Mesocosm Evaluation of the Safety in 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 non-conventional resources. Nevertheless, concern about the presence and possible negative effects of Contaminants of Emerging Concern (CECs) in reclaimed waters can put in danger the possible use of this water source. To clarify the guarantees that reclaimed water offers, a mesocosm study was carried out. Pots with a lettuce were watered with three different types of water (distilled, reclaimed and spiked with CECs). The results showed a low concentration of these compounds in leachates and in plant tissues when reclaimed water was used. However, their concentrations were high when pots were watered with spiked water, mainly for carbamazepine (CBZ), reaching a maximum of 2982 ng L^{-1} in leachates and 45.2 ng g^{-1} in plant roots. In lettuces watered with spiked water, CBZ was found in edible part at very low concentrations that did not imply any human risk. Finally, an acute toxicity test was performed with the leachates, which resulted only slightly toxic for spiked water. This work confirms that, with the current technical improvements of active sludge water treatment, reclaimed water can be used for irrigation without risk for CECs contamination.

1. Introduction

Due to the problems derived from water scarcity and the competition for a resource as necessary as drinking water, in arid and semiarid regions there has been a growing interest, for decades now, in search for new and alternative water sources to reduce the use of clean/potable water for diverse applications (Gil-Meseguer et al. 2019). Already in 1958, the UN Economic and Social Council (ECOSOC 1958) boosted water reuse by instructing not to consume high-quality water for uses that could tolerate lower qualities, such as for industrial activities or for soil irrigation. In the European Union (EU), the current tendency towards a circular economy has led to the promotion of further efforts to make a greater consumption of alternative water sources. However, at present, only 4% of all the water consumed in the world is reused and 80% of wastewaters return to the ecosystems, even without being treated (WWAP 2017). This data contrasts 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 (Scruggs et al. 2020). 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 (Ungureanu et al. 2020). Currently, Spain reuses 11.2% of its waters, but there is a great disparity in this reuse between north and south parts of the country. For example, in the Region of Murcia (SE Spain), 95.6% of the treated wastewater is used as for irrigation in the agriculture (INE 2018).

In the Region of Murcia, the annual average precipitation is approximately 300 mm per year, which is clearly insufficient for the agricultural production in the area, as there is a water deficit. The use in the local agriculture of water from different sources, including desalinated and reclaimed waters, is a key factor for its sustainability (Aldaya et al. 2019). This region reused 117 hm^3 $year^{-1}$ of treated water during 2020 (data from ESAMUR) being the leader in terms of the use of reclaimed water, and in agricultural production, exporting 2.5 million tons of fruit and vegetables per year (Martin-Gorriz et al.

2020). The use of reclaimed water has become part of the solution to prevent water scarcity and the effects produced by extreme climate events like droughts (WWAP 2017; WWDR 2020).

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 (Al-Baldawi et al. 2021, Golovko et al. 2021; Corrêa et al. 2021; Wang et al. 2021). Therefore, these compounds are candidates for being included in future regulations regarding reclaimed water reuse, as a function of their ecotoxicity, their occurrence in the different environmental compartments, etc. Examples within 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 tested and/or suspected of endocrine disruption. Several studies have determined their presence and ecological risk assessment in waters (Martínez-Alcalá et al. 2021; Corrêa et al. 2021; Molnar et al. 2021; Wang et al. 2021), in soil (Urra et al. 2019; Jauregi et al. 2021) and in plants (Keerthanan et al. 2021), receiving reclaimed water irrigation and/or sewage sludge on agricultural soils as fertilizer (Bourdat-Deschamps et al. 2017). Nevertheless, the concern about the possible soil contamination due to the use of reclaimed water (Chiaia-Hernández et al. 2020) and on the potential PPCPs transport to groundwater settings via runoff and leaching (Renau-Pruñonosa et al. 2020) persists.

The knowledge about the concentrations of CECs that can be found in wastewater treatment plants (WWTP) effluents, and the repercussions that could derive from the use of these effluents in crop irrigation, becomes prevalent to be able to refute any restriction that may arise on its use. The objective of this work was to assess, with an advisory, predictive and/or confirmatory nature, the possible consequences or ecotoxicological effects of the application of reclaimed water on the soil-organism-plant system. For that reason, water with CECs was used to irrigate plant crops in a mesocosm experiment and their leaching risk, accumulation in the leaves and roots of the test plants (lettuce) and remaining in the soil was determined. Also, an ecotoxicological evaluation of the regenerated and spiked waters was carried out and compared with clean control water.

2. Material And Methods

Mesocosm experiments were designed following the example of Carbonell et al. (2009). They consist of plastic pots of a height of 10 cm and an internal diameter of about 12 cm; a hole for drainage was made at the lower part of each pot where a nylon mesh was placed to avoid soil loss. Each pot had a system for the collection of leachates (a large beaker (600 mL) covered with tin 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; Fig. 1).

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

A total of 24 pots were settled in a growth chamber using a completely randomized design with 6 replicate pots per treatment. The treatments consisted in 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 plant (SW-P).

2.1. Chemicals, soil, plants, and waters

A total of 5 pharmaceutical compounds (1 psychiatric drug and 4 Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)) and a cosmetic preservative were included in the spiked water (Table S.I.1). The selection was based primarily on previous studies of the research group and their occurrence in treated wastewater (Martínez-Alcalá et al. 2017). The pharmaceutical compounds selected for the experiment were the anticonvulsant and mood stabilizing drug carbamazepine, four non-steroidal anti-inflammatory drugs (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 measurements although it can be considered a 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 (Kim et al. 2007). However, Shenker et al. (2011) 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 (S.I.1). Individual stock solutions were prepared in methanol at 500 mg L^{-1} and stored at -20 ºC in the dark. An intermediate combined solution was prepared in methanol with all the chemicals at a concentration of 2500 µg L^{-1}. The spiked water (SW) consisted on distilled water with5000 (ng L^{-1}) of each selected PPCPs (CBZ, DCF, IBU, KTP, NPX and TCS).

The reclaimed water (RW) was collected from the Murcia-East WWTP (UTMX (ETRS89): 670034, UTMY (ETRS89): 4207420), where an active sludge with modified A2O and disinfection treatment plant is running to 553451 population equivalents (data from 2019). 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 (IUSS 2015) 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 clay loam texture) was collected from the surface (top 20 cm), 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: 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% $CaCO_3$ concentration. Pots were filled with 500 g (dry weight) of soil and introduced in a growth chamber under controlled conditions (22/18 ± 2 ºC, environmental humidity of 60%, photoperiod of 16/8 h light/dark and illumination between 250 and 300 µmol $m^{-2}s^{-1}$).

Deionized water was added to the pots to adjust the overall moisture content to 35% of the water holding capacity (WHC) of the soil. The pots were irrigated 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 of 1 month old, acquired from Deitana nursery (Murcia, Spain), was placed in each pot. From that moment, the pots were irrigated everyday with 100 mL of the corresponding treatment water. The experiment was carried out for 21 days under the same conditions in the growth chamber. Then, the plants were harvested and the different soil and leachate samples collected.

2.2. Sampling procedure and PPCPs analysis.

After plant harvesting (21 days), 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 mortar, and then stored at -20°C until PPCPs extraction. Lettuce plants were split into roots and aerial part and rinsed with distilled water; half of the aerial part was weighted (fresh weight and dry weight after 24 h at 60 ºC in an aerated oven) and the other half was transferred to liquid nitrogen, freeze dried and ground to a fine powder, and then stored at -20°C until PPCPs extraction. The leachate samples (one sample per pot) were divided in two subsamples, one to analyze the PPCPs and another to perform an ecotoxicity bioassay with the aquatic crustacean *Thamnocephalus platyurus*.

PPCPs in soil were extracted following the method of Martín et al. (2010) with slight modifications. Briefly, 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 during 30 s, sonicated for 15 min and centrifuged at 4000 rpm (15.1 g) during 20 min. The supernatants from each extraction step were combined and evaporated to 0.2mL under a N_2 stream (TurboVap LV concentrator). The extract was diluted to 250 mL with distilled water acidified to pH 2 with sulfuric acid and subjected to a cleanup procedure, where the aqueous mixture was loaded onto a hydrophilic-lipophilic balance cartridge (HLB, 60 mg, Dublin, Ireland), which was preconditioned with 3 mL of acetone, 3 mL of methanol and 3 mL of deionized water acidified to pH 2 with sulfuric acid. 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 nitrogen stream and the evaporated extract was dissolved to a final volume of 1 mL with methanol.

Freeze dried and ground plant tissue samples were extracted and analyzed for PPCPs (Wu et al. 2012). Briefly, a 0.2 g (dry weight, DW) aliquot of 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 (50/60 Hz, Fisher) 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 N_2 stream at

30°C and re-dissolved in 1 mL methanol, followed by the addition of 20 mL distilled water. The aqueous mixture was then loaded onto a HLB cartridge (60 mg, Dublin, Ireland), which was 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 further condensed under a gentle N_2 stream and reconstituted to 1 mL with methanol.

In the leachates, PPCPs extraction was performed according to method described by Martínez-Alcalá et al. (2017). A volume of 500 mL of leachate samples was preserved by adjusting to pH 2 with concentrated sulfuric acid. Then the leachates were loaded onto HLB cartridges (60 mg, Dublin, Ireland), which were 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 extract was evaporated to dryness under vacuum at 40–50 ºC 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 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 section (S.I.2).

2.3. Estimation of human exposure and acute toxicity tests

The human exposure (HE) to PPCPs through the dietary intake was evaluated according to the corresponding concentrations in the plants produced in the different water treatments. The daily human exposure for the PPCPs accumulated in the aerial part of lettuce was calculated using the formula by Beltrán et al. (2020) (Eq. 1):

1. $HE = C \cdot 10^{-6} \times I$

Where HE is the human exposure (mg day^{-1}), C is the concentration of the corresponding PPCP (ng g^{-1}, Fresh Weight (FW)) in the edible part of the plant (data obtained in the present mesocosm experiment), while I is the romaine lettuce daily intake recommended values (edible portion in grams (uncooked weight) per day) from the U.S. EPA (2018).

The acute toxicity test with the crustacean *Thamnocephalus platyurus* (Thamnotoxkit F, MicroBioTest Inc., Gent, Belgium) was performed according to the ISO norm 14380 (2011), 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). So, for the analysis of the leachate samples, a 12 mL aliquot from each treatment was frozen and stored until its use. The tests were performed in 24-well plates, placing 10 crustaceans per well containing 1.0 mL of the corresponding test solution (leachates), in three replicates.

Serial dilutions from 0 to 100% of the solutions were tested and the results are presented as a percentage (%) of survival.

3. Results And Discussion

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 (Qiao-ling et al. 2020). Although these degradation processes can chief the PPCPs reduction or complete removal, it is also possible that transformation products, which can result more toxic and/or persistent than the parent contaminants, can be formed in the soil (Andreu and Picó 2004; Zheng et al. 2019). After degradation, the remaining (non-degraded) compounds can migrate to the groundwater system (especially hydrophobic PPCPs) and cause contamination. PPCPs retention and/or mobility in the soil is mainly affected by the properties of the soil components, of the particular PPCP and of the surrounding environment (Xu et al. 2021). Therefore, particular soil properties and composition play a very important role with respect to PPCPs migration and transformation (Qiao-ling et al. 2020). In the present experiment, the PPCPs contained in the different irrigation waters used were completely degraded in or leached from the soil, sincethe 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 S.I.3). Consequently, under the experimental conditions, irrigation both with the reclaimed water from the East Murcia WWTP and with the spiked water, did not produce any accumulation of the target compounds in the soil. PPCPs may have been either leached, photo-degraded under the light and temperature conditions of the growth chamber, and/or directly degraded by the action of soil microorganisms (Al-Baldawi et al. 2021).

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 S.I.2) and naproxen (NPX) was only detected in one replicate sample of the leachates from SW-P treatment (Table 1). These particular compounds seem to be well degraded in the soil. In agreement with this, Edwards et al. (2019) indicated that rapid rates of degradation and relatively slow rates of infiltration may explain why several analytes were not detected in 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 limiting their percolation to groundwater. Other authors (Stuart et al. 2012; Fram and Belitz 2011) have suggested that compounds with a log K_{ow} value of around 3–4 adsorb strongly onto soil particles, which may reduce their potential leaching and contamination of the groundwater. However, the fact that these compounds were not either detected in the soils suggests the complete degradation of these PPCPs, as these compounds are relatively easy to decompose in the soil (Martínez-Alcalá et al., 2017).

Table 1

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

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

*One replicated sample, other replicates were below detection limits.

Contrastingly, the anticonvulsant and mood stabilizing drug carbamazepine (CBZ, log K_{ow} of 2.45) was found in leachate samples from pots watered both with reclaimed (only in one replicate sample, with a concentration of 104 ng L^{-1}) and spiked water (1749 and 1943 ng L^{-1} with and without plant, respectively; Table 1). Several studies have shown that CBZ is highly stable in water (Martínez-Alcalá et al. 2017, 2021). Renau-Pruñonosa et al. (2020) conducted an experiment where CBZ was found in aquifers in all the samples analyzed, although at extremely low concentrations (< 0.2–1.9 ng L^{-1}). These authors calculated as 98.5% the percentage of degradation during the transfer of treated water from the WWTP to groundwater.

In this experiment, DCF was only detected in leachates from pots irrigated with spiked water (Table 1). Average concentrations of 1567 ng L^{-1} were detected in the presence of plant and 575 ng L^{-1} without plant, with a very high variability among replicate samples. These results agree with those reported by Renau-Pruñonosa et al. (2020), where DCF was found in the WWTP effluent, but only in some samples of the aquifers analyzed, at maximum concentrations of 4.6 ng L^{-1}, observing an almost complete degradation of this compound during the transfer of water from the WWTP to the groundwater level.

Regarding the NSAID ketoprofen (KTP) concentration 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 1). Ketoprofen has been previously found to be almost completely removed in WWTPs (Martínez-Alcalá et al. 2017). In the case of the spiked water, the concentration found in the leachates amounted for 2.8–10.6% of the original concentration, which indicates the high tendency of this compound to be degraded in the soil.

Finally, the triclosan (TCS) compound was only detected in SW-P leachates at very low concentrations (Table 1). TCS is highly hydrophobic, with a log K_{ow} of 4.90 (Table S.I.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 time of TCS biodegradation was calculated as 18 days under aerobic conditions and 70 days under anaerobic conditions (Ying et al., 2007), which indicate the easy biodegradation of TCS under the aerobic conditions prevailing in the soil. Also, in the case of TCS, the presence of plants provoked further degradation or retention of this compound since its concentration in the leachates from pots with lettuce were found to be below the limit of detection (Table S.I.4). Plants may excrete some substances capable of degrading or immobilizing triclosan, or this compound may have also been adsorbed on or absorbed by plant roots, preventing the leaching of this PPCP (Zhao et al. 2016).

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

3.2. PPCPs concentration in lettuces and human exposure risk assessment

Plants can remove soil/water PPCPs through mechanisms such as root absorption and the action of root exudates, but also the compounds absorbed 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 PPCPs physico-chemical properties as well as by soil and plant particular characteristics (Beltrán et al. 2020; Abril et al. 2021). In this experiment, the plants did not show any symptom of impaired growth in any of the treatments, and no significant differences were found for plant yield (fresh and dry weights) among the different treatments (Table 2).

Table 2

Plant fresh weight (FW) and dry weights (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) and 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			
RW	28.9 ± 2.2	3.1 ± 0.5	9.3 ± 1.3	37.1*		
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 replicated sample, other replicates were below detection limits.

The concentrations of some of the studied PPCPs (IBU, KTP, NPX and TCS) were below the corresponding DL (Table S.I.4) in the different plant tissues (aerial part or roots) coming from the different treatments. The low concentrations obtained for these compounds suggested a scarce tendency of plant absorption by plant roots and translocation to the aerial parts of the plants. However, CBZ (with relatively low hydrophobicity; Table S.I.1) was accumulated in both plant tissues (Table 2). The CBZ concentration in the roots of plants irrigated with reclaimed water (detected only in one replicate sample) was very close to that in the roots of plants irrigated with spiked waters, despite the huge difference in CBZ concentration in both irrigation waters.

Certain translocation of CBZ from the roots to the aerial part could occur when the plants were watered with spiked water, since the average concentration found in the aerial part (41.6 ng g^{-1}, DW) was similar to that determined in the roots of the plants from this water treatment (SW). In anexperiment with atenolol, carbamazepine and triclosan, Beltrán et al. (2020) found that, CBZ hadthe highest accumulation in lettuce, radish, and maize roots and leaves of the contaminants studied (atenolol, CBZ and TCS). They found that factors such as non-ionic property and relatively low hydrophobicity of CBZ could support this tendency, due to the absence of interaction with the organic colloids present in soil. This could also favor its potential for internal plant movement from the xylem to the phloem in the direction of the transpiration stream, and accumulation in leaves. In fact the same authors found translocation factors (root to leaves) for CBZ greater than 3 in maize and of 5 in radish irrigated with highly contaminated water. In a hydroponic experiment with lettuces, González-García et al. (2018) observed an accumulation of CBZ and DCF in plant leaves of up to 1456 and 82 ng g^{-1}, respectively. Those concentrations are much higher than the ones observed in the present experiment, likely as a consequence of the very high concentrations used in their spiked water (both compounds were added to the growing solution at a concentration of 210000 ng L^{-1}). This indicates the capacity of this plant to accumulate these compounds in its tissues. Nevertheless, these water concentrations are difficult to find in WWTPs effluents. In the spiked water, the concentration of those compounds was 5000 ng L^{-1}, which can be already considered high, since CBZ and DCF normal concentration observed in WWTPs of 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 (Martínez-Alcalá et al. 2017, 2021).

Regarding DCF, it was only detected in the roots of plants irrigated with spiked water (Table 2), with an average concentration of 14 ng g^{-1}. In a hydroponic experiment with lettuce (González-Garcia et al. 2018), a root concentration of DCF of 100 ng g^{-1} was found when the growing media was spiked with 30000 ng L^{-1} of DCF, a concentration 89% higher than that determined in the leaves. Diclofenac properties, like their high log K_{ow} (> 4, Table S.I.1), facilitate their absorption by roots, which generally show higher concentrations than leaves.

In light of these results, it can be said that this variety of lettuce, irrigated with reclaimed water under these experimental conditions, was not able to accumulate the compounds studied in the aerial part, and only some CBZ was retained in plant roots. However, when lettuces were watered with spiked water, CBZ and DCF were accumulated in the roots, and CBZ could even be translocated to the aerial and edible part of the plants. The plants did not absorb the rest of the contaminants studied (IBU, KTP, NPX and TCS), or maybe they were absorbed and transformed into other compounds that the analytical procedure used were not able to identify. In an experiment carried out by Abril et al. (2021) with radish, neither triclosan translocation or bioconcentration were measured, this fact being considered a consequenceof their metabolization after plant uptake. In addition, other authors (Kinney and Heuvel 2020) have attributed the low concentrations of PPCPs detected in plant tissues to their biodegradation by rhizosphere microorganisms.

Taking into account the concentrations obtained in the edible part of lettuce, the potential human exposure was evaluated. No PPCPs accumulation was observed in the aerial part of the lettuces watered with the reclaimed water, so they are safe for their consumption. Whereas, in lettuce plants watered with spiked water, a CBZ maximum concentration of 45.2 ng g^{-1} (DW) was obtained; if the average weight obtained for the edible part of lettuce is considered (Table 2), this corresponds to 4.51 ng g^{-1} (W/W), The daily intake of romaine lettuce (U.S. EPA 2018) is estimated to be 7.0 g day^{-1}. The human exposure (HE) estimated value per person for this PPCP was $31.6 \cdot 10^{-6}$ mg day^{-1}, lower than that found by Beltrán et al. (2020) in lettuces watered with spiked water (which ranged from $484 \cdot 10^{-6}$ to $6784 \cdot 10^{-6}$ mg day^{-1}). If the CBZ maximum permissible concentration for daily human intake (1200 mg $person^{-1}$ day^{-1}; Vademecum (2019)) is considered, the value obtained in the present experiment is several orders of magnitude below it, indicating no risk of human consumption of these lettuces, even when watered with spiked water.

4. Concluding Remarks

The irrigation with reclaimed water from the studied WWTP did not contribute to concentrate the studied PPCPs in leachates or the edible part of lettuces under our experimental conditions, while spiked water provoked the presence of CBZ, DCF, KTP and TCS in the leachates, and also some CBZ was detected in the edible part of lettuce. Nevertheless, this concentration may not imply human risk of consumption.

In the soils, PPCPs were not detected, not even when they were irrigated with the spiked water. These compounds were degraded or transformed in the soil, likely by soil microorganisms and/or by light induced degradation in the growth chamber. The acute toxicity test run indicated only some toxicity for spiked waters.

Therefore, it can be said that the use of reclaimed water from the studied WWTP is safe for watering crop species such as lettuce, since they do not imply any risk in terms of the transference of the compounds analyzed in this experiment to their edible part or to other water bodies or their accumulation in the soil.

Declarations

Data availability

All data are available for the submitted journal.

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Contributions

Conceptualization (Martínez-Alcalá, Lahora); data curation (Martínez-Alcalá, Pellicer-Martínez, Bernal); formal analysis (Martínez-Alcalá, Pellicer-Martínez, Clemente); funding acquisition (Martínez-Alcalá); investigation (Martínez-Alcalá, Lahora); methodology (Martínez-Alcalá, Bernal); project administration (Martínez-Alcalá); resources (Martínez-Alcalá, Bernal); supervision (Martínez-Alcalá); validation (Lahora, Clemente, Bernal); visualization (Bernal); writing, original draft (Martínez-Alcalá,); writing, reviewing and editing (Martínez-Alcalá, Pellicer-Martínez, Lahora, Clemente, Bernal).

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Ethical approval

This study has been approved by all the shared departments and the authors.

Consent to participate

Consent to participate has been signed and approved.

Consent to publish

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Abril C, Santos JL, Martín J, Aparicio I, Alonso E (2021) Uptake and translocation of multiresidue industrial and household contaminants in radish grown under controlled conditions. Chemosphere 268:128823. https://doi.org/10.1016/j.chemosphere.2020.128823
2. Al-Baldawi IA, Mohammed AA, Mutar ZH, Abdullah SRS, Jasim SS, Almansoory AF, Ismail I (2021) Application of phytotechnology in alleviating pharmaceuticals and personal care products (PPCPs) in wastewater: Source, impacts, treatment, mechanisms, fate, and SWOT analysis. J Clean Prod 319:128584. https://doi.org/10.1016/j.jclepro.2021.128584
3. Aldaya MM, Custodio E, Llamas R, Fernández MF, García J, Ródenas M (2019) An academic analysis with recommendations for water management and planning at the basin scale: A review of water planning in the Segura River Basin. Sci Total Environ 662:755–768. https://doi.org/10.1016/j.scitotenv.2019.01.266
4. Andreu V, Picó Y (2004) Determination of pesticides and their degradation products in soil: critical review and comparison of methods. TrAC Trends Anal Chem 23:772–789. https://doi.org/10.1016/j.trac.2004.07.008
5. Beltrán EM, Pablos MV, Fernández Torija C, Porcel M, González-Doncel M (2020) Uptake of atenolol, carbamazepine and triclosan by crops irrigated with reclaimed water in a Mediterranean scenario. Ecotoxicol Environ Safe 191:110171. https://doi.org/10.1016/j.ecoenv.2020.110171
6. Bourdat-Deschamps M, Ferhi S, Bernet N, Feder F, Crouzet O, Patureau D, Montenach D, Moussard GD, Mercier V, Benoit P, Houot S (2017) Fate and impacts of pharmaceuticals and personal care products after repeated applications of organic waste products in long-term field experiments. Sci Total Environ 607–608. https://doi.org/10.1016/j.scitotenv.2017.06.240
7. Carbonell G, Pro J, Gómez N, Babín MM, Fernández C, Alonso E, Tarazona JV (2009) Sewage sludge applied to agricultural soil: Ecotoxicological effects on representative soil organisms. Ecotoxicol Environ Safe 72:1309–1319. https://doi.org/10.1016/j.ecoenv.2009.01.007
8. Chiaia-Hernández AC, Scheringer M, Müller A, Stieger G, Wächter D, Keller A, Pintado-Herrera MG, Lara-Martin PA, Bucheli TD, Hollender J (2020) Target and suspect screening analysis reveals persistent emerging organic contaminants in soils and sediments. Sci Total Environ 740:140181. https://doi.org/10.1016/j.scitotenv.2020.140181
9. Corrêa JMM, Sanson AL, Machado CF, Aquino SF, Afonso RJCF (2021) Occurrence of contaminants of emerging concern in surface waters from Paraopeba River Basin in Brazil: seasonal changes and

risk assessment. Environ Sci Pollut Res 28:30242–30254. https://doi.org/10.1007/s11356-021-12787-z

10. Edwards QA, Sultana T, Kulikov SM, Garner-O’Neale LD, Metcalfe CD (2019) Micropollutants related to human activity in groundwater resources in Barbados, West Indies. Sci Total Environ 671:76–82. https://doi.org/10.1016/j.scitotenv.2019.03.314
11. Fram MS, Belitz K (2011) Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California. Sci Total Environ 409:3409–3417. https://doi.org/10.1016/j.scitotenv.2011.05.053
12. Gil-Meseguer E, Bernabé-Crespo MB, Gómez-Espín JM (2019) Recycled Sewage - A Water Resource for Dry Regions of Southeastern Spain. Water Resour Manage 33:725–737. https://doi.org/10.1007/s11269-018-2136-9
13. Golovko O, Örn S, Sörengård M, Frieberg K, Nassazzi W, Lai FY, Ahrens L (2021) Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. Sci Total Environ 754:142122. https://doi.org/10.1016/j.scitotenv.2020.142122
14. González-García M, Fernández-López C, Pedrero-Salcedo F, Alarcón JJ (2018) Absorption of carbamazepine and diclofenac in hydroponically cultivated lettuces and human health risk assessment. Agric Water Manage 206:42–47. https://doi.org/10.1016/j.agwat.2018.04.018
15. INE Instituto Nacional de Estadística (2018) Destino de las aguas residuales tratadas por comunidades y ciudades autónomas, lugar de destino y periodo. Estadística sobre el suministro y saneamiento del agua. Serie 2000–2018. Web. https://www.ine.es/jaxi/Datos.htm?path=/t26/p067/p01/serie/l0/&file=01006.px. AccessedFebruary14 2022
16. ISO 14380 (2011) Water Quality-Determination of the Acute Toxicity to *Thamnocephalusplatyurus*. Crustacea, Anostraca, International Organization for Standardization, Geneva, Switzerland
17. IUSS Working Group WRB, World Reference Base for Soil Resources (2015) 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. Rome, FAO.https://www.fao.org/3/i3794en/I3794en.pdf.Accessed February 12 2022
18. Jauregi L, Epelde L, Alkorta I, Garbisu C (2021) Agricultural soils amended with thermally-dried anaerobically-digested sewage sludge showed increased risk of antibiotic resistance dissemination. Front Microbiol. 12:666854.https://doi.org/10.3389/fmicb.2021.666854
19. Keerthanan S, Jayasinghe C, Biswas JK, Vithanage M (2021) Pharmaceutical and Personal Care Products (PPCPs) in the environment: Plant uptake, translocation, bioaccumulation, and human health risks. Crit Rev Environ Sci Technol 51:1221–1258. https://doi.org/10.1080/10643389.2020.1753634
20. Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA (2007) Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water res 41(5):1013–1021. https://doi.org/10.1016/j.watres.2006.06.034

21. Kinney CA, Heuvel B (2020) Translocation of pharmaceuticals and personal care products after land application of biosolids. CurrOpin Environ Sci Health 14:23–30. https://doi.org/10.1016/j.coesh.2019.11.004
22. Martín J, Santos JL, Aparicio I, Alonso E (2010) Multi-residue method for the analysis of pharmaceutical compounds in sewage sludge, compost and sediments by sonication-assisted extraction and LC determination. J Sep Sci 33:1760–1766. https://doi.org/10.1002/jssc.200900873
23. Martínez-Alcalá I, Guillén-Navarro JM, Fernández-López C (2017) Pharmaceutical biological degradation, sorption and mass balance determination in a conventional activated-sludge wastewater treatment plant from Murcia, Spain. Chem Eng J 316:332–340. https://doi.org/10.1016/j.cej.2017.01.048
24. Martínez-Alcalá I, Guillén-Navarro JM, Lahora A (2021) Occurrence and fate of pharmaceuticals in a wastewater treatment plant from southeast of Spain and risk assessment. J Environ Manage 279:111565. https://doi.org/10.1016/j.jenvman.2020.111565
25. Martin-Gorriz B, Gallego-Elvira B, Martínez-Alvarez V, Maestre-Valero JF (2020) Life cycle assessment of fruit and vegetable production in the Region of Murcia (south-east Spain) and evaluation of impact mitigation practices. J Clean Prod 265:121656. https://doi.org/10.1016/j.jclepro.2020.121656
26. Molnar E, Maasz G, Pirger Z (2021) Environmental risk assessment of pharmaceuticals at a seasonal holiday destination in the largest freshwater shallow lake in Central Europe. Environ Sci Pollut Res 28:59233–59243. https://doi.org/10.1007/s11356-020-09747-4
27. Qiao-ling Y, Zhi-ping LI, Lei-cheng LI, Shu-li W, Si-yu Y (2020) Pharmaceuticals and personal care products transference-transformation in aquifer system. J Groundw Sci Eng 8:358–365. https://doi/10.19637/j.cnki.2305-7068.2020.04.006
28. Renau-Pruñonosa A, García-Menéndez O, Ibáñez M, Vázquez-Suñé E, Boix C, Ballesteros BB, Hernáneez-García M, Morell I, Hernández F (2020) Identification of Aquifer Recharge Sources as the Origin of Emerging Contaminants in Intensive Agricultural Areas. La Plana de Castellón, Spain. Water 12:731. https://doi.org/10.3390/w12030731
29. Scruggs CE, Pratesi CB, Fleck JR (2020) Direct potable water reuse in five arid inland communities: an analysis of factors influencing public acceptance. J Environ Plann Manage 63:1470–1500. https://doi.org/10.1080/09640568.2019.1671815
30. Shenker M, Harush D, Ben-Ari J, Chefetz B (2011) Uptake of carbamazepine by cucumber plants–a case study related to irrigation with reclaimed wastewater. Chemosphere 82(6):905–910. https://doi.org/10.1016/j.chemosphere.2010.10.052
31. Stuart M, Lapworth D, Crane E, Hart A (2012) Review of risk from potential emerging contaminants in UK groundwater. Sci Total Environ 416:1–21. https://doi.org/10.1016/j.scitotenv.2011.11.072
32. U.S. EPA. Exposure Factors Handbook Chap. 9 (Update): Intake of Fruits and Vegetables. U.S. EPA Office of Research and Development, Washington, DC, EPA/600/R-18/098F, 2018. https://cfpub.epa.gov/ncea/efp/recordisplay.cfm?deid=341764. Accessed February10 2022

33. UN Economic and Social Council (ECOSOC) (1958) Establishment of the Executive Committee of the Programme of the United Nations High Commissioner for Refugees. UN Econ Soc CouncResolut 672. https://www.refworld.org/docid/3ae69eecc.html. Accessed February32022
34. Ungureanu N, Vlăduț V, Voicu G (2020) Water Scarcity and Wastewater Reuse in Crop Irrigation. Sustainability 12:9055. https://doi.org/10.3390/su12219055
35. Urra J, Alkorta I, Garbisu C (2019) Potential benefits and risks for soil health derived from the use of organic amendments in agriculture. Agronomy 9:542. https://doi.org/10.3390/agronomy9090542
36. Vademecum(2019) Web. https://www.vademecum.es.Accessed January 112022
37. Wang K, Reguyal F, Zhuang T (2021) Risk assessment and investigation of landfill leachate as a source of emerging organic contaminants to the surrounding environment: a case study of the largest landfill in Jinan City, China. Environ Sci Pollut Res 28:18368–18381. https://doi.org/10.1007/s11356-020-10093-8
38. Wu C, Spongberg AL, Witter JD, Sridhar BBM (2012) Transfer of wastewater associated pharmaceuticals and personal care products to crop plants from biosolids treated soil. Ecotoxicol Environ Safe 85:104–109. https://doi.org/10.1016/j.ecoenv.2012.08.007
39. WWAP (United Nations World Water Assessment Programme) (2017) The United Nations World Water Development Report 2017: Wastewater, The Untapped Resource. Paris, UNESCO. http://www.unesco.org/new/en/natural-sciences/environment/water/wwap/wwdr/2017-wastewater-the-untapped-resource/
40. WWDR (2020) The United Nations world water development report 2020: water and climate change. ISBN 978-92-3-100371-4. UNESCO. 219pp. https://unesdoc.unesco.org/notice?id=p::usmarcdef_0000372985
41. Xu Y, Yu X, Xu B, Peng D, Guo X (2021) Sorption of pharmaceuticals and personal care products on soil and soil components: Influencing factors and mechanisms. Sci Total Environ 753:141891. https://doi.org/10.1016/j.scitotenv.2020.141891
42. Ying G-G, Yu X-Y, Kookana RS (2007) Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. Environ Pollut 150:300–305. https://doi.org/10.1016/j.envpol.2007.02.013
43. Zhao C, Xie H, Xu J, Zhang J, Liang S, Hao J, Hao Ngo H, Guo W, Xu X, Wang Q, Wang J (2016) Removal mechanisms and plant species selection by bioaccumulative factors in surface flow constructed wetlands (CWs): In the case of triclosan. Sci Total Environ 547:9–16
44. Zheng Z, Peters GM, Arp HPH, Andersson PL (2019) Combining in silico tools with multicriteria analysis for alternatives assessment of hazardous chemicals: A case study of decabromodiphenyl ether alternatives. Environ Sci Technol 53:6341–6351. https://doi.org/10.1021/acs.est.8b07163

Figures

Figure 1

Picture of the mesocosm experiment.

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