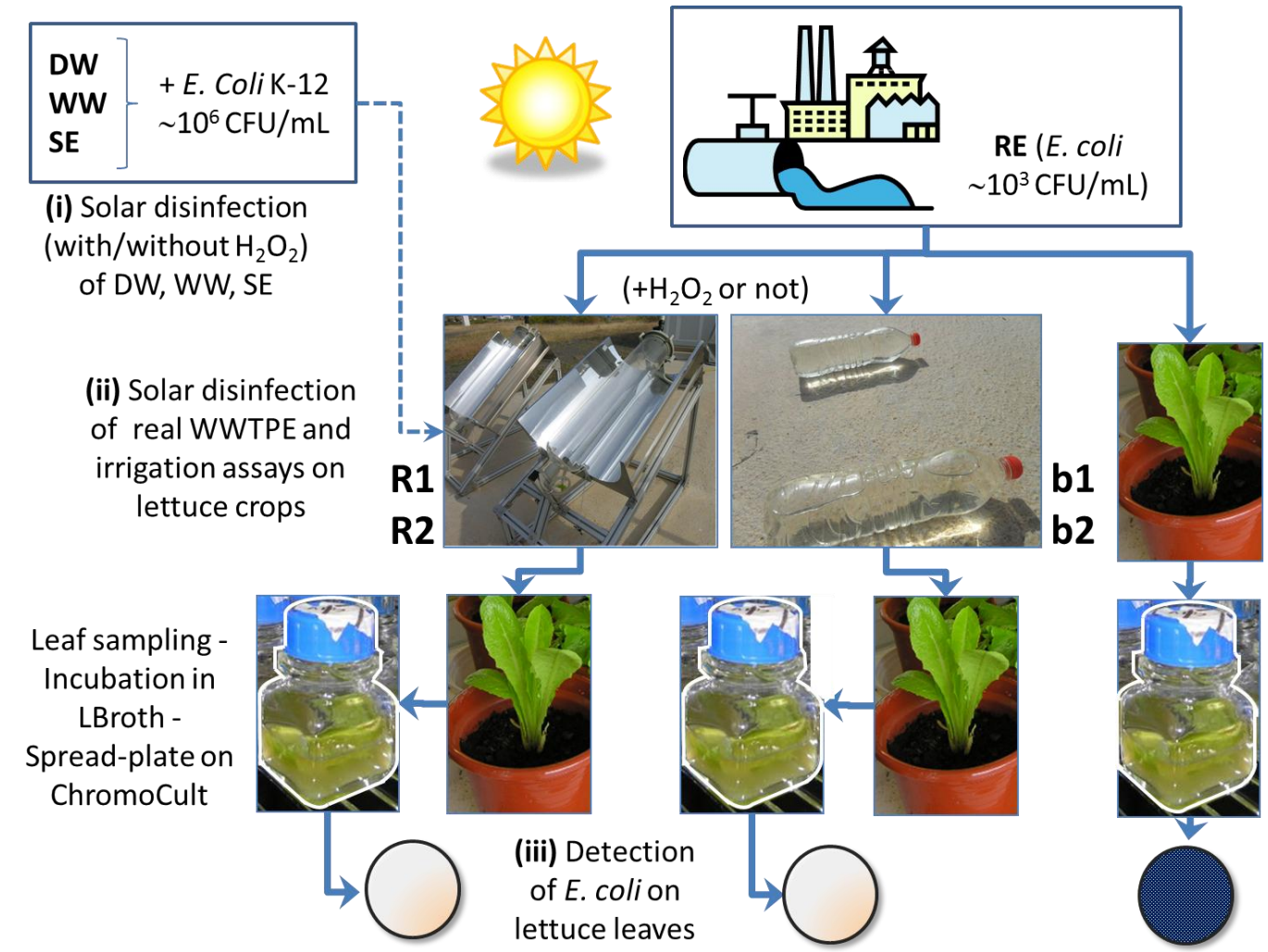

DW
WW
SE
+ E. Coli K-12
~10⁶ CFU/mL
(i) Solar disinfection (with/without H2O2) of DW, WW, SE
RE (E. coli ~10³ CFU/mL)
(+H2O2 or not)
(ii) Solar disinfection of real WWTPE and irrigation assays on lettuce crops
R1
R2
b1
b2
Leaf sampling - Incubation in LBroth - Spread-plate on ChromoCult
(iii) Detection of E. coli on lettuce leaves

Figure 2

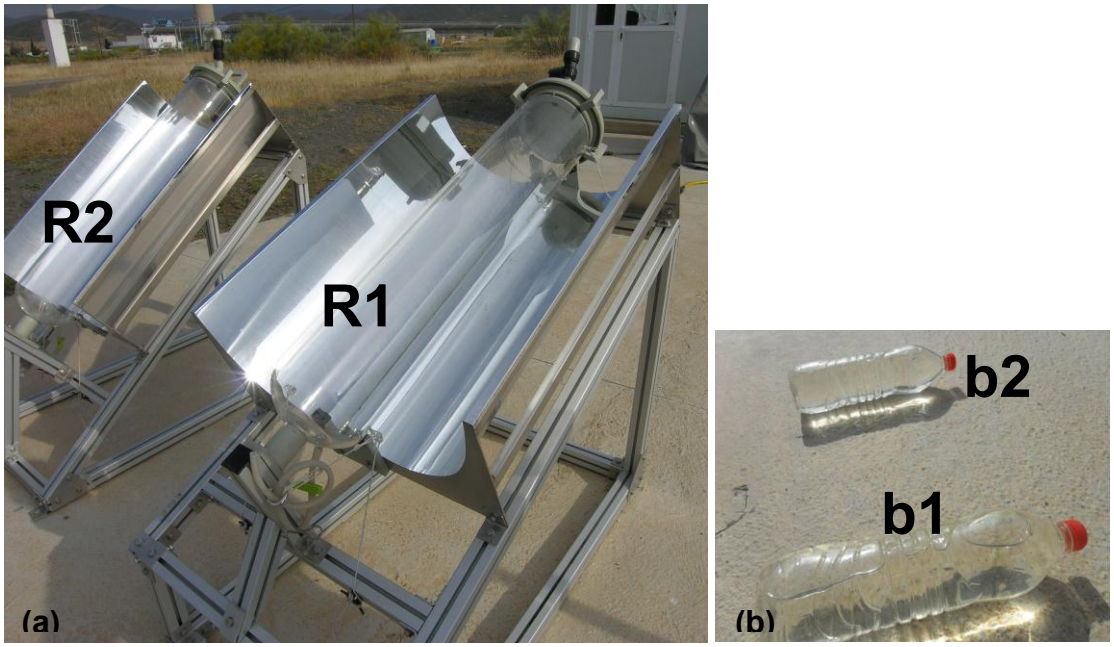


Figure 3

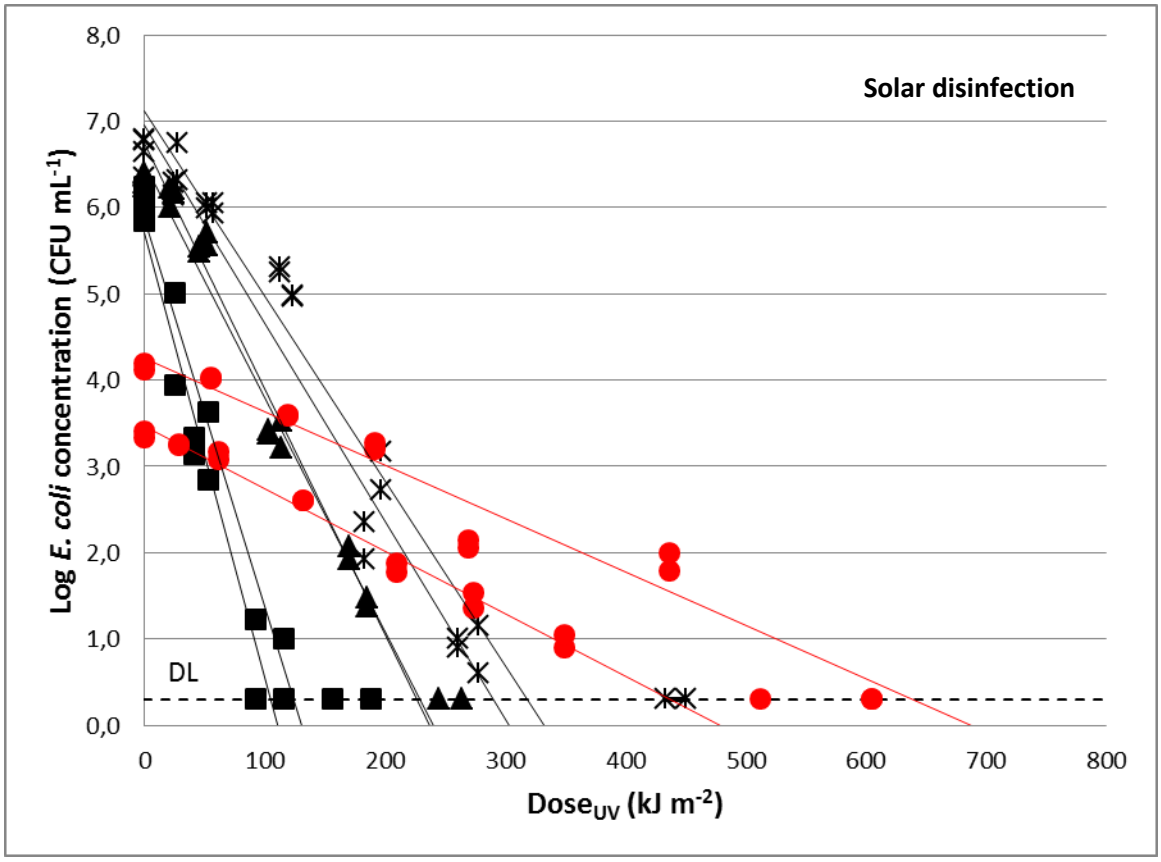

Solar disinfection
Log E. coli concentration (CFU mL-1)
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
DL
0
100
200
300
400
500
600
700
800
Dose UV (kJ m-2)

Figure 4

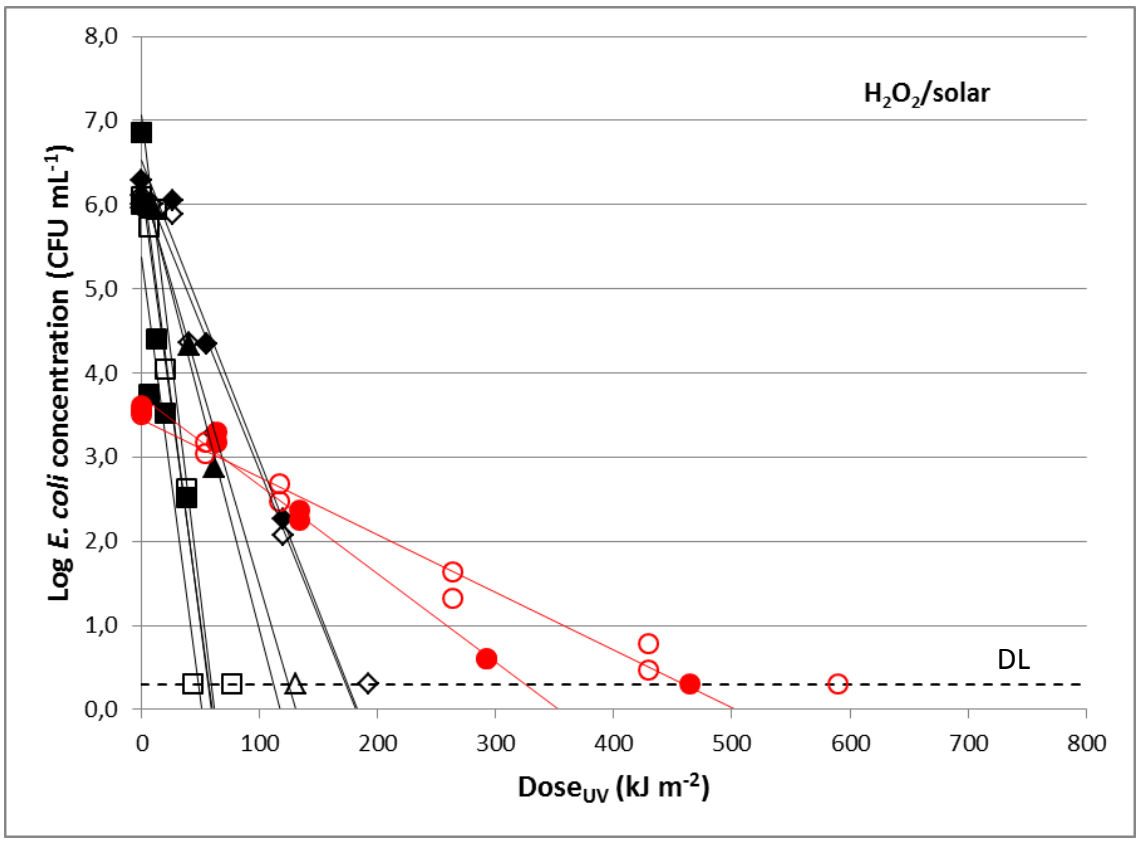
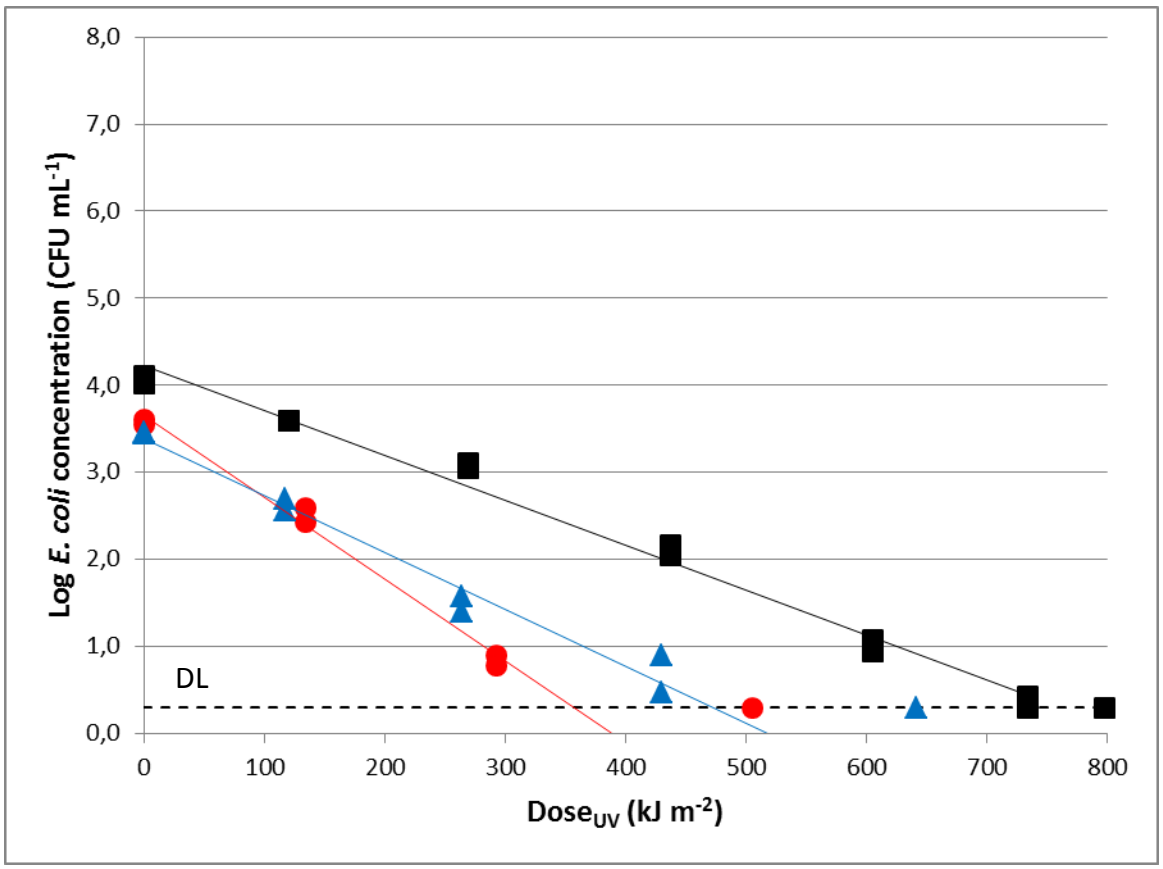

8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Log E. coli concentration (CFU mL-1)
H2O2/solar
DL
0
100
200
300
400
500
600
700
800
Dose UV (kJ m-2)

Figure 5


8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Log E. coli concentration (CFU mL-1)
DL
0
100
200
300
400
500
600
700
800
DoseUV (kJ m-2)