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Mesocosm Evaluation of the Safety of the Use of Reclaimed Water Regarding Emerging Pollutants in Murcia, Spain

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Abstract: The increasing shortage of conventional water for crop irrigation in arid and semiarid regions is encouraging the use of nonconventional water resources. Nevertheless, concern about the presence and possible negative effects of emerging contaminants in reclaimed water can cause individuals to avoid using these water sources. To clarify the guarantees that reclaimed water offers, a mesocosm study was carried out. Lettuce plants were cultivated in pots watered with three different types of water (distilled, reclaimed, and spiked with contaminants of emerging concern). The results showed low concentrations of contaminants in the leachates and plant tissues when the reclaimed water was used. However, their concentrations were high when the pots were watered with the spiked water; this was mainly true for carbamazepine, which reached a maximum of 2982 ng L^{-1} in the leachates and 45.2 ng g^{-1} in the plant roots. In the lettuce watered with the spiked water, carbamazepine was found in the edible part at very low concentrations that did not imply any human risk. Finally, an acute toxicity test was performed on the leachates, which were found to be only slightly toxic in the spiked water. This work indicates that with the current technical improvements in active sludge water treatment, reclaimed water can be used for irrigation without the risk of contamination by contaminants of emerging concern.

Keywords: reclaimed water; irrigation; WWTP; CECs; PPCPs; mesocosm; ecotoxicological assay

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1. Introduction

Due to the problems derived from water scarcity and the competition for resources as necessary as drinking water, there has been a growing interest in the search for new and alternative water sources to reduce the use of clean or potable water for diverse applications in arid and semiarid regions for decades now [1]. As early as 1958, the United Nations Economic and Social Council [2] boosted water reuse by stating that high-quality water should not be consumed in uses that could tolerate lower qualities, such as industrial activities or soil irrigation. In the European Union (EU), the current tendency towards a circular economy has led to further efforts to increase the consumption of alternative water sources. However, at present, only 4% of all the water consumed in the world is reused, and 80% of wastewaters are returned to ecosystems without being treated [3]. These data contrast with the high water demand in regions with a serious water deficit, where the number of cities that are reclaiming water for direct or indirect potable water use is steadily increasing [4]. In European regions, only 2.4% of treated wastewater is reused, while an increase is expected in the near future, mainly in the southern regions [5]. Currently, Spain reuses 11.2% of its water, but there is a great disparity in this reuse between the northern

and southern parts of the country. For example, in the Region of Murcia (SE Spain), 95.6% of the treated wastewater is used for irrigation in agricultural soils [6].

In the Region of Murcia, the annual average precipitation is approximately 300 mm, which is clearly insufficient for the agricultural production in the area as there is a water deficit (the annual evapotranspiration exceeds 700 mm). The use of water from different sources, including desalinated and reclaimed waters, in the agriculture in this area is a key factor for its sustainability [7]. This region reused 117 hm^3 of treated water during 2020 [8] and was the leader in terms of both the use of reclaimed water and agricultural production as it exported 2.5 million tons of fruit and vegetables per year [9]. The use of reclaimed water has become part of the solution to prevent water scarcity and the effects produced by extreme climate events such as droughts [10].

However, the possible presence of the so-called contaminants of emerging concern (CECs) in reclaimed waters has generated certain alarm as this may affect human and environmental health [11–14]. Therefore, these compounds are candidates for inclusion in future regulations regarding reclaimed water reuse due to their ecotoxicity, occurrence in different environmental compartments, etc. Examples from the list of CECs are surfactants, flame retardants, pharmaceutical and personal care products (PPCPs), gasoline additives and their degradation products, biocides, polar pesticides and their degradation products, and various compounds shown to cause or suspected of causing endocrine disruption. Several studies have determined their presence in water [13–16], soil [17,18], and plants [19] exposed to reclaimed water, and these studies have included an ecological risk assessment; additionally, concerns have been raised about agricultural soils receiving sewage sludge as fertilizer [20]. Also, worries persist about possible soil contamination due to the use of reclaimed water [21] and the potential transport of PPCPs to groundwater via runoff and leaching [22].

Most of the previous publications on CECs do not include a complete study of the soil–water–plant system. The best way to study this complex system is through mesocosm studies that can simplify the sampling of plants, soil, and leachates in the laboratory. A similar experimental design was used to evaluate the ecotoxicological effects of applying sewage sludge to an agricultural soil on representative soil organisms [23]. Furthermore, most of the studies consulted used an excessive doping of irrigation water to find out whether the species studied could take up these emerging pollutants; such high concentrations could only come from industrial discharges, not from conventional water purification systems.

The idea of this study is to confirm the hypothesis that the use of regenerated water for agricultural purposes is safe for consumers and the environment. To achieve this, knowledge about the concentrations of CECs in effluents of wastewater treatment plants (WWTP) and the repercussions that could derive from the use of these effluents in crop irrigation is paramount to be able to refute any restriction that may arise regarding their use. The main aim of this work was to assess, with an advisory, predictive, and/or confirmatory nature, the possible consequences or ecotoxicological effects of the use of reclaimed water for irrigation on the soil–organism–plant system. For this, control, regenerated, and spiked (with CECs) water were used to irrigate crop plants in a mesocosm experiment. Then, the CECs' leaching risk, their accumulation in the leaves and roots of the test plants (lettuce), and the CECs remaining in the soil were evaluated. Also, an ecotoxicological evaluation of the regenerated and spiked waters was carried out and compared with the clean control water. The novelty of this study is that by using typical wastewater treatment plant effluents, it allows for the evaluation of the possibility that emerging contaminants can be retained by the plants (the aerial part or root) and in the soil and/or can contaminate groundwater through leaching, which might result in an ecotoxicological risk to organisms that are vulnerable to contamination. Water spiked with CECs at realistic concentrations that could be found in the effluents of WWTP and that are not easily removed was used to determine what could happen in the worst-case scenario; additionally, uncontaminated water was used as a control.

2. Materials and Methods

The mesocosm experiments were designed based on the example of Carbonell et al. [23]. They consisted of plastic pots with a height of 10 cm and an internal diameter of about 12 cm; a hole for drainage was made in the lower part of each pot, where a nylon mesh was placed to prevent soil loss. Each pot had a system for the collection of leachates (a large beaker (600 mL) covered with aluminum foil to prevent light exposure). The pots were filled with unpolluted agricultural soil, and lettuce seedlings were planted in the soil (one plant per pot; Figure 1).

Figure 1. Picture of the mesocosm experiment.

In this type of mesocosm system, the soil–air interface, the water transport, and the kinetics of absorption/degradation are reproduced better than in standard soil bioassays. The system allows for a realistic incorporation of CECs such as PPCPs, which resembles the conditions expected due to agricultural practices involving the use of reclaimed water.

A total of 24 pots were placed in a growth chamber using a completely randomized design with six replicate pots per treatment. The treatments consisted of irrigation with distilled water (CW), reclaimed water (RW), spiked water with 5000 ng L^{-1} of PPCPs (SW), and spiked water with 5000 ng L^{-1} of PPCPs but without a plant (SW-P).

2.1. Chemicals, Soil, Plants, and Waters

A total of five pharmaceutical compounds (one psychiatric drug and four Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)) and a cosmetic preservative were included in the spiked water (Table 1). The selection was based primarily on previous studies [24]. The pharmaceutical compounds selected for the experiment were the anticonvulsant and mood stabilizing drug carbamazepine, four NSAIDs (diclofenac, ibuprofen, ketoprofen, and naproxen), and the cosmetic preservative triclosan (CBZ, DCF, IBU, KTP, NPX, and TCS, respectively).

The spiked concentration of each compound was 5000 ng L^{-1} to facilitate the measurements, although this can be considered severe contamination in treated water. Lower concentrations of PPCPs such as carbamazepine, diclofenac, and naproxen have been previously detected (around 500 ng L^{-1}) in treated wastewater [25]. However, Shenker et al. [26] reported that in Israel, the level of carbamazepine in treated wastewater used for irrigation could be as high as 3000 ng L^{-1}. The sources of the chemicals and the deuterated standards are described in the Supplementary Information S1. Individual stock solutions were prepared in methanol at 500 mg L^{-1} and stored at −20 °C in the dark. An intermediate

combined solution was prepared in methanol with all the chemicals at a concentration of 2500 μg L^{-1}, from which the final dilution was prepared. The spiked water (SW) consisted of distilled water with 5000 ng L^{-1} of each of the selected PPCPs (CBZ, DCF, IBU, KTP, NPX, and TCS).

Table 1. List of target compounds with their Chemical Abstracts Service (CAS) number and net charge at soil pH, water solubility at 25 °C, octanol–water partition coefficients (log K_{OW}), and acid dissociation constants (pK_a).

Family	Compound	CAS Number	Solubility (mg L^{-1})	Family Log K_{OW}	pK_a
Psychiatric drug	CBZ	298-46-4	18	2.45	3.80–15.96
NSAIDs	DCF	15307-79-6	2.37	4.51	4.15
	IBU	15687-27-1	21	3.97	4.91–5.30
	KTP	22071-15-4	51 (22 °C)	3.12	4.45
	NPX	22204-53-1	15.9	3.18	4.15
Biocide	TCS	3380-34-5	10 (20 °C)	4.76	7.9

https://pubchem.ncbi.ninlm.nih.gov/ (accessed on 20 July 2022).

The reclaimed water (RW) was collected from the Murcia-East WWTP (UTMX (ETRS89): 670034, UTMY (ETRS89): 4207420), which includes an active sludge with modified A2O and a disinfection treatment plant running 553451 population equivalents [8]. The modified A2O process consisted of one anaerobic stage, two anoxic stages, and two oxic stages. The reclaimed water contained 25.6, 27.3, and 134 (ng L^{-1}) of CBZ, DCF, and KTP, respectively.

An uncontaminated calcaric fluvisol [27] was taken from the facilities of the Catholic University of Murcia (UTM coordinates: X: 659338; Y: 4206255, SE Spain) for this study. The soil (with a clay loam texture) was collected from the surface (0–20 cm depth), air dried, and passed through a 2 mm sieve before its use in the experiment and the corresponding analyses. The main physicochemical characteristics of the soil were a silt loam texture (29.0% sand, 55.8% silt, 15.2% clay), pH 7.63, Eh 226 mV, 3.1% organic matter content, 0.22% total nitrogen, and 33.0% $CaCO_3$. The soil had a water-holding capacity (WHC) of 24.3%. The pots were filled with 500 g (dry weight) of soil and were placed in a growth chamber under controlled conditions (22/18 ± 2 °C, environmental humidity of 60%, photoperiod of 16 h, and illumination between 250 and 300 μmol m^{-2} s^{-1}). Distilled water was added to the pots to adjust the overall moisture content to 35% of the WHC of the soil. The pots were watered with distilled water every 2 days before planting.

After 14 days of stabilization, the pots were wetted to 80% of their WHC with distilled water, and then a mini romaine lettuce (*Lactuca sativa* var. Jabera) seedling (1 month old), acquired from the Deitana nursery (Murcia, Spain), was placed in each pot. From that moment, the pots were watered every day with 100 mL of the corresponding treatment water. The experiment was carried out for 21 days in the growth chamber under the conditions described above. Then, the plants were harvested, and the different soil and leachate samples were collected.

2.2. Sampling Procedure and PPCPs Analysis

After harvesting the plants, soil samples were taken from the pots, mixed to homogeneity, freeze dried (Freeze Dryer Christ alpha 1–2/LD plus), ground to a fine powder with a porcelain mortar, and then stored at −20 °C until the PPCPs extraction. The lettuce plants were split into the roots and aerial part and rinsed with distilled water; half of the aerial part was weighed (fresh weight and dry weight after 24 h at 60 °C in an aerated oven) and the other half was transferred to liquid nitrogen, freeze dried, ground to a fine powder, and then stored at −20 °C until the PPCPs extraction. The leachate samples (one sample per pot) were divided into two subsamples; one was used to analyze the PPCPs and the other was used to perform an ecotoxicity bioassay with the aquatic crustacean

Thamnocephalus platyurus. At least three replicates of the samples were used to ensure the representativity of the results.

The PPCPs in the soil were extracted by following the method of Martín et al. (2010) [28] with slight modifications. Aliquots (2 g) of lyophilized soil samples were accurately weighed directly in centrifuge tubes (12 mL). Afterwards, the samples were successively extracted with 5 and 2 mL of methanol and 2 mL of acetone. In each extraction step, the sample was vigorously shaken for 30 s, sonicated for 15 min, and centrifuged at 4000 rpm (15.1 g) for 20 min. The supernatants from each extraction step were combined and evaporated to 0.2 mL under a N_2 stream (TurboVap LV concentrator). The extract was diluted to 250 mL with distilled water acidified to a pH of 2 with sulfuric acid and was subjected to a cleanup procedure where the aqueous mixture was loaded onto a hydrophilic–lipophilic balance cartridge (HLB, 60 mg, Dublin, Ireland) that was preconditioned with 3 mL of acetone, 3 mL of methanol, and 3 mL of deionized water acidified to a pH of 2 with sulfuric acid. The samples were percolated through the cartridges using a vacuum manifold system (Waters) connected to a vacuum pump. The loaded cartridges were rinsed with 6 mL of water/methanol (95:5 v/v) and 3 mL of n-hexane. The elution was performed with three aliquots of 1 mL of acetone. The combined aliquots were evaporated to dryness by a gentle N_2 stream, and the evaporated extract was dissolved to a final volume of 1 mL with methanol.

The freeze-dried and ground plant tissue samples were extracted and analyzed to determine the concentration of PPCPs [29]. A 0.2 g (dry weight, DW) aliquot of each plant sample was placed in a 50 mL glass centrifuge tube, spiked with deuterated PPCPs as recovery surrogates, and then extracted with 20 mL of methyl tert-butyl ether (MTBE) in an ultrasonic water bath for 20 min, followed by centrifugation at 3000 rpm (15.1 g) for 20 min. The supernatant was decanted into a 40 mL glass vial and the residue was extracted once more using 20 mL of acetonitrile. The combined extracts were dried under a N_2 stream at 30 °C and were redissolved in 1 mL of methanol, followed by the addition of 20 mL of distilled water. The aqueous mixture was then loaded onto an HLB (60 mg) cartridge (Waters, Dublin, Ireland) that had been preconditioned with 7 mL of methanol and 7 mL of distilled water. After the cartridge was dried with N_2, the analytes were eluted using 7 mL of methanol. The extract was concentrated further under a gentle N_2 stream and was reconstituted to 1 mL with methanol.

In the leachates, a PPCPs extraction was performed according to the method described in [24]. Leachate samples of 500 mL were adjusted to a pH of 2 with concentrated sulfuric acid. Then, they were loaded onto HLB (60 mg) cartridges (Waters, Dublin, Ireland) that had been preconditioned with 5 mL of MTBE, 5 mL of methanol, and 5 mL of distilled water. After that, the cartridges were rinsed with reagent water and eluted with 5 mL of 10/90 (v/v) methanol/MTBE followed by 5 mL of methanol. The resulting extracts were evaporated to dryness under vacuum at 40–50 °C using a TurboVap LV concentrator. Finally, the extracts were brought to a volume of 1 mL using methanol.

All the samples were filtered through polytetrafluoroethylene (PTFE) filters (13 mm, 0.2 µm, Millipore, Carrigtwohill, Cork, Ireland) before the instrumental analysis. The final samples were analyzed using an ACQUITY UPLC Waters I-Class system (Waters Corporation, Milford, MA, USA) coupled to a Bruker Daltonics QToF-MS mass spectrometer (maXis impact Series, Bruker Daltonics, Bremen, Germany). Details of the instrumental analysis and quality control are provided in the Supplementary Information S2.

2.3. Estimation of Human Exposure and Acute Toxicity Tests

The human exposure (HE) to PPCPs through dietary intake was evaluated according to the corresponding concentrations in the plants produced by using the different water treatments. The daily human exposure to the PPCPs accumulated in the aerial part of the lettuce was calculated using the formula of Beltrán et al. [30] (Equation (1)):

$$HE = C \cdot 10^{-6} \times I \quad (1)$$

where HE is the human exposure (mg day^{-1}), C is the concentration of the corresponding PPCP (ng g^{-1}, converted to fresh weight (FW)) in the edible part of the plant (data obtained in the present mesocosm experiment), and I is the value recommended for the daily intake of romaine lettuce (edible portion in grams (uncooked weight) per day) by the U.S. EPA [31].

The acute toxicity test with the crustacean *Thamnocephalus platyurus* (Thamnotoxkit F, MicroBioTest Inc., Ghent, Belgium) was performed on the leachates from the pots according to the ISO norm 14380 [32] by using larvae hatched from cysts 20–22 h before the assay in diluted (1:8) standard freshwater at 25 °C under continuous illumination (3000–4000 lx). The tests were performed on 24-well plates, whereby we placed 10 crustaceans in each well containing 1.0 mL of the corresponding test solution (leachates) with three replicates. Serial dilutions from 0 to 100% of the solutions were tested, and the results are presented as the percentage (%) survival (number of surviving individuals in each dilution).

3. Results

3.1. PPCPs Concentrations in Leachates and Soils

Once PPCPs are introduced into soil, they can suffer both biotic (biodegradation and/or phytodegradation) and abiotic (soil adsorption, hydrolysis, photolysis, volatilization, etc.) transformations [33]. Although these degradation processes can lead to a decline in the concentration of PPCPs or a complete removal, it is also possible that transformation products, which can be more toxic and/or persistent than the parent contaminants, will accumulate in the soil [34,35]. The remaining (nondegraded) compounds can migrate to the groundwater system (especially hydrophilic PPCPs) and cause contamination. The retention and/or mobility of PPCPs in the soil are mainly affected by the properties of the soil components, the particular PPCP, and the surrounding environment [36]. Therefore, the particular soil properties and composition play a very important role in the migration and transformation of PPCPs [33]. In the present experiment, the PPCPs contained in the different irrigation waters used were completely degraded in or leached from the soil since the concentrations of the different PPCPs in the soil extracts were all below the corresponding limits of detection (DL) of the equipment used for the analyses (Table S1). Consequently, under these experimental conditions, irrigation with the reclaimed water from the East Murcia WWTP or the spiked water did not produce an accumulation of the target compounds in the soil. The PPCPs may have been leached, degraded under the light and temperature conditions of the growth chamber, and/or directly degraded by the action of the soil microorganisms [10].

Ibuprofen (IBU) was not detected in any of the leachate samples studied (the limits of detection and quantification in our experimental conditions can be found in Table S2), and naproxen (NPX) was only detected in one replicate sample of the leachates from the SW-P treatment (Table 2). These particular compounds seem to be well degraded in the soil. In agreement with this, Edwards et al. [37] indicated that the rapid rates of degradation and the relatively slow rates of infiltration may explain why several analytes were not detected in the groundwater. Also, the fact that both are relatively hydrophobic (log K_{ow} of 3.97 and 3.18, respectively) could favor their adsorption to soil, which limits their percolation to groundwater. Other authors [38,39] have suggested that compounds with a log K_{ow} value between three and four are strongly absorbed by soil particles, which may reduce their potential leaching and contamination of the groundwater. However, the fact that IBU and NPX were not detected in the soils suggests the complete degradation of these PPCPs as their decomposition in the soil is relatively straightforward [24].

Contrastingly, the anticonvulsant and mood-stabilizing drug carbamazepine (CBZ, log K_{ow} of 2.45) was found in leachate samples from the pots watered with reclaimed (only in one replicate sample with a concentration of 104 ng L^{-1}) or spiked water (1749 and 1943 ng L^{-1} with and without a plant, respectively; Table 2). Several studies have shown that CBZ is highly stable in water [14,24]. Renau-Pruñonosa et al. [22] found CBZ in all the aquifer samples they analyzed, although at extremely low concentrations (<0.2–1.9 ng L^{-1}).

These authors found that 98.5% was the percentage CBZ degradation during the transfer of treated water from WWTP to groundwater.

Table 2. Concentrations (ng L^{-1}) of detected compounds in the leachates from the pots watered with distilled (CW), reclaimed (RW), or spiked water (with a plant (SW) or without a plant (SW-P)): carbamazepine (CBZ), diclofenac (DCF), ketoprofen (KTP), naproxen (NPX), triclosan (TCS).

	CBZ	DCF	KTP	NPX	TCS
CW	BDL	BDL	BDL	BDL	BDL
RW	104 *	BDL	BDL	BDL	BDL
SW	1749 ± 1590	1567 ± 1677	532 ± 222	BDL	BDL
SW-P	1943 ± 978	575 ± 345	140 ± 92	225 *	11.5 ± 0.7

* One replicate sample, the other replicates were BDL (Below Detection Limits).

In this experiment, DCF was detected only in the leachates from the pots irrigated with the spiked water (Table 2). An average concentration of 1567 ng L^{-1} was detected in the water when in the presence of a plant and 575 ng L^{-1} when the water was not in the presence of a plant, and there was a very high variability among the replicate samples. These results agree with those reported by Renau-Pruñonosa et al. [22], whereby DCF was found in the WWTP effluent, but only in some samples of the aquifers analyzed; the DCF was found at a maximum concentration of 4.6 ng L^{-1}, and they observed an almost complete degradation of this compound during the transfer of the water from the WWTP to the groundwater.

Regarding the concentration of the NSAID ketoprofen (KTP) in the leachates, all the samples showed concentrations below the DL (40 ng L^{-1}), except those collected from pots watered with spiked water (Table 2). Ketoprofen was previously found to be almost completely removed by the treatments applied in WWTP [24]. In the case of the spiked water, the concentration found in the leachates amounted to 2.8–10.6% of the original concentration, which indicates the high tendency of this compound to be degraded in the soil.

Finally, triclosan (TCS) was only detected in the SW-P leachates at very low concentrations (Table 2). TCS is highly hydrophobic with a log K_{ow} of 4.90 (Table 1), so it is expected to be retained in the soil, but it also seems to be easily degraded, especially when compared to other PPCPs such as CBZ. The half-life of the TCS biodegradation was calculated as 18 days under aerobic conditions and 70 days under anaerobic conditions [40], which indicates the easy biodegradation of TCS under the aerobic conditions prevailing in the soil of the present work. Also, in the case of TCS, the presence of a plant provoked the further degradation or retention of this compound since its concentrations in the leachates from the pots with a lettuce plant were below the limit of detection (Table S3). Plants may excrete some substances that are capable of degrading or immobilizing TCS, or this compound may have also been adsorbed or taken up by plant roots, which prevented its leaching [41].

Regarding the acute toxicity test, the larvae of *T. platyurus* used barely showed any negative effects when exposed to the leachates from the different treatments. In both the distilled (CW) and reclaimed (RW) water, no mortality was observed, while with the spiked water (SW) leachates, some larvae died when exposed to the nondiluted (100%) samples. The calculated mortality of *T. platyurus* in the spiked water was 10% while the reclaimed water did not show toxicity towards this organism.

3.2. PPCPs Concentrations in Lettuces and Human Exposure Risk Assessment

Plants can remove soil/water PPCPs through mechanisms such as root uptake and the action of root exudates, but also the compounds taken up by the roots can be translocated to the aerial part of the plants or the fruits. Nevertheless, these processes are complicated since they are governed by the physicochemical properties of the PPCPs as well as by particular soil and plant characteristics [30,42]. In this experiment, the plants did not show any symptoms of impaired growth when treated by any of the treatments, and no

significant differences were found regarding the plant yield (fresh and dry weights) among the different treatments (Table 3).

Table 3. Plant fresh weight (FW) and dry weight (DW) (g) and concentrations (ng g^{-1} DW) of detected compounds: CBZ and DCF in the roots and aerial part (AP) of the lettuces irrigated with distilled (CW), reclaimed (RW), or spiked water (SW).

	FW	DW	FW/DW	CBZ_{Root}	CBZ_{AP}	DCF_{Root}
CW	31.1 ± 3.8	3.5 ± 0.2	8.9 ± 0.9	BDL	BDL	BDL
RW	28.9 ± 2.2	3.1 ± 0.5	9.3 ± 1.3	37.1 *	BDL	BDL
SW	34.1 ± 4.1	3.4 ± 0.6	10.2 ± 0.9	40.0 ± 20.5	41.6 ± 4.1	13.9 ± 7.8

* One replicate sample; the other replicates were BDL (Below Detection Limits).

The concentrations of some of the studied PPCPs (IBU, KTP, NPX, and TCS) were below the corresponding DL (Table S3) in the different plant tissues (the aerial part or roots) among the different treatments. These low concentrations suggest that uptake by plant roots and translocation to the aerial part were minimal. However, CBZ (with relatively low hydrophobicity; Table 1) was accumulated in both plant tissues (Table 3). The CBZ concentration in the roots of the plants receiving the reclaimed water (detected only in one replicate sample) was very close to that in the roots of the plants receiving the spiked water, despite the huge difference in the CBZ concentration between these waters.

The use of spiked water caused some translocation of CBZ from the roots to the aerial part of the plants as the average concentration found in the aerial part (41.6 ng g^{-1}, DW) was similar to that determined in the roots of the SW plants. In an experiment conducted by Beltrán et al. [30], CBZ had the highest accumulation in lettuce, radish, and maize roots and leaves among the contaminants studied (atenolol, CBZ, and TCS). The authors found that the nonionic character and relatively low hydrophobicity of CBZ could explain this tendency, as there was no interaction with the organic colloids present in the soil. This could also allow for movement within the plant from the xylem to the phloem and an accumulation in the leaves. In fact, Beltrán et al. found that the translocation factors (root to leaves) for CBZ were greater than three in maize and five in radish when the plants were watered with highly contaminated water. González-García et al. [43] observed a similar phenomenon in a hydroponic experiment with lettuce, where CBZ and DCF accumulation in plant leaves reached 1456 and 82 ng g^{-1}, respectively. These concentrations are much higher than those observed in the present experiment, which was likely due to the very high concentrations used in their spiked water (210,000 ng L^{-1} for both compounds). This indicates the capacity of lettuce to accumulate these compounds in its tissues. Nevertheless, these concentrations in spiked water are difficult to find in the effluents of WWTP. In our spiked water, the concentration of these compounds was 5000 ng L^{-1}, which can also be considered high since the normal concentrations observed in WWTP in the same area are between 10.1 and 98 ng L^{-1} for CBZ and between 6.64 and 307 ng L^{-1} for DCF [14,23].

DCF was only detected in the roots of the plants exposed to the spiked water (Table 3), and it had an average concentration of 14 ng g^{-1}. González-García et al. [43] found a root concentration of DCF of 100 ng g^{-1} in a hydroponic experiment with lettuce when the growing medium was spiked with 30,000 ng L^{-1} of DCF, a concentration 89% higher than that found in the leaves. The high log K_{ow} (>4, Table 1) of DCF facilitated its uptake by the roots, which generally had higher concentrations than the leaves.

Based on these results, it can be concluded that this variety of lettuce, when watered with reclaimed water under these experimental conditions, was not able to accumulate the studied compounds in its aerial part. There was some accumulation of CBZ in the roots of the plants exposed to the reclaimed water, but when the plants were watered with spiked water, CBZ and DCF accumulated in the roots, and some CBZ was translocated to the aerial and edible parts of the plants. The plants did not take up the other contaminants studied (IBU, KTP, NPX, and TCS), or they could have been taken up and transformed into other compounds that the analytical procedure could not identify. Abril et al. [42] did not detect

TCS translocation and bioconcentration in radish, which was attributed to its metabolism after plant uptake. Other authors [44] have suggested that low concentrations of PPCPs in plant tissues were due to biodegradation by rhizosphere microorganisms.

Taking into account the concentrations found in the edible part of lettuce, the potential daily human exposure (HE) was evaluated. No accumulation of PPCPs was observed in the aerial part of the lettuce plants watered with the reclaimed water, so they are safe for consumption. By contrast, in the lettuce plants watered with spiked water, a maximum concentration of CBZ of 45.2 ng g^{-1} (DW) was obtained; taking into account the average dry and fresh weights of the edible part of the lettuce (Table 3), this corresponds to 4.51 ng g^{-1} (FW). The daily intake of romaine lettuce [29] is estimated to be 7.0 g day^{-1}. Then, based on the calculation of the HE established in Section 2.3., the exposure to this PPCP would be $31.6 \cdot 10^{-6}$ mg day^{-1}, which is lower than the values found by Beltrán et al. [30] in lettuces watered with spiked water (which ranged from $484 \cdot 10^{-6}$ to $6784 \cdot 10^{-6}$ mg day^{-1}). If the CBZ treatment prescribed for children (the most vulnerable population) usually starts with a minimum dose of 100 mg per day [45], then the value obtained in the present experiment ($31.6 \cdot 10^{-6}$ mg day^{-1}) is almost $3 \cdot 10^{7}$ times lower than this dose, which indicates that these lettuces pose no risk to humans even when watered with spiked water.

4. Conclusions

The results of our study indicate that the use of reclaimed water from the studied WWTP did not lead to increased concentrations of the studied pharmaceutical and personal care products (PPCPs) in leachates or the edible parts of lettuce. However, when the lettuce plants were watered with spiked water, CBZ, DCF, KTP, and TCS were detected in the leachates, and CBZ was also found in the edible part of the plant, although this concentration may not pose a human health risk.

No PPCPs were detected in the soil, even when it received spiked water. These compounds were likely degraded or transformed in the soil by microorganisms or underwent light-induced degradation in the growth chamber. The acute toxicity test showed that only the spiked water exhibited some toxicity.

Therefore, it can be concluded that the reclaimed water from the studied WWTP is safe for watering lettuce and other crops as there is little or no risk of transfer of the compounds analyzed in this study to the edible parts or to water bodies, or of their accumulation in soil.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/su15054536/s1: Supplementary Information S1. Materials; Supplementary Information S2. Instrumental analysis and quality control: Table S1: Detection and quantification limits, extraction efficiency, and linearity in soils obtained with the method used by the UPLC Acquity I-Class System (DW); Table S2: Detection and quantification limits, extraction efficiency, and linearity in water obtained with the method used by the UPLC Acquity I-Class System; Table S3: Detection and quantification limits, extraction efficiency, and linearity in vegetables obtained with the method used by the UPLC Acquity I-Class System (DW).

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