

Reclamation of Real Urban Wastewater Using Solar Advanced Oxidation Processes: An Assessment of Microbial Pathogens and 74 Organic Microcontaminants Uptake in Lettuce and Radish

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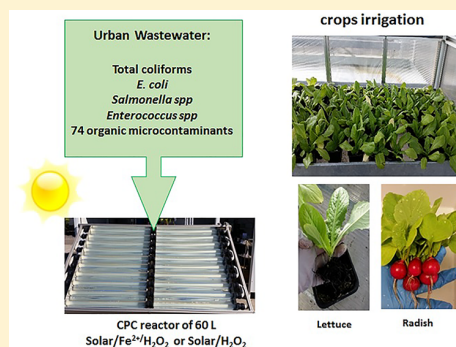
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Supporting Information

ABSTRACT: In this study, disinfection of urban wastewater (UWW) with two solar processes (H_2O_2 –20 mg/L and photo-Fenton 10 mg/L- Fe^{2+} /20 mg/L- H_2O_2 at natural water pH) at pilot scale using a 60 L compound parabolic collector reactor for irrigation of two raw-eaten vegetables (lettuce and radish) has been investigated. Several microbial targets (total coliforms, *Escherichia coli*, *Salmonella* spp., and *Enterococcus* spp.) naturally occurring in UWW and 74 organic microcontaminants (OMCs) were monitored. Disinfection results showed no significant differences between both processes, showing the following inactivation resistance order: *Salmonella* spp. < *E. coli* < total coliforms < *Enterococcus* spp. Reductions of target microorganisms to concentrations below the limit of detection (LOD) was achieved in all cases with cumulative solar UV energy per volume (Q_{UV}) ranged from 12 to 40 kJ/L (90 min to 5 h). Solar photo-Fenton showed a reduction of 66% of OMCs and solar/ H_2O_2 of 56% in 5 h treatment. Irrigation of radish and lettuce with solar treated effluents, secondary effluents, and mineral water was performed for 6 and 16 weeks, respectively. The presence of bacteria was monitored in surfaces and uptake of leaves, fruit, and also in soil. The bacterial concentrations detected were below the LOD in the 81.2% (lettuce) and the 87.5% (radish) of the total number of samples evaluated. Moreover, uptake of OMCs was reduced above 70% in crops irrigated with solar treated effluents in comparison with secondary effluents of UWW.



1. INTRODUCTION

The increase of water scarcity in arid zones has forced a search for alternative water sources, for example, wastewater, for use in sectors like agriculture, the largest water-consuming human activity; this activity has become a widespread practice.¹ The employment of treated wastewater in agriculture involves important health risks, especially for the consumption of raw crops, due to the presence of several chemical (micro-pollutants) and microbiological hazardous contaminants.^{2,3} Consequently, different agencies have established guidelines to control the microbial load for agricultural irrigation. Meanwhile, organic microcontaminants (OMCs) have not been included in these regulations. OMCs refer to chemical organic substances, which have been identified on water environments in the range of ng/L to $\mu\text{g/L}$. They belong to different chemical families with diverse physical–chemical characteristics and include priority substances, already regulated in the EU (Directive, 2013/39/EC), such as polycyclic aromatic

hydrocarbons, polychlorinated biphenyls, etc., as well as contaminants of emerging concern (CECs), which are unregulated, poorly characterized in terms of occurrence, and have the potential to cause adverse ecological or human health impacts. These include personal care products, pharmaceuticals, drugs, UV-filters, transformation products (TPs), etc., which in many cases are still unknown.⁴

The aforementioned guidelines are based on the control of the concentration of *Escherichia coli* as main indicator of faecal contamination. They include defined different contamination categories depending on the final use of the reclaimed wastewater. For example, <1000 CFU/100 mL for restricted irrigation according to the World Health Organization.⁵ Other

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regulations specifically describe the *E. coli* concentration for raw crop irrigation for unprocessed purposes, <100 CFU/100 mL in Spanish legislation⁶ and ISO recommendation⁷ and no detectable (<1 CFU/100 mL) by United States Environmental Protection Agency.⁸ Recently, the European Commission published a proposal to regulate wastewater reuse, which include minimum quality requirements for water reuse in agricultural irrigation,⁹ which reports a maximum concentration of <10 CFU/100 mL for *E. coli* for the same reuse.

The persistence of trace levels of OMCs after a conventional secondary treatment and the presence of high microbial load (ca. 10⁵ CFU/100 mL) require the application of a tertiary treatment to achieve the quality levels required by the reuse guidelines. The continuous discharge of pharmaceuticals in the environment and their possible incorporation in the human food chain represents one of the greatest threats to human health. This is also recognized as a clear route to proliferation of antimicrobial resistant microorganisms and genes in the environment.¹⁰ Conventional tertiary treatments (O₃, UV–C, chlorination) can achieve the microbial quality required for irrigation reuse, although they have some drawbacks that are still unsolved, as for example the low removal of OMCs, high cost of the treatments, and the generation of undesired disinfection byproducts (DBPs).¹¹

Solar water treatment may represent a sustainable alternative wastewater treatment for reuse as it allows minimizing the cost of the treatment by using sunlight and also avoids the generation of DBPs during the process.¹² Solar advanced oxidation processes (AOPs) have been proven to be an effective alternative tertiary wastewater treatment due to their high effectiveness for disinfection and decontamination based on the capacity to generate hydroxyl radicals (HO•), which are nonselective and very powerful oxidants (2.8 eV). Several studies on these solar AOPs show high OMCs degradation and bacterial inactivation rates.¹²

Among the different solar AOPs, photo-Fenton is one of the most investigated processes due to its simplicity and high disinfection and decontamination capability using low reagent concentrations (iron and H₂O₂). The process is based on the generation of HO• through a photocatalytic cycle between iron ions, hydrogen peroxide (H₂O₂), and UV–vis radiation.¹³ Furthermore, solar disinfection processes based on photo-inactivation of bacteria, assisted or not by H₂O₂ (solar/H₂O₂), have also demonstrated high disinfection efficiencies.^{13–16} These treatments may have promising applications in highly solar irradiated areas. Previous investigations have demonstrated the capability of solar/H₂O₂ treatment to improve the microbial quality of secondary effluents. These works also demonstrated that irrigated lettuce crops with the solar/H₂O₂-treated wastewater reduced the microbial contamination risk.^{17,18} Nevertheless, there is still a lack of comprehensive studies about the capability of these processes as tertiary treatment and on the further translocation and accumulation of OMCs and their TPs in vegetables, as a result of irrigation practices.

The aim of this study was to evaluate solar/H₂O₂ and solar photo-Fenton process at natural pH for reducing the load of microorganisms and OMCs of real UWW effluents using a solar compound parabolic collector (CPC) pilot reactor. Following the treatment, the microbial and chemical quality of irrigated lettuce and radish crops was assessed in an experimental greenhouse. Total coliforms (TC), *E. coli*, *Salmonella* spp., and *Enterococcus* spp. and 74 OMCs present

in real UWW were monitored. To our knowledge, this is the first time that the efficiency of solar/H₂O₂ and solar photo-Fenton at natural water pH is investigated to simultaneously reduce the microbial risk of naturally occurring pathogens and the presence of OMCs in real urban wastewater effluents. A multiresidue analytical approach has been applied to detect OMCs and some of their TPs, in both water and vegetable samples, to evaluate their potential uptake by raw-eaten crops.^{17–19}

2. MATERIALS AND METHODS

2.1. Water Matrixes. Two types of water sources were used: (i) secondary effluents from the urban wastewater (UWW) treatment plant of El Bobar (Almeria, Spain). This secondary effluent was directly used as positive control for irrigation assays and also it was solar treated (as described below) and used for irrigation assays; and (ii) commercial mineral water (Agua del Marquesado S.L., Spain) was used as negative control in the irrigation experiments.

Chemical characterization of UWW and mineral water is shown in Table S1 (Supporting Information (SI)). Total organic carbon (TOC) was analyzed using a Shimadzu TOC-5050 (Shimadzu, Japan). Ion concentrations were measured by ionic chromatography (Model 850, Metrohm, Switzerland). Turbidity was determined using a turbidity-meter (model 2100 N Hach, Loveland, CO) and conductivity with a conductivity sensor (GLP31, CRISON, Spain).

2.2. Microbial Enumeration and OMCs Quantification. **2.2.1. Bacterial Assessment.** Total coliforms, *E. coli*, *Salmonella* spp., and *Enterococcus* spp. naturally occurring in UWW effluents were detected and enumerated using the pour plate technique by spreading appropriate sample volumes (50, 250, or 500 μ L, depending on the bacterial load) in selective and specific agar media (limit of detection (LOD): 2 CFU/mL). Total coliforms and *E. coli* were simultaneously enumerated using the chromogenic ChromoCult® Coliform Agar (Merck KGaA, Germany) which distinguishes between total coliforms and *E. coli* by the color of the colonies formed in the agar media, red and violet colonies, respectively. *Salmonella* spp. was enumerated on Salmonella-Shigella agar (Scharlau, Spain) and *Enterococcus* spp. on Slanetz Bartley agar (Scharlau, Spain). Colonies were counted after incubation for 24–48 h at 37 °C. Concentrations of pathogens detected in secondary effluent are shown in SI Table S1.

2.2.2. Organic Microcontaminants. This work screened 74 OMCs (mainly pharmaceuticals and some of their transformation products (TPs)) due to their frequent identification in UWW effluents.^{20,21} The analysis was performed with a 1200 LC system (Agilent Technologies, Foster City, CA) with a XDB C18 50 $\times$ 4.6 mm and 1.8 μ m particle size analytical column (Agilent Technologies, Santa Clara, CA), coupled to a hybrid quadrupole-linear ion trap-mass spectrometer (QqLIT) 5500 QTRAP from Sciex Instruments (Foster City, CA) equipped with a TurboIon Spray source featuring electrospray ionization (ESI), operating in positive (ESI+) and negative (ESI–) modes. The source settings were ionspray voltage, 4500 V; curtain gas, 25 (arbitrary units); GS1, 50 psi; GS2, 40 psi; and temperature, 550 °C. N₂ served as nebulizer, curtain, and collision gas. Samples were directly injected (10 μ L) and analyzed by a multiresidue analytical method previously reported.²¹ The OMCs uptake was analyzed in the vegetable matrices by a multiresidue method as reported elsewhere.²² Briefly, it consists of the extraction of OMCs based on a

Table 1. Organic Microcontaminants (OMCs) Detected in Secondary Effluents and Solar Treated Effluents^a

compound	concentration range in secondary effluents (ng/L)	average in secondary effluents (ng/L)	degradation (%) solar/ H ₂ O ₂	degradation (%) solar photo-Fenton
4-AA	233–428	304	100	100
4-AAA	3117–4254	3590	54	61
4-FAA	6106–6897	6541	73	75
4-MAA	48–64	54	100	100
acetaminophen	50	50	–	100
antipyrine	294–322	308	100	100
atenolol	626–770	681	0	6
azithromycin	259–476	363	32	51
caffeine	55–68	61	37	40
carbamazepine	90–107	97	11	19
carbamazepine epox	22–24	23	60	62
citalopram	187–209	198	2	14
clarithromycin	16–30	23	0	0
diazepam	10–15	12	0	0
famotidine	13–24	18	100	100
fenofibric acid	26–97	66	78	89
gemfibrozil	457–852	573	77	88
hydrochlorothiazide	438–1015	593	83	49
ketoprofen	40–87	66	100	100
lincomycin	72–130	96	100	100
mepivacaine	9–22	16	96	47
metoclopramide	18–31	26	97	100
metoprolol	7–17	13	0	3
metronidazole	20–52	30	100	78
naproxen	50–62	54	100	–
pentoxifylline	24–90	51	0	11
primidone	51–104	84	0	0
propranolol	30–58	50	95	94
ranitidine	178–484	340	100	100
sotalol	20–37	32	100	100
sulfamethoxazole	51–112	83	71	63
sulfapyridine	34–76	58	91	87
trimethoprim	25–64	45	54	47
venlafaxine	126–287	233	0	4
total average load (ng/L)		14 832	56%	66%

^aOMCs with a 100% degradation are presented in bold. (–): No data. ^bMetabolites of metamizole: 4-AA (4-aminoantipyrine); 4-AAA (N-acetyl-4-aminoantipyrine); 4-FAA (N-formyl-4-aminoantipyrine); 4-MAA (N-methyl-4-aminoantipyrine).

modification of QuEChERS acetate sample extraction protocol followed by LC-MS/MS analysis.

2.3. Solar Water Treatments. **2.3.1. Compound Parabolic Collector (CPC) Reactor.** The solar CPC photoreactor used in this work was described elsewhere.²³ It consists of two CPC mirror modules with 10 borosilicate-glass tubes per module placed on an anodized-aluminum platform tilted at 37°. CPC mirrors are made of highly reflective anodized aluminum (MiroSun, Alanod, Germany), with a concentration factor of 1, 4.5 m² of total irradiated surface, and illuminated water volume is 45 L over a total volume of 60 L. The flow rate used was 30 L/min. pH, dissolved oxygen, and temperature were continuously monitored using several probes inserted on pipes, and data were recorded by data acquisition software.

2.3.2. Reagents. Ferrous sulfate heptahydrate (FeSO₄·7H₂O, PANREAC, Spain) was used as the source of Fe²⁺ for photo-Fenton experiments. Iron concentration was measured in water and in soil samples from crops irrigation tests. The measurement was done according to ISO6332, with a limit of quantification (LOQ) of 0.1 mg/L.¹³

Hydrogen peroxide aqueous solution was used at 30% (w/v) (Riedel-de Haën, Germany), and diluted directly into the reaction mixture. H₂O₂ concentration was measured with a spectrophotometer at 410 nm according to DIN 38409 H15, limit of quantification (LOQ) 0.1 mg/L.¹³

2.3.3. Solar Radiation Measurements. Solar UV irradiance was measured using a global UV pyranometer (295–385 nm, model CUV4, Kipp & Zonen, Netherlands), providing data of incident radiation in terms of W_{UV}/m². The accumulated UV energy in the solar reactor per unit of treated water volume and time (Q_{UV}, kJ/L) was estimated to evaluate the bacterial inactivation during solar processes. Treatment time was also used to describe the effectiveness of the solar processes. Q_{UV} allows the comparison of results under different weather conditions and reactors characteristics, and it is calculated by eq 1:

$$Q_{UV,n} = Q_{UV,n-1} + \frac{\Delta t_n UV_{G,n} A_r}{V_t}; \Delta t_n = t_n - t_{n-1} \quad (1)$$

where Q_{UV,n} and Q_{UV,n-1} is the cumulative UV energy per liter (kJ/L) at times *n* and *n*–1; UV_{G,n} is the average incident

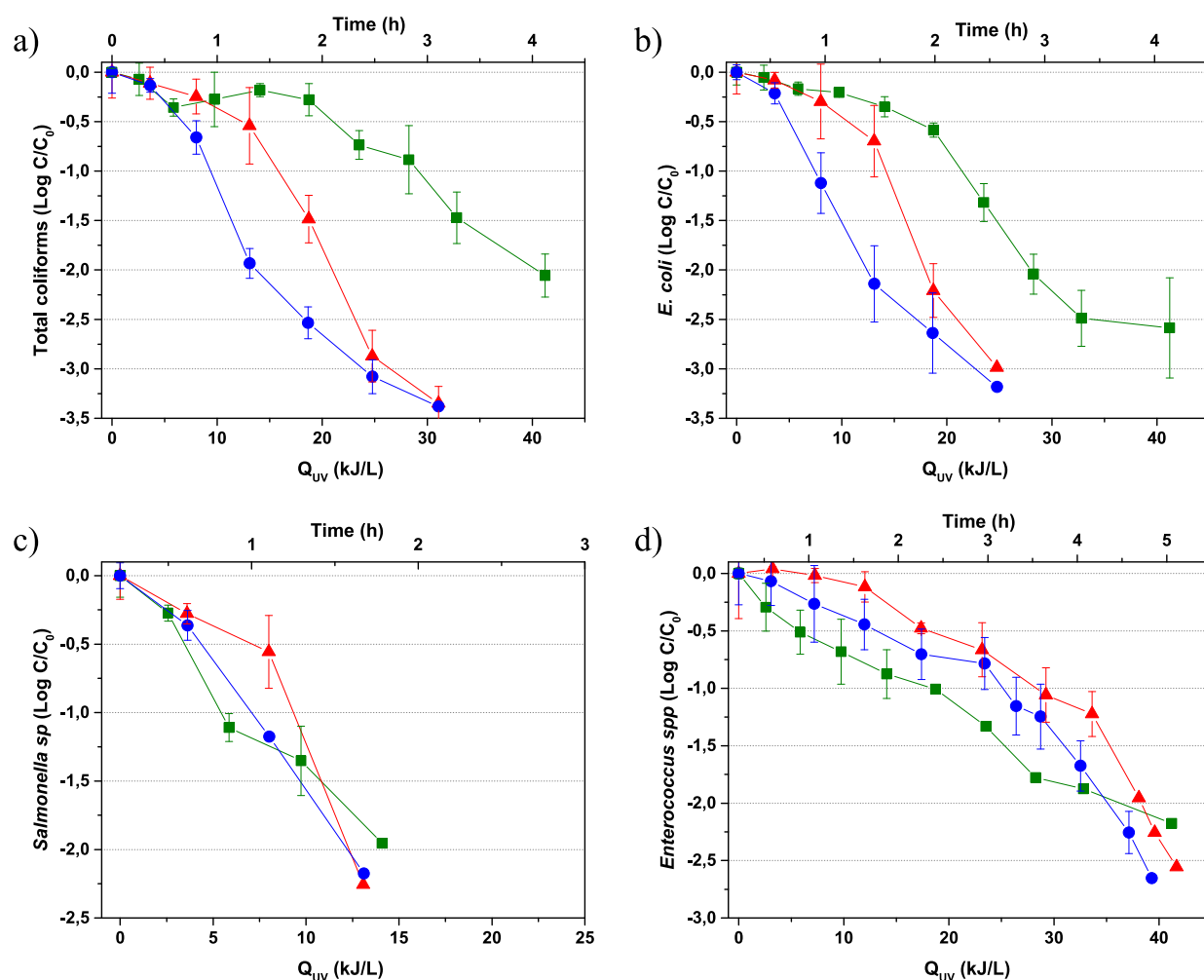


Figure 1. Inactivation profile of (a) Total coliforms, (b) *E. coli*, (c) *Salmonella* spp., and (d) *Enterococcus* spp. in UWW effluents by solar photoinactivation (—■—), solar/H₂O₂ (20 mg/L) (—●—), and solar photo-Fenton (10/20 mg/L of Fe²⁺/H₂O₂) (—▲—).

radiation on the irradiated area (W/m^2), Δt_n is the experimental time of samples (s), A_i is the illuminated area of collector (m^2), and V_t is the total volume of water treated (L).²³

2.3.4. Solar Experiments. All experiments were conducted at Plataforma Solar de Almeria (Spain) on completely sunny days. They started at the same local time (10:30 am) and lasted 5 h in consecutive days, so that water temperature (ranged from 27.1 to 39.2 °C) and solar UV irradiance (ranged from 23 to 45 W/m^2) were similar for all experiments. Three solar treatments were investigated: (i) solar photoinactivation, (ii) solar/H₂O₂ with 20 mg/L of H₂O₂, and (iii) solar photo-Fenton at natural water pH with 10/20 mg/L of Fe²⁺/H₂O₂. The reagent concentrations herein used were selected based on optimal concentrations for the same conditions as reported elsewhere.^{13,15,18}

Solar experiments were carried out as follows; the photo-reactor tank was filled with 60 L of UWW effluent. When required, the reagents were added and recirculated in the dark for homogenization during 15 min.²³ After that, the reactor was uncovered, and 10 mL of samples were taken at regular intervals during the solar experiment for bacterial, OMCs, and reagent quantification. Two batches of UWW effluents per solar treatment were sampled and monitored to obtain the

average monitoring results. The average of the bacterial inactivation result is reported along with an error equal to the standard deviation. Average values of the OMCs concentrations and their degradation during these treatments are also reported (Table 1).

2.4. Irrigation Assays. 2.4.1. Experimental Greenhouse.

The irrigation assays were performed under controlled conditions at Plataforma Solar de Almeria using a 30 m^2 experimental greenhouse, designed and built by Suministros D.R. (Spain). It consists of four individual areas (7.5 m^2 each) equipped with temperature and humidity sensors connected to Ambitrol software for controlling these parameters by cooling (Fisair, Spain) and heating (Gabarrón, Spain) systems and also automatic windows located in the roof of each area. Average temperature during the experiments was 25 ± 5 °C and humidity varied daily from 50 to 90%.

2.4.2. Crops. Lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) crops were selected as they are raw-eaten vegetables with relatively fast growing, that is, 8–16 and 4–6 weeks from seeding to harvesting, respectively. Both seeds were obtained from a local provider and grown on propylene pots (9 × 9 × 10 cm) filled with commercial and regular peat as substrate. According to the manufacturer, it contains a N–P–K ratio (w/v) of 13–14–13 g/L, respectively, pH 7, and 120 mS/m of

conductivity. 100 pots per each type of crop and irrigation condition were used for statistical purposes.

2.4.3. Crops Irrigation Experiments. Lettuce and radish irrigation tests were done simultaneously, with similar growing conditions. Four sets of 100 pots of lettuce and radish pots were planted, one per water sample evaluated (negative control, positive control, solar/H₂O₂ treated effluent, and solar photo-Fenton treated effluent). Each set was placed in an individual area of the greenhouse to avoid potential risk of cross contamination between solar treated and secondary effluents. Two batches of solar treated effluents were collected and stored at 4 °C to provide sufficient irrigation water along the irrigation period. Analysis of bacterial regrowth of the selected pathogens in solar treated effluents during storage was evaluated at 24, 48, and 72 h post-treatment; no positive regrowth was found in any case. Each pot was regularly watered with 50 mL of corresponding type of water as reported elsewhere.^{18,22}

2.4.4. Detection of Pathogens on Crops and Soil. After the irrigation period, 15 out 100 samples/pot of each irrigation test were randomly selected and analyzed to detect and quantify the pathogens on surfaces of lettuce and radish leaves, radish fruit, and on soil samples following reported methodology.^{17,18} Briefly, it consists of the following steps:

- Leaves of each lettuce or radish samples are weighed (3 ± 0.5 g), cut in small (<1 cm²) pieces, then mixed with 20 mL of saline solution and homogenized in a Stomacher 400 (Seward, UK) at 260 rpm for five min.
- Radish fruit: follows the same procedure as leaves, but with sample weights of 7.0 ± 0.1 g.
- Peat: the soil around each plant (1 cm around) was collected and weighed to obtain samples of 5.0 ± 0.5 g. Then, they were mixed with 45 mL of saline solution in a 100 mL container and homogenized manually.

The enumeration of pathogens in crops and soils was performed following the pour plate counting procedure described in Section 2.2 and additionally, 5 mL of the generated extract samples was spread in 140 mm Petri dishes with the corresponding culture media. The LOD was reduced to 20 CFU/100 mL in this case.

2.4.5. Quantification of OMCs on Crops. Composite samples of lettuce and radish (leaves and fruit) were washed with mineral water prior to trituration to remove any deposition in the surface of the crop and detect only true OMCs absorption.²³ After that, the extraction of the composite samples was performed in triplicate. Results are shown as the average of the concentrations calculated (concentrations expressed in wet weight, w.w.).

3. RESULTS AND DISCUSSION

3.1. Solar Treatment of UWW Effluents. **3.1.1. Bacterial Inactivation.** The inactivation profile of total coliforms, *E. coli*, *Salmonella* spp., and *Enterococcus* spp. in effluents treated by solar processes at near neutral pH is shown in Figure 1. Water temperature never exceeded 40 °C, excluding, therefore, the thermal effect ($T > 45$ °C) as a key parameter for bacterial inactivation in these results.²⁴

Reductions of target microorganisms to concentrations below the limit of detection were attained in all cases requiring different solar cumulated UV energy and treatment times depending on the type of pathogen. As expected, the disinfection results using both solar processes were very similar

and much faster than solar photoinactivation.^{13,14,18} Nevertheless, in the case of *Enterococcus* spp., the solar/H₂O₂ treatment was slightly faster than for the rest of microorganisms. The inactivation order for both solar oxidation processes was *Salmonella* spp. (12 kJ/L and 90 min) > *E. coli* (23 kJ/L and 2.5 h) > total coliforms (31 kJ/L and 3 h) > *Enterococcus* spp. (39 kJ/L and 41 kJ/L, or 4–5 h for solar/H₂O₂ and photo-Fenton, respectively).

These results show the capability of both treatments for reducing the microbial load in UWW effluents. Similar results have been reported.^{13,25} The mechanisms explaining this behavior have been already described. In brief, the bactericidal effect of small concentrations (mM range) of H₂O₂ and sunlight is mainly attributed to the oxidative effect of internal photo-Fenton reactions assisted by the internal iron and the diffused H₂O₂ inside the cell, leading to cell lethal damages.^{13,25,26} The results of photo-Fenton at natural water pH are also in agreement with previous results in UWW, where effects of oxidant concentrations, pH, and organic matter as key parameters for bacterial inactivation have been extensively investigated and correlated with the inactivation mechanisms, which are directly related to the generation of HO•.^{13,16}

The final H₂O₂ concentration measured was 6.13 mg/L for photo-Fenton and 7.45 mg/L for solar/H₂O₂. In photo-Fenton, H₂O₂ (20 mg/L) was added at the beginning and after 60 min of the experiment to balance the consumed H₂O₂ during the process. This residual concentration (<20 mg/L) can be considered innocuous for the crops, as their growth is not compromised, as observed during the experiments. Even, the spontaneous H₂O₂ breakdown in the natural water to water and oxygen will continue happening and decreasing the residual H₂O₂.^{18,26,27} Moreover, the residual presence of an oxidizing agent in the solar treated effluent could be beneficial for avoiding a possible bacterial regrowth (no regrowth was observed in our study) during the post-treatment water storage.²⁸

3.1.2. Organic Microcontaminants Removal. The concentration of OMCs (commonly found in UWW) detected in secondary effluents and solar treated effluents by solar/H₂O₂ and photo-Fenton treatment and their removal percentages are shown in Table 1. The results revealed the presence ($>LOQ$) of 34 OMCs out of the 74 investigated belonging to different classes of pharmaceutical compounds.

The OMC concentrations obtained in secondary effluents ranged from 7 to 6897 ng/L with a total load of 14 832 ng/L, highlighting the high average concentration of 4-FAA (6541 ng/L), 4-AAA (3590 ng/L), atenolol (681 ng/L), hydrochlorothiazide (593 ng/L), and gemfibrozil (573 ng/L). These results are in line with previously reported results^{21,22} and confirm the common presence of OMCs, which remain in water after physical and conventional biological treatments due to their high water stability and solubility.²⁹

Considering the total load of the detected OMCs (14 832 ng/L), solar/H₂O₂ and solar photo-Fenton showed a removal efficiency of 56% and 66%, respectively. Looking at specific OMCs, 10 of them were completely removed using both treatments (final concentration $< LOQ$); whereas 9 and 6 OMCs were degraded above 70% by H₂O₂/solar and solar/photo-Fenton, respectively. In the case of hydrochlorothiazide, metronidazole, mepivacaine, sulfamethoxazole, sulfapyridine, and trimethoprim, H₂O₂/solar was more efficient than photo-Fenton, whereas the opposite happened in the rest of the OMCs.

Table 2. Detection of Pathogens on Lettuce and Radish Crop Irrigated with Secondary Effluents and Solar Treated Effluents^a

crop sample	UWW sample	total coliforms ^b	<i>E. coli</i> ^b	<i>Salmonella</i> spp. ^b	<i>Enterococcus</i> spp. ^b
Lettuce					
Leaves	secondary effluent	15/15 (400)	5/15 (200)	15/15 (200)	9/15 (200)
	treated (H ₂ O ₂ /solar)	3/15 (200)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	treated (photo-Fenton)	3/15 (200)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
Soil	secondary effluent	15/15 (15500)	15/15 (1200)	15/15 (200)	0/15 (<LOD)
	treated (H ₂ O ₂ /solar)	11/15 (900)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	treated (photo-Fenton)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
Radish					
Leaves	secondary effluent	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	treated (H ₂ O ₂ /solar)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	treated (photo-Fenton)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
Fruits	secondary effluent	10/15 (4400)	10/15 (3900)	15/15 (200)	15/15 (23300)
	treated (H ₂ O ₂ /solar)	1/15 (1800)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	treated (photo-Fenton)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
Soil	secondary effluent	12/15 (6300)	12/15 (400)	15/15 (200)	0/15 (<LOD)
	treated (H ₂ O ₂ /solar)	10/15 (400)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	treated (photo-Fenton)	1/15 (100)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)

^a(<LOD): below limit of detection: 20 CFU/100 mL. ^bNumber of positive detected samples/total samples analyzed, that is, 15. Numbers in brackets show the maximum concentration in CFU/mL detected.

These results can be explained by the mechanisms involved in the OMCs degradation in both processes. In the case of solar/H₂O₂, the partial removal of OMCs is due to the oxidation by H₂O₂ in combination with solar photons. Although the generation of HO• under solar light can be discarded, as this process requires photons with wavelengths under 290 nm, which are practically nonexistent in the solar spectrum at the Earth's surface,¹² the generation of a small amount of HO• cannot be completely discarded considering the chemical complexity of the water matrix (secondary effluents) investigated, which may account for the OMCs degradation obtained in this work. The effect of H₂O₂ in the dark at this concentration over the OMCs can be also discarded, as confirmed by previous research.^{30–33}

Previous investigations in similar conditions confirm similar degradation rates of pharmaceuticals in UWW. Ferro et al. (2015)¹⁸ used solar/H₂O₂ for inactivation of antimicrobial resistant *E. coli* and *E. faecalis* and for the removal of spiked pharmaceuticals (100 µg/L). Carbamazepine, flumequine, and thiabendazole were removed at 36.9%, 68.3%, and 99.9% within 5 h of treatment, respectively. Moreira et al. (2018)³⁴ reported the removal of spiked pharmaceuticals (100 µg/L) in UWW, 40% carbamazepine, 45% sulfamethoxazole, after 5 h, and 100% diclofenac in 3 h of solar exposure. Higher antibiotic concentrations have also removed partially in UWW, as reported by Rizzo et al., (2018),³⁰ where, for example, 71% removal of chloranphenicol was attained with a solar UV dose of 1173 kJ/L. Nevertheless, the initial pharmaceutical concentration (25 000 µg/L) in this work³⁰ is much higher than common amount of OMCs usually found in UWW samples, from 0.002 to 6.9 µg/L, as shown in our results (Table 1).

Conversely, for solar photo-Fenton, the well-known generation of HO• is responsible for a higher oxidant capacity, although limited degradation was obtained. Recent studies report on the limited efficiency of “mild photo-Fenton”, that is, mM concentrations of reagents and near neutral pH, for the degradation of OMCs (most cases with spiked pollutants in real WW samples).^{30,34} They claim the main reason is the

formation of iron sludge due to the precipitation of iron hydroxide at neutral pH. For example, Moreira et al. (2018) report degradations of only 20% for carbamazepine and sulfamethoxazole, and 100% for diclofenac.³⁴

The present contribution reports, for the first time, on the disinfection and degradation of OMCs in real UWW effluents by solar/H₂O₂ and solar photo-Fenton at a pilot scale. Both solar treatments reduce more than 55% the total OMCs load determined in the effluents as well as the selected microbial pathogens to below the detection limit (2 CFU/mL). The lower chemicals consumption for solar/H₂O₂ makes this process more suitable for the reuse application under study. Further research is needed to improve the disinfection results to meet the irrigation quality criteria-restricted use (<1/100 CFU/mL).

3.2. Microbiological Assessment of Irrigated Crops.

The microbiological results obtained for crops irrigated with mineral water (negative control) showed, as expected, negative results in all analyzed samples, that is, the absence of bacteria in lettuce, radish, radish leaves, and soil samples. Table 2 shows the presence and absence of the selected pathogens in the crop samples irrigated with secondary effluents and solar treated effluents by the two solar processes.

The samples irrigated with secondary effluents showed the presence of all the selected bacteria in 60% of the samples analyzed (leaves, fruit, and soil), with concentrations ≥200 CFU/100 mL. Nevertheless, the radish leaves showed complete absence of all bacteria, which may be explained by the high hydrophobicity of the leaves surface, reducing, therefore, the adhesion of bacteria. Although radish leaves are not eaten by humans, they can be used for animal feed; therefore, control of their quality may be also important. *E. coli* was detected in only 33% of the analyzed lettuce leaves samples, which may be attributed to the low capability of survival of this bacterial strain far from their environmental conditions (water or humidity and nutrients).³⁵ Total absence of *Enterococcus* spp. in soil samples was also observed. This can be explained by the lack of required more complex nutrients for these bacterial strains.³⁶ Moreover, *Enterococcus* spp. has a

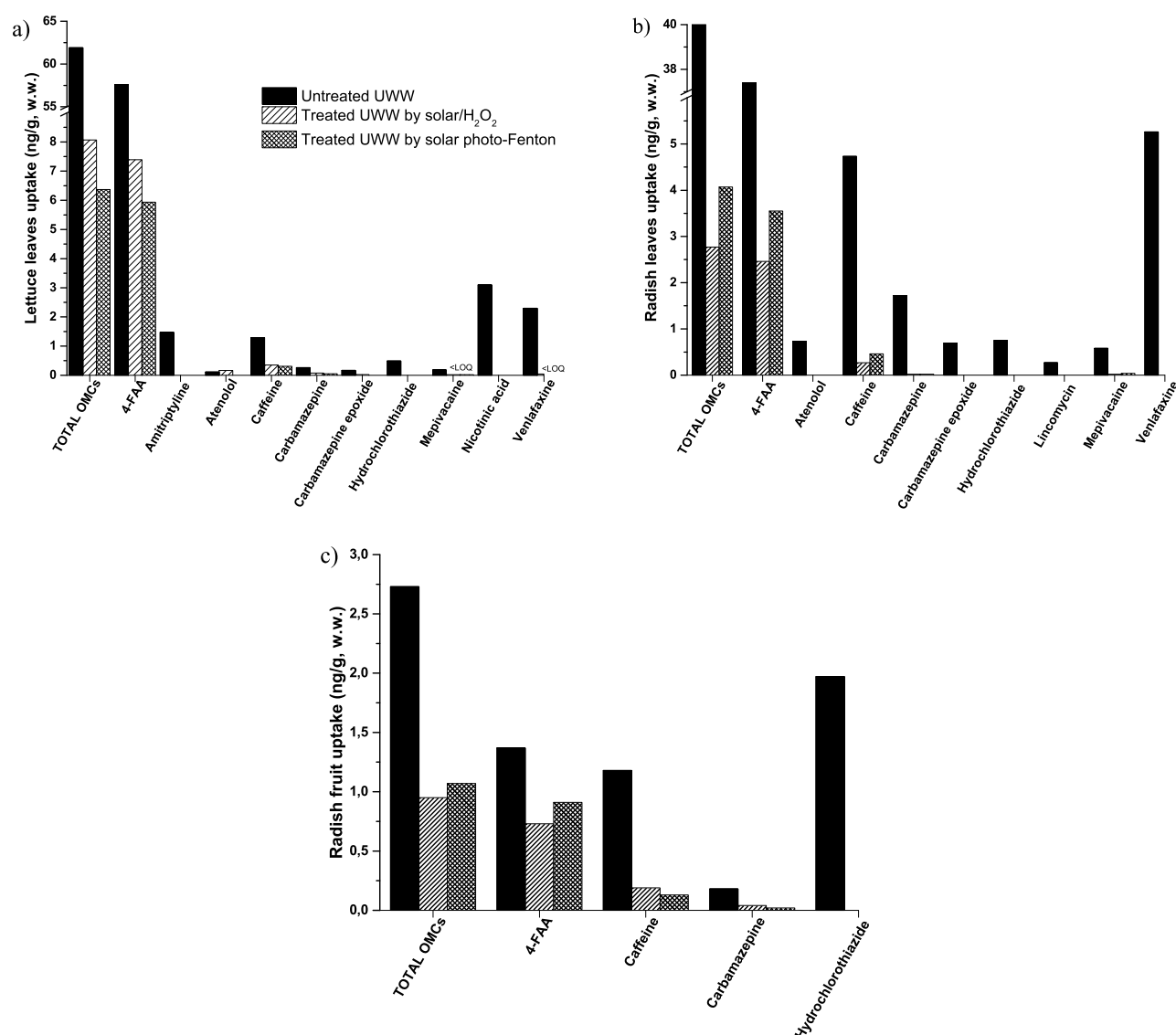


Figure 2. Concentration (ng/g, w.w.) of target analytes found in (a) lettuce, (b) radish leaves, and (c) radish fruit irrigated with secondary effluents and solar treated effluents. Experimental data of secondary effluents obtained from ref 22.

lower survival capacity in soils after watering (rainfall) compared with other Gram-negative bacteria, for example, *E. coli*.³⁷ These results confirmed the high health risk associated with direct reuse of secondary effluents for human consumed crops.^{2,3} According to the guidelines for the restricted reuse of wastewater, the presence of *E. coli* is, in all cases, over the permitted concentration for irrigation of unprocessed raw eaten crops (<100 CFU/100 mL^{6,7} and <1 CFU/100 mL⁸).

The microbiological quality results from the analysis of lettuce leaves, radish fruit and leaves, and soil irrigated with treated effluents by solar/H₂O₂ and solar photo-Fenton show the complete absence of *E. coli*, *Salmonella* spp., and *Enterococcus* spp. (Table 2). Regarding total coliforms, a substantial reduction in lettuce leaves using both treatments was observed. Only 20% of the samples showed positive results in the case of lettuce, that is, 3 out of 15 samples, with a concentration of 200 CFU/100 mL, and none for radish samples, including leaves and fruits. On the other hand, in soil samples, the detection of total coliforms showed that, in the

case of solar/H₂O₂, 10–11 (radish soil-lettuce soil) out of 15 samples were positive, with a maximum concentration of 900 CFU/100 mL; whereas photo-Fenton showed zero (lettuce soil) or one (radish soil) out of 15. These differences observed in soil samples can be attributed to the presence of a certain amount of iron in the soil due to the consecutive irrigation events. The iron concentration measured in water/soil samples used during irrigation protocol (using treated effluents by solar photo-Fenton) revealed a slightly higher concentration (0.46 mg/L) than in those irrigated by solar/H₂O₂ (<0.1 mg/L). This iron in soil may react with the residual H₂O₂ of the solar treated effluent through Fenton and Fenton-like reactions producing a bactericidal effect. Several articles show that Fenton processes have been used in the remediation of pesticide-contaminated soils including pendimethalin, DDT, diuron, 2,4-dichlorophenol, and pentachlorophenol.³⁸ However, some authors claim that Fenton applied for soil remediation is very harmful to the microbes in the soil.³⁸

These results show the absence (with exceptions due to the plant substrate surface) of all pathogens under study when the UWW is disinfected up to the desired level ($<2\text{CFU/mL}$). This is coherent with previous findings; Bichai et al. (2012) reported the improvement of the microbiological quality of lettuce irrigated with treated effluents by solar/ H_2O_2 with consideration of naturally occurring *E. coli*.¹⁷ Ferro et al. (2015) investigated the cross contamination of lettuce by antibiotic resistant *E. coli* and *Enterococcus* spp. in UWW also treated by solar/ H_2O_2 .¹⁸

3.3. Crop Uptake of OMCs. Figure 2 shows the OMC concentrations detected in lettuce and radish crops irrigated with solar-treated effluents. The OMCs uptake in the crops irrigated with secondary effluents—same methodology—have been used for comparison purposes in Figure 2.²² The irrigation with secondary effluents led to the uptake of 12 OMCs in the plant samples assessed (4-AAA, 4-FAA-dipyrone metabolites-, amitriptyline, atenolol, caffeine, carbamazepine, carbamazepine epoxide-carbamazepine metabolite-, hydrochlorothiazide, lincomycin, mepivacaine, nicotinic acid, and venlafaxine). At harvest, the concentrations ranged from 0.11 ng/g (atenolol in lettuce) to 57.6 ng/g (4-FAA in lettuce).²²

The results obtained using solar treated effluents evidence the capability of the solar processes under study to reduce the amount of OMCs available for the crop uptake (Figure 2a). The total OMCs uptake in lettuce leaves when irrigated with secondary effluents, 61.9 ng/g, was reduced to 8.1 ng/g and 6.4 ng/g for solar/ H_2O_2 and solar photo-Fenton, respectively.

The results obtained for the 10 detected OMCs uptaken by lettuce crops irrigated by solar treated effluents are shown in Figure 2a. Significant level of 4-FAA (57.6 ng/g) in lettuce samples was strongly reduced to 7.4 ng/g and 5.9 ng/g when solar/ H_2O_2 and solar photo-Fenton treated effluents were used, respectively. Nicotinic acid (3.1 ng/g), amitriptyline (1.5 ng/g), hydrochlorothiazide (0.49 ng/g), venlafaxine (2.3 ng/g), and mepivacaine (0.18 ng/g) were reduced to levels below the LOQ when treated effluents (via both solar treatments) were used for irrigation.

Regarding the results of radish leaves uptake, 10 OMCs were detected and quantified (Figure 2b). The OMCs with higher concentrations detected were 4-FAA (37 ng/g), venlafaxine (5.2 ng/g), and caffeine (4.7 ng/g), followed by carbamazepine (1.7 ng/g), carbamazepine epoxide, hydrochlorothiazide and atenolol (0.7 ng/g), mepivacaine (0.6 ng/g), and lincomycin (0.3 ng/g). Irrigation with solar treated effluents resulted in undetectable levels for most except for the highly concentrated OMCs, that is, 4-FAA, which decreased the uptake levels up to 2.5 (solar/ H_2O_2) and 3.5 ng/g (photo-Fenton) and for caffeine below 0.5 ng/g with both treatments.

Figure 2c shows the accumulation of only four OMCs (4-FAA, caffeine, carbamazepine, and hydrochlorothiazide) in radish fruit irrigated with UWW. These concentrations are lower than those found in leafy parts (lettuce leaves or radish leaves). This can be due to the fact that OMCs are translocated by the transpiration stream at the leafy parts, which normally presents a greater water flow.¹⁹ The total OMCs found in radish was also reduced in a large percentage by the solar treatments: from 2.7 ng/L (secondary effluents) to 0.9 (65% of reduction) and 1.1 (60% of reduction) for solar/ H_2O_2 and solar photo-Fenton, respectively. However, in radish fruit, only one OMC: the diuretic drug hydrochlorothiazide, was reduced under the limit of quantification in both cases.

The evaluation of the OMCs intake by the roots and their subsequent translocation to other plant organs is a difficult task in which many factors are involved. Biotic parameters, such as physiological state of the plant, surrounded microfauna, the crop's genotype, and other abiotic factors including the typology of soil, the organic matter present, the environmental stress, and even the irrigation method can influence the process.¹⁰ The physical-chemical properties of the micro-contaminants also play an important role in this complex process. The OMCs root uptake and their translocation to aboveground parts of plants is usually evaluated taking into account compound lipophilicity ($\log K_{ow}$), pK_a values, and electrical charge, which are fundamental to understand their transport capabilities. Typically, polar OMCs in neutral species ($-1 < \log K_{ow} < 5$) and cationic analytes in a wide range of plant physiology pH values ($\sim 5.5 < \text{pH} < \sim 7.5$) are more likely to be uptaken by plant roots and then transported through the vascular plant system.³⁹ Nevertheless, anions are more likely to be retained in cell roots due to diverse interactions such as ion trapping and, therefore, less transported.⁴⁰

In SI Table S2, the different lipophilic coefficients ($\log K_{ow}$ for neutral compounds, pH-dependent $\log K_{ow}$, $\log D_{ow}$ for ions), pK_a , and molecular charge (soil pore solution pH 7.5) of the identified OMCs are listed (PubChem Database (www.pubchem.ncbi.nlm.nih.gov),⁴¹). Predominantly, moderate to strong bases ($pK_a \geq 7$) in neutral form (4-AAA, 4-FAA, amitriptyline, atenolol, caffeine, carbamazepine, and carbamazepine epoxide) and weak bases ($pK_a < 6$) in their cationic or partially ionized species (hydrochlorothiazide, lincomycin, mepivacaine, and venlafaxine) were found in leaves and radish roots. Only an acidic analyte in its neutral form was detected in lettuce leaves (nicotinic acid). Anionic forms of OMCs were not detected in any plant tissue. These results are in agreement with the literature, where the higher capability of neutral and cationic molecules to translocate from roots to other plant organs in comparison to anions is demonstrated.^{39,40}

The values of $\log K_{ow}$ for neutral species and $\log D_{ow}$ for cations at pH 7.5, were from -0.62 to 4.92 (SI Table S2). This range covers from low to medium lipophilic values, which represents diverse affinities to lipid tissues and agrees with the reported data.³⁹

Our results revealed that leaves of lettuce and radish showed a higher uptake capacity of OMCs than radish roots, in agreement with other articles.^{22,39,42,43} This behavior has been attributed to the transport properties of the OMCs by the plant transpiration-derived mass flow. Therefore, the OMCs tend to accumulate at higher concentrations in leafy parts than in roots.^{10,43}

In summary, both solar water treatments have demonstrated a high purification capability to reduce both OMCs and pathogens from UWW secondary effluents, as well as to reduce the presence of pathogens ($>$ limit of guidelines) and the uptake of OMCs in lettuce and radish (fruit and leaves) crops irrigated with solar treated effluents.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b00748.

Table S1. Physico-chemical and microbiological characterization of water matrixes used in this study. Table S2.

Physicochemical properties of the OMCs found in real samples: log of the acid dissociation constant (pK_a), log of the octanol–water partition coefficient ($\log K_{ow}$), and log of the pH-dependent octanol–water partition coefficient ($\log D_{ow}$) in the soil solution (pH 7.5) and predominant state. Table S3. Concentration in ng/L of OMCs quantify in secondary effluents before and after solar treatments. Two replicates per solar treatment are shown (PDF)

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Notes

The authors declare no competing financial interest.

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