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Soil and Water Management Factors That Affect Plant Uptake of Pharmaceuticals: A Case Study

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Abstract: Water and food security are of global concern. Improving knowledge on crops' potential uptake of pharmaceutical compounds (PhCs) is necessary to guarantee consumer health and improve the public's perception of reclaimed water reuse. This study aimed to determine how water management (bottom-up applied for being supplied by Subsurface Drip Irrigation) and the plant rhizosphere effect on the uptake of PhCs. Five PhCs were mixed: atenolol, carbamazepine, diclofenac, ibuprofen and valsartan. A total of 5 treatments were considered: 3 concentrations of PhCs in agricultural volcanic soil: 0.1, 10 and 100 $\mu\text{g}\cdot\text{L}^{-1}$; 0.1 $\mu\text{g}\cdot\text{L}^{-1}$ in sterilized soil; and a blank with three plant replications at 30, 45, and 60 days after emerging. The maximum quantity of the added PhCs was 100 $\mu\text{g}\cdot\text{kg soil}^{-1}$. A variant of the QuEChERS method was followed to extract PhCs from samples. The limits of quantification were between 10 $\text{ng}\cdot\text{L}^{-1}$ and 100 $\text{ng}\cdot\text{L}^{-1}$ in extracts. No PhCs over the limits of detection were detected (0.06–0.6 $\mu\text{g}\cdot\text{kg}^{-1}$ of dry plant sample). Hence, the described water reuse methodology poses a negligible consumer risk, which contrasts with hydroponic systems in which this risk has been shown. The results are discussed in terms of the effects of irrigation system, water management and the soil-plant barrier.

Keywords: pharmaceuticals; plant uptake; reclaimed water; soil; Subsurface drip irrigation



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1. Introduction

Water and food security are of global concern and key to fulfilling Sustainable Development Goals [1]. Water scarcity is the main factor that affects agriculture in arid and semiarid regions, and is strongly influenced by climate change trends. Reclaimed water (RW) irrigation can significantly contribute to strengthening resilience and adapting to climate change capabilities by enhancing the adaptive capacity to address additional desertification and land degradation risks [2]. As nonconventional water resources are one of the alternatives to alleviate the hydrological imbalance between water use and renewable resource availability [3], reuse is necessary to guarantee economic and environmental sustainability in semiarid regions [4]. Treated wastewater reuse can be considered a reliable water supply, and is quite independent of seasonal drought and weather variability that is able to cover water demand peaks [5].

The following RW irrigation barriers have been identified in Europe: environmental and health risks, water treatment cost, and the public not readily accepting treated water-irrigated products [6]. One of these environmental and health risks could be emerging pollutants, which are usually detected at low concentrations and are practically ubiquitous [7] given their marked persistence because, despite their fast degradation, they are continuously released to the environment [8,9]. They are also substances for which many aspects of their behavior and effect on the water/soil/rhizosphere/plant/aquifer system

are unknown. To ensure consumer health and improve how the public perceives water reuse, it is necessary to improve knowledge about their potential uptake by crops as wastewater treatment plants (WWTPs) are not capable of eliminating all (100%) these substances. Indeed, numerous pharmaceutical compounds (PhCs) and their metabolites have been detected in RW because they are not completely removed by conventional treatment technologies [10–14]. Estevez et al. [15] determined low concentrations of carbamazepine and atenolol in desalinated RW. Later [16,17], the continuous presence of other emerging pollutants and pesticide waste was also determined, even in deep groundwater.

Wastewater has been the most widely studied aquatic matrix given the wide-ranging PhCs that reach wastewater and the subsequent implications of PhCs when they enter the environment because they are not completely removed. This issue has been identified not only by the scientific community but also by some legislators such as the European Commission [18]. Since 2018, the Watch Lists of the Water Framework Directive, which include organic compounds susceptible to analysis by Member States, have included several pharmaceuticals. They include anti-inflammatories (for example, diclofenac), some antibiotics (for example, amoxycillin or ciprofloxacin [18]), as well as antifungal agents [19]. The new EU regulation on minimum water reuse requirements for agricultural irrigation purposes was published in 2020 [5]. As emerging pollutants are not regulated by this reuse regulation, predicting the translocation of organic pollutants to plants is crucial to ensure the quality of agricultural goods and to assess the risk of human exposure via the food web [20]. Of all the EU reuse regulation indications, drip irrigation systems are considered the safest. Subsurface drip irrigation (SDI) stands out, in which reuse safety relies more on management than on the water treatment level [21,22]. SDI systems, in which water moves by differential water potentials can, therefore, not only modify the distribution of dissolved substances along the soil profile [23] but also lower the concentration of emerging pollutants, which may reach the rhizosphere.

The possibility of emerging pollutants entering the food chain is one of the highest risks perceived by the population. It also acts as a barrier to commercialize the products irrigated with RW because the public does not readily accept treated water-irrigated products [6]. The uptake process by roots from soil depends on pollutants' chemical speciation, plant species, soil type and water management. Recent studies have shown the effect of micropollutant properties on their ability to be absorbed by roots, and to translocated and bioconcentrated in the rest of the plant [24]. Indeed, very few studies mention the uptake of pharmaceuticals in field experiments [25,26] under similar conditions to those of agricultural practices, apart from hydroponic ones, where roots come into direct contact with pharmaceutical solutions [27]. Christou et al. [28] point out the need to study the uptake of pollutants by plants during agricultural practices. Understanding the uptake of emerging pollutants in semiarid regions is an essential challenge, where plants are frequently irrigated by drip irrigation systems and contaminants emerge in the environment as multicomponent mixtures.

The aim of this study was to determine the effects of both frequent water management from bottom- to up-irrigated and plant rhizosphere uptake of a mixture of PhCs. Studying several pharmaceuticals at environmental concentrations and much higher ones will point out soil/plant capacity to act as a barrier, and to assess the potential risk of consuming plants irrigated by SDI.

2. Materials and Methods

2.1. Experimental Conditions and Sample Collection

The simulation experiment was conducted in a greenhouse of “Granja Agrícola Experimental del Cabildo de Gran Canaria” in June and July 2018, with a mean of 10.6 h of sunlight. Outside the greenhouse, the mean parameters measured by an Automatic Weather Station were: temperature of 19.4 °C (22.1–17.7), relative humidity of 76.4%, 3.8 mm ET and net radiation of 12.3 (MJ·m⁻²). Plastic pots (200 × 140 × 200 mm, Ø_{sup}, Ø_{inf} and height, respectively) were filled with a composite soil sample without sieving. These 4.54-L

pots were filled with 5 kg of soil to grow alfalfa. This experiment was run with sterilized and non-sterilized soil. Air-dried soil was autoclaved at 121 °C at 1.1 atm for 20 min and for 3 days. Between each autoclaved cycle, the soil was incubated at 25 °C. Ten alfalfa seedlings were planted in each pot. After emerging (1 week after seeding), alfalfa plants were irrigated using water with a mixture of PhCs (Table 1). A total of 5 different treatments were carried out:

- T0: The control treatment: untreated soil and irrigation with conventional water;
- T1: Nonsterilized soil and irrigation with conventional water and a mixed concentration of each PhC ($0.1 \mu\text{g}\cdot\text{L}^{-1}$);
- T2: Nonsterilized soil and irrigation with conventional water and a mixed concentration of each PhC ($10 \mu\text{g}\cdot\text{L}^{-1}$);
- T3: Nonsterilized soil and irrigation with conventional water and a mixed concentration of each PhC ($100 \mu\text{g}\cdot\text{L}^{-1}$);
- T4: Sterilized soil and irrigation with conventional water and a mixed concentration of each PhC ($0.1 \mu\text{g}\cdot\text{L}^{-1}$).

Table 1. Total quantity of the water and selected PhCs added to each pot during the experiment.

Days	Total Water (L)	Added PhCs ($\mu\text{g kg}^{-1}$ Soil)		
		T1/T4 ($0.1 \mu\text{g}\cdot\text{L}^{-1}$)	T2 ($10 \mu\text{g}\cdot\text{L}^{-1}$)	T3 ($100 \mu\text{g}\cdot\text{L}^{-1}$)
30	2.65	0.053	5.3	53
45	3.95	0.079	7.9	79
60	5.05	0.101	10.1	101

The concentrations of PhCs were set by considering that the lowest concentration ($0.1 \mu\text{g}\cdot\text{L}^{-1}$) was above the environmental concentrations reported in other studies. Accordingly, the average concentration previously detected in the desalinated RW used on the Gran Canaria Island (Spain) was $10 \text{ ng}\cdot\text{L}^{-1}$ and the maximum values of frequent pharmaceuticals were $695 \text{ ng}\cdot\text{L}^{-1}$ [15]. Moreover, higher concentrations of $10 \mu\text{g}\cdot\text{L}^{-1}$ and $100 \mu\text{g}\cdot\text{L}^{-1}$ were selected by assuming that both were above the worst case of semiarid region scenarios, as considered by Beltrán et al. [29].

Five pots were set as the total pool of each treatment. As three pots were always analyzed, there were always enough replicates available to compensate for any failures. Ten plants were grown in each pot. A total of 3 plant replications were sampled from each pot per treatment at 30, 45 and 60 days after emerging. The last sample coincided with the harvest interval of this species in warm regions.

Water was applied bottom-up on a small plastic plate placed under the pot using a syringe. As water was added, it was moving by capillary rise, exactly as it moves in SDI. The dose was varied at day 3 per week between 150 mL and 300 mL per pot depending on the weather conditions. The total water and quantity of PhCs that were added to each pot during the experiment are presented in Table 1.

After harvest at 30, 45 and 60 days, the whole alfalfa stems and leaves were carefully frozen until plant samples were lyophilized by a vacuum-freeze dryer for 48 h at 0.16 mbar and -48°C . The lyophilized plant samples were then ground and stored at -80°C .

2.2. PhCs

Some PhCs, previously detected in both RW and groundwater as previously mentioned, were selected for this study. Their characteristics appear in Table 2. As shown, the selected PhCs had different lipophilicity (octanol water partition coefficient: K_{ow}), solubility in water and acid ionization constant (pK_a) values.

Table 2. Identifiers, pharmaceutical class and physico-chemical characteristics of the target PhCs.

PhC	CAS	Pharmaceutical Class	Solubility (mg·L ⁻¹) in H ₂ O 25 °C	Log K _{OW} *	pKa *
Atenolol (ATE)	29122-68-7	Beta blocker. antihypertensive, antianginal	13.3	0.16	9.6
Carbamazepine (CBZ)	298-46-4	Anticonvulsant and analgesic	18	2.45	13.9
Diclofenac sodium salt (DIC)	15307-79-6	NSAID (nonsteroidal anti-inflammatory drug)	2.37	4.51	4.15
Ibuprofen sodium salt (IBU)	31121-93-4	NSAID	25	3.97	4.9
Valsartan (VAL)	137862-53-4	Antihypertensive	1.4	4.00	3.6

* Log K_{OW} and pKa values obtained from <https://pubchem.ncbi.nlm.nih.gov/> (accessed on 23 March 2022).

2.3. Soil Characterization

Table 3 presents the initial conditions of the main properties of the clay soil used in this experiment. Total Carbon (TC, %) and Total Nitrogen (TN, %) were determined by dry combustion with a LECO TruMac CN 2000 analyzer. Organic Matter values were indirectly deduced by applying the ‘Van Bemmelen’ factor of 1.724 to Organic Carbon (OC) after subtracting Inorganic Carbon (measured by a soil calcimeter) from TC. Soluble salts were estimated by electrical conductivity EC1:5 (soil:water ratio; dS·m⁻¹). Available nitrate was determined by soil extraction at the 1:5 ratio with 0.01 M calcium chloride, and was analyzed by ionic chromatography. Available soil P (mg·kg⁻¹) was determined by sodium bicarbonate extraction according to the method of Olsen and Sommer [30] and the UV-spectrophotometry molybdenum blue method of Murphy and Riley [31]. Exchangeable cations (K, Ca, Mg and Na, meq·100 g⁻¹) were extracted with buffered 1 M ammonium acetate, pH 7, B in hot water (mg·kg⁻¹). Metals (Fe, Cu, Mn and Zn, mg·kg⁻¹) were DTPA-extracted, pH 7. They were all analyzed by ICP-OES. Soil texture was determined by the Bouyoucos method [32]. Andic properties were characterized by Phosphate retention and Oxalate-extractable Al and Fe (P retention and Alo, Feo), determined according to Blakemore et al. [33]. All the analyses were determined at the Laboratorio Agroalimentario del Cabildo de Gran Canaria.

Table 3. Relevant properties of the studied soil.

pH	EC1:5	OM	TN	C/N	Nitrate	P	K	Ca	Mg	Na	B	Cu	Fe	Mn	Zn
	dS m ⁻¹		%		mg kg ⁻¹			meq 100 g ⁻¹				mg kg ⁻¹			
8.14	1.50	3.64	0.24	8.8	53	54	4.7	23.8	10	7.2	9.6	1.8	3.9	22.6	5.64
Sand	Silt	Clay	Pret ⁽¹⁾	Alo+ $\frac{1}{2}$ Feo ⁽²⁾											
			%												
20.2	33	46.8	24.35	0.32											

⁽¹⁾ Pret: P retention ⁽²⁾ amorphous Al and Fe.

2.4. Chemicals and Solvents

The reference standards (purity, ≥98%) for the quantitative analysis of all the compounds listed in Table 2 were purchased from Sigma-Aldrich. Isotopically labeled compounds (atenolol-d7 and carbamazepine-d10) were used as internal standards and were

supplied by Sigma-Aldrich. The individual standard solutions of the targeted compounds were prepared at concentrations between 1000 and 4000 mg·L⁻¹ in methanol (MeOH) and stored at -20 °C in the dark. Working standard solutions were prepared daily by the dilution of standard solutions in MeOH:water (10:90, v/v).

The HPLC-methanol used in the extraction procedure was obtained from Sigma-Aldrich. Sodium sulfate (Na₂SO₄), sodium chloride (NaCl), trisodium citrate dihydrate (C₆H₅Na₃O₇·2H₂O) and disodium hydrogen citrate sesquihydrate (C₆H₆Na₂O₇·1.5H₂O), used during the QuEChERS extraction, came from PanReac AppliChem, Merck (Denmark) and Sigma-Aldrich, respectively. The reversed phase silica gel C18 and primary-secondary amine (PSA), employed in the QuEChERS clean-up step were purchased from Supelco (Bellefonte, PA, USA). For the chromatographic analysis, LC/MS-grade acetonitrile (MeCN) and MeOH were supplied by Scharlau (Spain). Formic acid (CH₂O₂, purity ≥ 98%) and ammonium fluoride (NH₄F) were purchased from Merck (Germany).

2.5. Sample Extraction and Clean Up

A variant of the QuEChERS method, based on European Standard Method EN Code 15662, was followed. Briefly, 0.5 g. of the freeze-dried sample was placed in a 50 mL polypropylene centrifuge tube with 10 mL of a MeOH and MeCN mixture (1:1, v/v). Then 0.5 g of NaCl, 2 g of Na₂SO₄, 0.5 g of C₆H₅Na₃O₇·2H₂O and 0.5 g C₆H₆Na₂O₇·1.5H₂O were added. The tube was shaken for 10 min in an orbital shaker at 200 rpm. Then samples were extracted in an ultrasonic bath for 15 min and centrifuged at 3500 rpm for 30 min.

After the extraction step, a clean-up step based on dispersive solid phase extraction, d-SPE was applied. An aliquot of 5 mL of the supernatant was transferred to a polypropylene 50 mL centrifuge tube to be cleaned up by adding 0.750 g of Na₂SO₄, 0.125 g of PSA and 0.125 g of C18. Tubes were shaken in a vortex for 1 min and centrifuged at 3500 rpm for 30 min.

Finally, 1 mL of the supernatant after the clean-up step was transferred to a glass tube and evaporated in a centrifugal vacuum concentrator at room temperature. Once dried, extracts were reconstituted with 1 mL of a 90:10 (v/v) water-methanol mixture. To eliminate particulate after reconstitution, extracts were centrifuged at 13,100 rpm for 5 min and the supernatant was transferred to chromatographic phials.

2.6. The HPLC-MS/MS Separation and Determination Procedure

The quantification of samples was carried out by the method developed by Santiago-Martín et al. [26]. Briefly, samples were analyzed using a 1200 series liquid chromatograph coupled to a triple quadrupole, equipped with an electrospray ionisation interface (ESI). Both were from Agilent Technologies (USA). Analyses were carried out in the positive mode for ATE, CBZ, DIC and VAL. The negative mode was used for IBU. For those compounds determined in the positive mode, a Kinetex biphenyl column (50 × 3 mm, 2.7 µm, Phenomenex) was used, while a Poroshell 120 EC-C18 column (50 × 3 mm, 2.7 µm, Agilent Technologies) was employed for those compounds determined in the negative mode. In both modes, chromatographic separation was performed in the gradient mode at a flow rate of 0.6 mL·min⁻¹. The injection volume was set at 20 µL. [26]. The quantification method showed appropriate instrumental analytical parameters. In line with this, the instrumental limits of quantification (LOQ) were determined as the lowest compound concentration with a signal-to-noise ratio (S/N) that equaled 10 by maintaining the abundance criteria between mass transitions. For the target PhCs, the LOQ were 10 ng·L⁻¹ for ATE and CBZ, and 100 ng·L⁻¹ for DIC, IBU and VAL. Regarding reproducibility, the instrumental relative standard deviations at the LOQ concentrations for the target compounds were below 10% in all cases.

3. Results and Discussion

3.1. Extraction Procedure Validation

By following a similar procedure to that by Beltran et al. [29], validation was performed with the plant samples irrigated with clean water, which gave undetectable concentrations of PhCs. To evaluate any matrix effects, the peak area of 2 deuterated compounds (atenolol-d7 and carbamazepine-d10) were also measured on the external and internal calibration curves. No differences were observed between the calibration curves prepared in pure solvents and sample extracts. Quantification was performed by building internal calibration curves with bulk alfalfa samples irrigated with freshwater without PhCs and fortified with target PhCs after freeze-drying at the same concentrations as the external calibration curves. The linearity of the calibration curves was demonstrated by the correlation factors (R^2) over 0.996. The limits of detection (LOD) and LOQ for the five PhCs were calculated as the concentration that gave a peak with an S/N ratio of 3 and 10, respectively. The instrumental LOQs were $50 \text{ ng}\cdot\text{L}^{-1}$ for CBZ, ATE and their deuterated compounds. LOQs were $100 \text{ ng}\cdot\text{L}^{-1}$ for IBU, DIC and VAL. To control the sensitivity of the chromatography system while determining compounds, a standard of the target analytes was injected regularly in the batch analysis. Blank injections were programmed in the injection batch to confirm the absence of carryover effects during the chromatographic analysis. Extraction recoveries were calculated at four concentration levels for CBZ and ATE: LOQ, 2·LOQ, 10·LOQ and 20·LOQ and LOQ, 5·LOQ and 10·LOQ for DIC, IBU and VAL. Recoveries were over 83% (except for valsartan > 65%). The method's LOQ (MLOQ) were between 0.2 and $2 \text{ }\mu\text{g}\cdot\text{kg}^{-1}$ (dry sample). It is important to highlight that these MLOQ were calculated using the lyophilized samples. As the studied alfalfa had about 80% water, the MLOQ calculated from 0.04 to $0.4 \text{ }\mu\text{g}\cdot\text{kg}^{-1}$ (fresh sample) were similar to the LOQ reported in other studies [26,34]. The repeatability of the methodology was studied by calculating the relative standard deviation (RSD) of the triplicate of each sample at the same concentration levels as the recovery tests. The RSD values were below 15% in most cases.

3.2. Pharmaceuticals in the Plant Samples

Despite the high concentrations used, mostly above the concentrations measured under environmental conditions, the highest value of $100 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ was below the toxic concentration of emerging pollutants; for example, ACT (1 and $5 \text{ mg}\cdot\text{L}^{-1}$) or antibiotics ($1 \text{ mg}\cdot\text{L}^{-1}$), which could affect plant roots [35,36]. Consequently, the grown alfalfa plants were healthy. Emerging pollutants were not detected above the MLOQ in any of the plant samples harvested after 30, 45, and 60 days, or in either the non-sterilized or sterilized soils (Table 4).

Table 4. Concentrations of the target PhCs in the plant samples obtained in the different tested treatments.

Pharmaceutical	Tested Treatments				
	T0	T1	T2	T3	T4
Atenolol, ATN	n.d. ^a	n.d.	n.d.	n.d.	n.d.
Carmabazepine, CBZ	n.d.	n.d.	n.d.	n.d.	n.d.
Diclofenac, DIC	n.d.	n.d.	n.d.	n.d.	n.d.
Ibuprofen, IBU	n.d.	n.d.	n.d.	n.d.	n.d.
Valsartan, VAL	n.d.	n.d.	n.d.	n.d.	n.d.

^a not detected.

These unexpected results indicate several factors that are not generally considered in studies about PhCs uptake by plants, such as soil activity and water management. Besides, potential metabolite products were not quantified and our results focused on parent compounds. However, we cannot rule out the possibility of the transformation of some parent compounds in our study. These transformations are demonstrated by some authors [37].

In most cases, emerging pollutants are detected in plants; for example, CBZ has been previously reported as being stable in soils, and is easily taken up, accumulated and metabolized in plant leaves [38]. Nevertheless, experimental works are generally conducted under hydroponic conditions and very few works have grown plants in soil. Under hydroponic conditions, the characteristics of PhCs are the main factor to govern possible PhCs uptake by plants. Briggs et al. [39] proposed that the uptake of neutral chemicals by plants would imply a maximum translocation at a $\log K_{ow} \sim 1.78$ compared to particularly hydrophobic (high $\log K_{ow}$) and hydrophilic (low $\log K_{ow}$) conditions. Plant uptake depends on both species and compounds [34], and root uptake from soil and/or water is the major pathway [20] for most hydrophobic organic compounds ($\log K_{ow} > 3.0$). As CBZ is hydrophilic in nature ($\log K_{ow} = 2.45$), it appears more in the aqueous phase than being attached to soil particles. Therefore, high CBZ concentrations in pore water may play a crucial role in the marked uptake that many studies indicate. Zhang et al. [36] report organic compounds with $\log K_{ow}$ values between 0.5 and 3 because they may generally and simultaneously display hydrophilic and lipophilic behaviors, which would allow for translocation through the lipid bilayer of cell membranes by facilitating their transfer to plant aerial organs. In fact, it would appear that CBZ uptake is passive and not restricted by root membranes because relatively low CBZ hydrophobicity enables it to be transported by the mass flow from roots and to concentrate in mature older leaves [29]. Accordingly, Kodešová et al. [38] found negative relations between CBZ sorption coefficients in soil and its concentration in roots. The other PhCs studied herein fell within a $\log K_{ow}$ range from 0.16 (ATE, hydrophilic) to 4.51 (DIC, hydrophobic).

The remarkable study by Beltran et al. [29] indicate different bioaccumulation and translocation potentials of ATN, CBZ, and triclosan (TCS) in the aerial organs of three plant species after applying similar PhCs concentrations to those used in our experiment. The above authors report a maximum for CBZ in leaves, which varies as follows: 424.8 ± 153.0 , 142.9 ± 43.2 , and $9.3 \pm 0.7 \text{ ng} \cdot \text{g}^{-1} \text{ w.w.}$ for radish, maize and lettuce, respectively, and with lower concentrations for ATN (10.5 ± 1.9 , 5.7 ± 0.9 and $1.8 \pm 0.8 \text{ ng} \cdot \text{g}^{-1} \text{ w.w.}$, respectively). Their results reveal efficient root uptake for the three PhCs regardless of plant species and fortification levels. Those authors conclude that the characteristics of PhCs and physiological plant variables can be critical for crops irrigated with RW in regions with long dry seasons, high solar incidence and low annual rainfall, as in the Mediterranean rim where plants face high transpiration rates. Although we obtained similar LOD to those reported by Beltran et al., none of the PhCs were detected. A remarkable difference between the experiments in both works was that Beltran et al. [29] used an artificial mixture of sieved soil, mulch and river sand, which they used in weekly irrigations. Those authors did not study the effect of natural soil, the irrigation system and water management which, as our results suggest, can act as governing factors for not detecting uptake by the plants in our experiment.

As we previously mentioned, apart from the characteristics of PhCs and physiological plant variables, adsorption phenomena, biodegradation and root barrier activity in healthy agricultural soils must also be considered and are discussed below.

3.2.1. Biodegradation

Soil sterilization had no apparent effect on PhCs uptake and, thus, no emerging pollutants were detected in the plant samples cultivated in sterilized soil at $0.1 \text{ } \mu\text{g} \cdot \text{L}^{-1}$ of pollutants. Although plant degradation in these samples cannot be ruled out, characteristics of PhCs, soil adsorption phenomena and abiotic degradation, along with the physical root barrier component, could explain the obtained results because soil biota was previously eliminated. Moreover, only $0.1 \text{ } \mu\text{g} \cdot \text{kg}^{-1}$ of each PhC in sterilized soil was added, and this quantity is clearly below the quantity that the soil can retain; this explains why it was not detected in stems and leaves.

Despite CBZ being included for the range of values within which maximum uptake has been frequently detected by many studies in leafy plant parts, it was not detected in

the plant samples of our experiment, not even after applying very high concentrations of PhCs. Consequently, the soil food web can play an important role in the biodegradation and root barrier of micropollutant substances. Eggen and Lillo [40] conclude that both the root system and rhizosphere act as a barrier for micropollutants to enter the plant interior. Besides, plants present diverse mechanisms of response to PhCs. Leitão et al. [35] report an antioxidative response induced by ACT pollution as plants presented significantly increased anthocyanin contents in leaves with both exposure time and ACT concentration.

Although contaminant absorption is driven mainly by diffusion, an energy-dependent symplastic route, constituted by transporters and channels, may also facilitate PhCs uptake [41]. The very interesting study by Zhang et al. [36], who added respiration inhibitors, demonstrate that antibiotics uptake is an active process. They also conclude that, although some substances are unable to enter roots through water channels, their translocation is associated with aquaporin activity. These authors mention that the higher root concentration factor of antibiotics can be obtained in hydroponic experiments and not under realistic soil conditions. Consequently, the high risk associated with hydroponic cultivation questions the suitability of this growing technique when reusing RW.

3.2.2. Effect of Soil Characteristics

The adsorption potential of micropollutants, whose concentration lowers in dissolution, depends on both the characteristics of these compounds and soil properties [42,43], which highlights the type and contents of clay, organic matter, among others. Adsorption strength depends on chemisorption mechanisms and the extent of the soil surface area (texture). Besides, the molecules that access soils and root surfaces are controlled by soil porosity. Volcanic soils, especially Andic soils from recent materials, generally have a very important binding capacity due to both high-surface-area amorphous aluminum-silicates and organo-mineral compounds. Some functional groups of organic molecules (carboxylic, phosphonate, etc.) can have a very high affinity for the $-AlOH$ and $-FeOH$ groups that are common in amorphous aluminosilicates and iron and aluminum oxy-hydroxides. Estevez et al. [44] studied the adsorption behavior of IBU in four volcanic soils from the Canary Islands. They found that sorption maxima varied from 4 to 200 $mg \cdot kg^{-1}$, and K_d values (0.04 – $0.5 L \cdot kg^{-1}$) were related to andic properties (amorphous Al and Fe) and organic matter contents. The lowest S_{max} and K_d values were for loam soil, with only 0.67% OM, and also for the 1:1 S/W ratio, which came close to the field conditions (1:1) in irrigated soils. When comparing these results to the larger added PhCs amounts in this experiment ($0.101 mg \cdot kg^{-1}$, 40-fold lower than the lower S_{max}), and considering irrigation water's upward movement, PhCs can be expected to be adsorbed in soil on their way to the roots, which would decrease plant uptake. Along these lines, Muñoz Carpena et al. [45] report a high adsorption coefficient of glyphosate ($K_d = 307 L \cdot kg^{-1}$) in basaltic soils rich in iron oxides. Although the soil selected for the present study is of volcanic origin (basalt), it has attenuated Andic properties (Table 3), probably because of the age of both parent materials and agronomic practices (blockage of anion sorption capacity).

Another important aspect of PhCs retention in volcanic soils is microporosity. In volcanic soils of banana crops in the French West Indies, allophanic soils (Andosols) retain more chlordecone (organochlorine insecticide) than non allophanic soils containing crystalline clays because of their higher contaminant trapping capacity and, therefore, lower contaminant availability [46]. Those authors put forward the notion that the fractal and tortuous allophane clay microstructure would favor chlordecone retention in soils and hinder its transfer to water and plants.

3.2.3. Effect of Water Management

As mentioned earlier, studies regarding PhCs uptake by plants have been generally conducted under hydroponic conditions, and have rarely considered which irrigation system is involved. Under hydroponic conditions, the characteristics of PhCs are the main factor which governs the transportation and possible uptake by plants. In our study,

irrigation is performed by capillary rise and soil moisture displays periodic variations. Consequently, the soil solution in micropores can intermittently concentrate [47], which can enhance the adsorption of sorptive solutes by soil surfaces and can, thus, not only lower their concentration in solution, but would also limit plant availability.

It is noteworthy that the herein followed irrigation method shares some characteristics with SDI as to the way that water is transported from the source (emitter). Moreover, no saturation conditions were reached at the low water dose applied. This would thus minimize the risk of pollutants being absorbed by plant roots.

3.2.4. Human Exposure

In spite of the large quantities of each PhC being added to soil over the 60-day period ($101 \mu\text{g}\cdot\text{kg}^{-1}$), all of the above-mentioned factors permitted plants to remain clean of PhCs. Therefore, we conclude a negligible risk for animals and humans. Our results indicate that if all consumed crops are grown in soil and irrigated as used by SDI, and despite crops containing the selected PhCs at concentrations as high as $100 \text{ ng}\cdot\text{L}^{-1}$, neither animals nor humans would consume levels above the acceptable daily intake for any of the pharmaceuticals herein selected. Likewise, Beltran et al. [29] suggest a negligible risk for humans when consuming edible plants irrigated with RW samples for which the presence of PhCs was low. However, their study revealed efficient root uptake.

4. Conclusions

The non-detection of PhCs in our study suggests the importance of soil, biota and water management in plants' uptake of PhCs. The irrigation procedure (from bottom-up and as used in SDI), as well as frequency and water quantity, differ from those described in other articles; these factors might explain our infrequent results. Moreover, the high risk of PhCs uptake by plants grown in hydroponic systems questions the suitability of this growing technique when reusing RW.

Considering that plant uptake also largely depends on transpiration rates in arid and semiarid zones, water management and soil characteristics are critical factors to control PhCs for irrigation with treated wastewater.

Further studies are necessary to profoundly understand the effect of irrigation system and water management on the risk for animals and humans when using RW in the presence of PhCs, and to also confirm this promising result, which suggests a very safe procedure for reusing RW by means of SDI.

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