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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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 60L-Compound Parabolic Collector reactor for irrigation of 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 (Q_{UV}) ranged from 12 to 40 kJ/L (90 min to 5 hours). Solar photo-Fenton showed a reduction of 66% of OMCs and solar/H_2O_2 of 56% in 4 hours 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.

Keywords: Bacterial inactivation, solar photo-Fenton, u~~U~~rban wastewater, plant uptake, organic microcontaminants.

1. Introduction

The increase of water scarcity in arid zones has forced to search alternatives water sources like wastewater for use in sectors like agriculture, the largest water consumer human activity, turning this activity into a widespread practice [1]. The employment of treated wastewater in agriculture involves important health risks, especially for raw consumption crops, due to the presence of several chemical (micropollutants) 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-μg/L. They belong to different chemical families with diverse physic-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 (TP), etc., which in many cases are still unknown [4].

The aforementioned guidelines are based on the control of the concentration of *E. 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/100mL for restricted irrigation according to the World Health Organization [5]; and in other regulations, it is specifically described the *E. coli* concentration for raw crops irrigation for unprocessed purposes, <100 CFU/100mL in Spanish legislation [6] and ISO recommendation [7], and no detectable (< 1 CFU/100mL) by USEPA [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/100mL 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. 10^5 CFU/100mL) require the application of a tertiary treatment to achieve the quality levels required by ~~of~~ the reuse guidelines. The continuous discharge of pharmaceuticals in the environment and their possible incorporation in the human ~~food-chain~~ food chain represents one of the greatest threats to human health. This is also recognised as a clear route to proliferation of antimicrobial resistant microorganisms and genes in the environment [10]. Conventional tertiary treatments (O_3, UV-C, chlorination, etc.) 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 by-products (DBPs) [11].

Solar water treatments may represent a sustainable alternative wastewater treatment for reuse as they ~~it~~ allow~~s~~ minimizing the cost of the treatment using sunlight and they also avoid~~ing~~ the generation of DBPs during the process [12]. Solar advanced oxidation processes (AOPs) have been proven effective alternative tertiary wastewater treatment due to their high effectiveness for disinfection and decontamination based on the capacity to generate hydroxyl radicals ($HO^{\bullet}$), which are non-selective and very powerful oxidants (2.8 eV). Several studies on these solar AOPs show high OMCs degradation ~~of OMCs~~ 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 reagents concentrations (iron and H_2O_2). The process is based on the generation of $HO^{\bullet}$ through a photocatalytic cycle between iron ions, hydrogen peroxide (H_2O_2) and UV-vis radiation [13]. Furthermore, solar disinfection processes based on photo-inactivation of bacteria assisted or not by H_2O_2 (Solar/H_2O_2) 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/H_2O_2 treatment to improve the microbial quality of secondary effluents. These works also demonstrated that irrigated lettuce crops with the solar/H_2O_2 treated wastewater reduced 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/H_2O_2 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/H_2O_2 and solar photo-Fenton at natural water pH is investigated to simultaneously reduce the microbial risk of natural occurring pathogens and the presence of OMCs in real effluents of urban wastewater. 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 (Almería, 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 (Aguas 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 (Supplementary information). Total Organic Carbon (TOC) was analysed using a Shimadzu TOC-5050 (Shimadzu, Japan). Ionic concentrations was measured with a Dionex DX-600 (Dionex, USA) IC system for anions, and with a Dionex DX-120 system for cations. Turbidity was determined using a turbidity-meter (Model 2100 N Hach, USA) and conductivity with a conductivity sensor (GLP31, CRISON, Spain).

2.2. Microbial enumeration and OMCs quantification ~~in water~~

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 µL, 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 permits to distinguish between total coliforms and *E. coli* by the colour of the colonies formed in the agar media, corresponding to 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 Table S1 (Supplementary information).

2.2.2 Organic microcontaminants

74 OMCs (pharmaceuticals and some of their transformation products (TPs)) were screened in this work due to their frequent identification in UWW effluents [20-21]. The analysis was performed by a 1200 LC system (Agilent Technologies, Foster City, CA, USA) -with a XDB C18 50 x 4.6 mm and 1.8 μm particle size analytical column (Agilent Technologies, CA, USA), coupled to a hybrid quadrupole-linear ion trap-mass spectrometer (QqLIT) 5500 QTRAP® from Sciex Instruments (Foster City, CA, USA) 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 ºC. N_2 served as nebulizer, curtain and collision gas. Samples were directly injected (10 µL) and analysed by a multi-residue analytical method previously reported [21]. The OMCs uptake was analysed in the 3 vegetable matrices by a multi-residue method as reported elsewhere [22]. Briefly, it consists on the extraction of OMCs based on a 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-aluminium platform titled at 37°. CPC mirrors are made of highly reflective anodized aluminium (MiroSun, Alanod, Germany), with a concentration factor of 1, 4.5 m^2 of total irradiated surface, and illuminated water volume is 45L over a total volume of 60L. The flow rate used was 30 L/min. pH, dissolved oxygen, and temperature were continuously monitored by several probes inserted on pipes and data were recorded by data acquisition software.

2.3.2. Reagents

Ferrous sulphate heptahydrate ($FeSO_4 \bullet 7H_2O$, PANREAC, Spain) was used as the source of Fe^{2+} for photo-Fenton experiments. Iron concentration was measured in water ~~samples~~ 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. H_2O_2 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}/m^2). 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):

$$Q_{uv,n} = Q_{uv,n-1} + \frac{\Delta t_n UV_{G,n} A_r}{V_t}; \qquad \Delta t_n = t_n - t_{n-1} \qquad \text{Eq (1)}$$

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 radiation on the irradiated area (W/m^2), Δt_n is the experimental time of samples (s), A_r 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) was similar for all experiments. Three solar treatments were investigated: i) solar photo-inactivation, ii) solar/H_2O_2 with 20 mg/L of H_2O_2 and iii) solar photo-Fenton at natural water pH with 10/20 mg/L of Fe^{2+}/H_2O_2. The reagents 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 re-circulated in the dark for homogenisation during 15 min [23]. After that, the reactor was uncovered and 10-mL samples were taken at regular intervals during the solar experiment for bacterial, OMCs and reagents quantification. 2 batches of UWW effluents per solar treatment were sampled and monitored to obtain the average monitoring results. The average of the bacterial inactivation results 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 4 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. Averaged temperature during the experiments was 25±5 ºC 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 relative fast growing, i.e., 8-10 and 4-6 weeks from seeded to harvested, respectively. Both seeds were obtained from a local provider and grown on propylene pots (9x9x10 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. 4 sets of 100 pots of lettuce and radish pots were planted, one per water sample evaluated (negative control, positive control, solar/H_2O_2 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. 2 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 24h, 48h and 72h 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 analysed 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:

i) Leaves of lettuce or radish: each sample weights (3±0.5) g, it is cut in small (<1 cm^2) pieces, then mixed with 20 mL of saline solution, and homogenized in a Stomacher 400 (Seward, UK) at 260 rpm for five min.

ii) Radish fruit: follows the same procedure as leaves, but with samples of (7.0±0.1) g.

iii) Peat: the soil around each plant (1cm around) was collected and weighted to obtain samples of (5.0±0.5) g. Then, they were mixed with 45 mL of saline solution in 150 mL container and homogenized manually.

The enumeration of pathogens in crops and soils were performed following the pour plate counting procedure described in section 2.2 and additionally 5 mL of the generated extract samples was spread in 140mm 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 lettuces and radish (leaves and fruit) were washed with mineral water prior trituration to remove any deposition in the surface of the crop and detect only true CECs absorption [23]. After that, the extraction of the composite samples was made per 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 showed in Figure 1. Water temperature never exceeded 40ºC, excluding therefore the thermal effect (T>45ºC) as a key parameter for bacterial inactivation in these results [24].

Reductions of target microorganisms to concentrations below our limit of detection ~~was~~ 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 photo-inactivation [13-14,18]. Nevertheless, in the case of *Enterococcus* spp, the solar/H_2O_2 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 for solar/H_2O_2 and photo-Fenton, respectively).

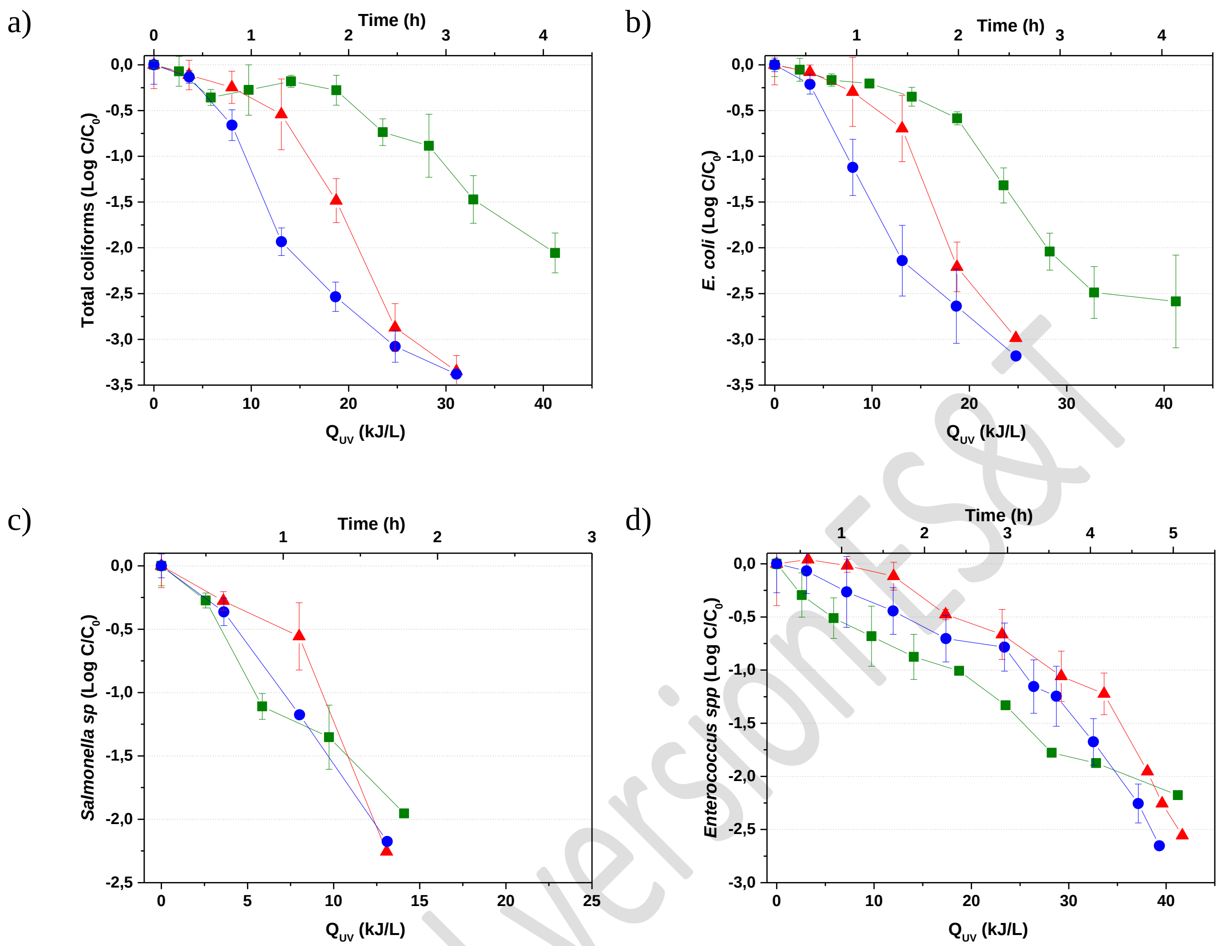


Figure 1. Inactivation profile of (a) Total coliforms, (b) *E. coli*, (c) *Salmonella sp* and (d) *Enterococcus sp.* in UWW effluents by: solar photo-inactivation (-■-), solar/H_2O_2 (20 mg/L) (-●-) and solar photo-Fenton (10/20 mg/L of Fe^{2+}/H_2O_2) (-▲-).

These results show the capability of both treatments for reducing the microbial load in ~~urban~~ UWW effluents. Similar results have been reported [13, 25]. The mechanisms explaining this behaviour have been already described. In brief, the bactericidal effect of small concentrations (mM range) of H_2O_2 and sunlight is mainly attributed to the oxidative effect of internal photo-Fenton reactions assisted by the internal iron and the diffused H_2O_2 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 $HO^{\bullet}$ [13,16].

The final H_2O_2 concentration measured was 6.13 mg/L for photo-Fenton and 7.45 mg/L for solar/H_2O_2. In photo-Fenton, H_2O_2 (20 mg/L) was added at the beginning and after 60 min of the experiment to balance the consumed H_2O_2 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 H_2O_2 breakdown in the natural water to water and oxygen will continue happening and decreasing the residual H_2O_2 [18, 26-27]. Moreover, the residual presence of an oxidizing agent in the solar treated effluent could be beneficial to 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 74 OMCs (commonly found in UWW) detected in secondary effluents and solar treated effluents by solar/H_2O_2 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 OMCs concentration obtained in secondary effluents ranged from 10 to 6897 ng/L with a total load of 14832 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].

Table 1. Organic microcontaminants (OMCs) detected in secondary effluents and solar treated effluents. OMCs with a100% degradation are presented in bold.

Compound	Concentration range in secondary effluents (ng/L)	Average in secondary effluents (ng/L)	Degradation (%) solar/H_2O_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)		**14832**	**56%**	**66%**

LOQ: Limit of quantification. (-): No data.
*Metabolites of metamizole: 4-AA (4-Aminoantipyrine); 4-AAA (N-acetyl-4-aminoantipyrine); 4-FAA (N-formyl-4-aminoantipyrine); 4-MAA (N-methyl-4-aminoantipyrine).

Considering the total load of the detected OMCs (14832 ng/L), solar/H_2O_2 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); while 9 and 6 OMCs were degraded above 70% by H_2O_2/solar and solar/photo-Fenton, respectively. In the case of hydrochlorothiazide, metronidazole, mepivacaine, sulfamethoxazole, sulfapyridine and trimethoprim, H_2O_2/solar was more efficient than photo-Fenton, while the opposite happened in the rest of the OMCs.

These results can be explained by the mechanisms involved in the OMCs degradation in both processes. In the case of solar/H_2O_2, the partial removal of OMCs is due to the oxidation by H_2O_2 in combination with solar photons. Although the generation of $HO^{\bullet}$ under solar light can be discarded, as this process requires photons with wavelengths under 290 nm, which are practically inexistent in the solar spectrum at the Earth´s surface [12], the generation of a small amount of $HO^{\bullet}$ 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.

Previous investigations in similar conditions confirm similar degradation rates of pharmaceuticals in UWW. Ferro et al. (2015) [18] used solar/H_2O_2 for inactivation of antimicrobial resistant *E. coli* and *E. faecalis*, and the removal of spiked pharmaceuticals (100 μg/L). Carbamazepine, flumequine and thiabendazole were removed 36.9%, 68.3% and 99.9% within 5 hours of treatment, respectively. Moreira et al. (2018) [30] reported the removal of spiked pharmaceuticals (100 μg/L) in UWW, 40% carbamazepine, 45% sulfamethoxazole, after 5 hours, and 100% diclofenac in 3 hours of solar exposure. Higher antibiotic concentrations have also removed partially in UWW, as reported by Rizzo et al., (2018) [31], 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 work [31] is much higher than the commonly 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 $HO^{\bullet}$ is responsible for a higher oxidant capacity, although limited degradation was obtained. Recent studies report on the limited efficiency of 'mild photo-Fenton' – i.e. mM concentrations of reagents and near neutral pH - for the degradation of OMCs (most of cases with spiked pollutants in real WW samples) [30-31]. They claim as the main reason 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 [30].

The present contribution reports for the first time on the disinfection and degradation of 74 OMCs in real UWW effluents by solar/H_2O_2 and solar photo-Fenton at pilot scale. Both solar treatments reduce more than 55% the total OMCs load determined in the effluents as well as the selected microbial pathogens below to the detection limit (2 CFU/mL). The lower chemicals consumption for solar/H_2O_2 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 analysed samples, i.e., 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 ~~untreated~~ 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 analysed (leaves, fruit and soil), with concentrations ≥ 200

CFU/100mL. 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 human 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 analysed 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) [32]. 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 bacteria strain [33]. Moreover, *Enterococcus spp*. has a lower survival capacity in soils after watering (rainfall) compared with other gram-negative bacteria, i.e. *E. coli* [34]. 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/100mL [6-7] and < 1 CFU/100mL [8]).

The microbiological quality results from the analysis of lettuce leaves, radish fruit and leaves, and soil irrigated with treated effluents by solar/H_2O_2 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 the 20% of the samples showed positive results in the case of lettuce, i.e., 3 out 15 samples, with concentration of 200 CFU/100mL, 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/H_2O_2, 10-11 (radish soil-lettuce soil) out of 15 samples were positive, with a maximum concentration of 900 CFU/100mL; while 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/H_2O_2 (< 0.1 mg/L). This iron in soil may react with the residual H_2O_2 of the solar treated effluent thought Fenton and Fenton-like reactions producing a bactericidal effect. Several articles show that Fenton processes have been used in the remediation of pesticides contaminated soils including pendimethalin, DDT, diuron, 2,4-dichlorophenol and pentachlorophenol [35]. However, some authors claim that Fenton applied for soil remediation is very harmful to the microbes in the soil [35].

Table 2. Detection of pathogens on lettuce and radish crop irrigated with secondary effluents and solar treated effluents.

Crop sample	UWW sample	Total coliforms*	*E. coli**	*Salmonella spp**	*Enterococcus spp**
Lettuce					
Leaves	secondary effluent ~~Untreated~~	15/15 (400)	5/15 (200)	15/15 (200)	9/15 (200)
	Treated (H_2O_2/solar)	3/15 (200)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	Treated (PhotoFenton)	3/15 (200)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
Soil	secondary effluent ~~Untreated~~	15/15 (15500)	15/15 (1200)	15/15 (200)	0/15 (<LOD)
	Treated (H_2O_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 ~~Untreated~~	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	Treated (H_2O_2/solar)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)
	Treated (PhotoFenton)	0/15 (<LOD)	0/15 (<LOD)	0/15 (<LOD)	0/15(<LOD)
Fruits	secondary effluent ~~Untreated~~	10/15 (4400)	10/15 (3900)	15/15 (200)	15/15 (23300)
	Treated (H_2O_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 ~~Untreated~~	12/15 (6300)	12/15 (400)	15/15 (200)	0/15 (<LOD)
	Treated (H_2O_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)

* Number of positive detected samples / Total samples analized, i.e., 15. Numbers in brackets show the maximum concentration in CFU/mL detected.
(< LOD): below limit of detection: 20 CFU/100mL.

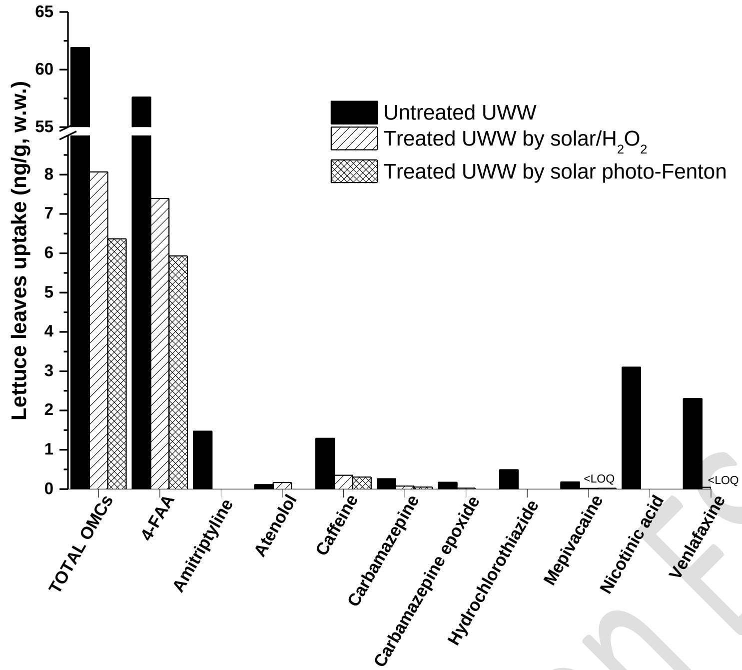
These results shows the absence (with exceptions due to the plant substrate surface) of all pathogens under study when the UWW is disinfected up to the desired level (< 2CFU/mL). This is coherent with previous findings; Bichai et al (2012) [17] reported the improvement of the microbiological quality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

3.3 Crop uptake of OMCs

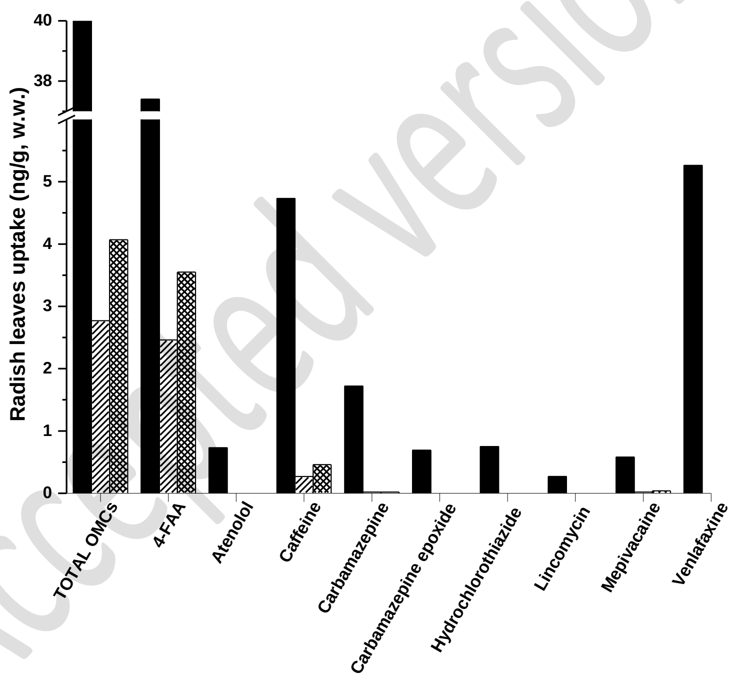
Figure 2 shows the OMC concentrations detected in lettuce and radish crops irrigated with solar treated effluents. The OMCs uptake in the crops irrigated with secondary effluents--same methodology- have been used for comparison purposes in Figure 2 [22]. The irrigation with secondary effluents led to 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 crops uptake (Fig 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.

a)


65
60
55
8
7
6
5
4
3
2
1
0
Lettuce leaves uptake (ng/g, w.w.)
Untreated UWW
Treated UWW by solar/H_2O_2
Treated UWW by solar photo-Fenton
<LOQ
<LOQ
TOTAL OMCs
4-FAA
Amitriptyline
Atenolol
Caffeine
Carbamazepine
Carbamazepine epoxide
Hydrochlorothiazide
Mepivacaine
Nicotinic acid
Venlafaxine

b)


40
38
5
4
3
2
1
0
Radish leaves uptake (ng/g, w.w.)
TOTAL OMCs
4-FAA
Atenolol
Caffeine
Carbamazepine
Carbamazepine epoxide
Hydrochlorothiazide
Lincomycin
Mepivacaine
Venlafaxine

c)

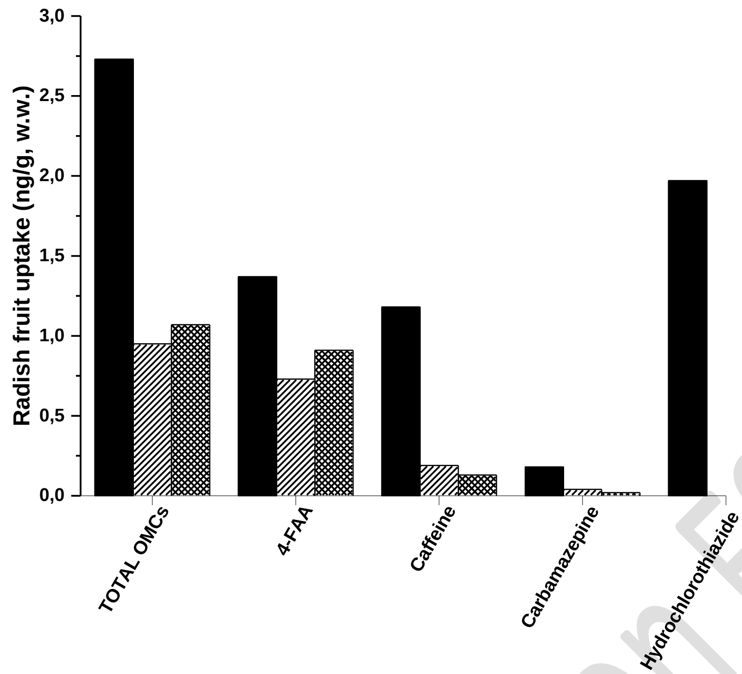


Figure 2. Concentration (ng/g, w.w.) of target analytes found in a) lettuces, b) radish leave and c) radish fruit irrigated with secondary effluents and solar treated effluents. Experimental data of secondary effluents obtained from [22].

The results obtained for the 10 detected OMCs uptake 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) was used for irrigation.

Regarding the results of radish leaves uptake, 10 OMCs were detected and quantified (Fig. 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 made undetectable the levels of most of them, except for the highly concentrated OMCs, i.e. 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 4 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 uptake 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 micro-fauna, 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 physic-chemical properties of the microcontaminants play also 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

($\sim 5.5 < pH < \sim 7.5$) are more likely to be uptaken by plant roots and then transported through the vascular plant system [36]. Nevertheless, anions are more likely to be retained in cell roots due to diverse interactions such as ion trapping and, therefore, less transported [37].

In 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), 38]. Predominantly, moderate to strong bases ($pKa \geq 7$) 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 [36-37].

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 (Table S2). This range covers from low to medium lipophilic values, which represents diverse affinities to lipid tissues and agrees with the reported data [36].

Our results revealed that leaves of lettuce and radish showed a higher uptake capacity of OMCs than radish roots, in agreement with other articles [22,36,39-40]. 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 [10,40].

In summary, both solar water treatments have demonstrated a high purification capability to both reduce the initial load (OMCs and microbial pathogens) of 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.

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Supporting Information.

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 (pKa), log of the octanol-water partition coefficient (log Kow) and log of the pH-dependent octanol-water partition coefficient (log Dow) in the soil solution (pH=7.5) and predominant state

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