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Fate, modeling, and human health risk of organic contaminants present in tomato plants irrigated with reclaimed water under real-world field conditions

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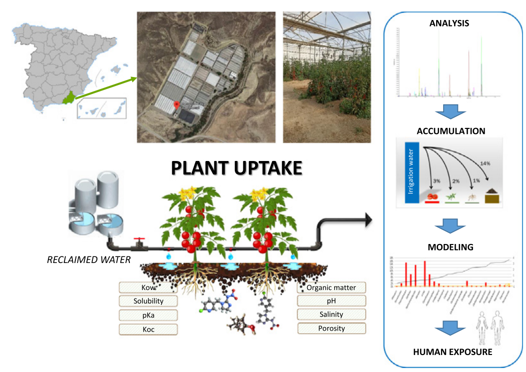
HIGHLIGHTS

- Soil samples exhibited the highest content of CECs followed by fruit ≥ leaf ≥ roots.
- We identify CECs with the highest potential for plant uptake and soil accumulation.
- We propose mathematical models to estimate the CECs uptake in soil and plants.
- A correlation was found between CECs uptake and log*Kow*.
- Daily human intake was estimated considering the worst-case scenario.

GRAPHICAL ABSTRACT



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ABSTRACT

Using reclaimed water to irrigate crops can be an important route for organic contaminants of emerging concern (CECs) to be introduced into agricultural production and thus find their way into the food chain. This work aims to establish accumulation models for the different parts of a crop (fruit/leaves/roots) and the soil of some of the most commonly detected CECs in reclaimed water, through field trials in greenhouses. For this, tomato plants were permanently irrigated under realistic agricultural conditions with a mixture of the selected compounds at approx. 1 μg/L. A total of 30 contaminants were analyzed belonging to different compound categories. A modified QuEChERS extraction method followed by liquid chromatography coupled to tandem mass spectrometry was the procedure used. The study revealed the presence of 21 target contaminants in the tomatoes, and 18 CECs in the leaves, roots, and soil. The average total concentration of pesticides detected in the tomatoes was 3 μg/kg f.w., whereas the average total load of pharmaceuticals was 5.8 μg/kg f.w. after three months, at the time of crop harvesting. The levels of pharmaceutical products and pesticides in the non-edible tissues were up to 3.5 and 2.1 μg/kg f.w., respectively, in the leaves and up to 89.3 and 31.3 μg/kg f.w., respectively, in the roots. In the case of the soil samples, the pesticide concentration found after crop harvesting was below 11.4 μg/kg d.w., and less than 3.0 μg/kg d.w. for pharmaceuticals. Overall, the concentration levels of CECs detected in the tomatoes, which were permanently irrigated with contaminated reclaimed water, do not pose a risk to human health via dietary intake.

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1. Introduction

Climate change, intensive agriculture and the increasing population are significantly contributing to the great pressure on water resources, leading to water scarcity and deterioration in water quality. The use of reclaimed wastewater for agricultural irrigation is increasing around the world and is a potential alternative for combating water scarcity. Water reuse has much less impact on the water cycle compared, for example, with water transfers from rivers; it also offers important environmental benefits (extending the life cycle of the water and zero discharge) as well as economic and social benefits (Ait-Mouheb et al., 2018). In this sense, the use of treated water constitutes a strategic resource that can reduce the structural water deficit suffered by many regions, such as countries in southern Europe. According to the data from Eurostat Statistics, Spain accounted for about a third of the total volume of EU water reuse (347 Mm^3/year) in 2019, followed by Italy (233 Mm^3/year) and Germany (42 Mm^3/year). In Spain, approx. 71% of reclaimed water is used for agricultural irrigation, 17% for landscape irrigation, 11% for recreational/urban use and only 0.3% for industrial processes (Eurostat Statistics Explained, 2019).

The European Union has recently published a new Regulation (EU) 741/2020, concerning the minimum requirements for water quality and control to ensure the safe use of treated urban wastewater (The European Parliament and the Council, 2020). This Regulation aims to guarantee that reclaimed water is safe to use and to provide a high level of human, animal, and environmental health protection, in addition to tackling water scarcity.

To date, hundreds of scientific papers have reported the presence of organic contaminants of emerging concern (CECs), such as pharmaceuticals, personal care products, or pesticides, in treated water or irrigation water (Calderón-Preciado et al., 2011; Martínez Bueno et al., 2012; Quintana et al., 2019; Renau-Pruñonosa et al., 2020). These studies provide clear evidence that conventional wastewater treatment plants (WWTPs) are poorly effective to comprehensively remove of most CECs and advanced treatment steps are needed to effectively remove CECs (Krzeminskia et al., 2019). Therefore, the use of reclaimed water in crop irrigation can be an important route by which emerging pollutants are introduced into agricultural production and subsequently enter the food chain; this could be hazardous to human health and to the environment. For example, Calderón-Preciado et al. (2011) detected a total of 26 chemical contaminants in reclaimed water used for agricultural crop irrigation. The average concentration for pesticides ranged between 0.05 and 0.1 μg/L, while pharmaceutical products were found at concentrations between 0.03 and 0.7 μg/L.

Contaminant uptake in plants can be influenced by a wide variety of factors, both biotic and abiotic. The main biotic factors affecting adsorption are the plant itself (the species, variety, and physiological state) and soil microorganisms, which are the principal cause of contaminant biodegradation/biotransformation in the soil. Climatic conditions (such as temperature, UV radiation, salinity and wind speed), the contaminants' physicochemical properties (hydrophobicity, polarity and solubility in water) and the structure and composition of the soil constitute the main abiotic factors influencing the plants' uptake potential (Christou et al., 2019). In spite of some scientific works related to the study of the mechanism by which plants absorb organic pollutants have recently been published, it is still relatively unknown. Most of these works have been focused on evaluating the accumulation of pharmaceutical and personal care products (PPCPs) in crops irrigated with treated wastewater. For example, Wu et al. (2014) investigated the accumulation of 19 PPCPs in eight types of vegetables irrigated with treated wastewater under field conditions. The total concentration of PPCPs detected in the edible tissues was in the 0.01–3.87 μg/kg range. In a recent study, Picó et al. (2019) evaluated the potential for plant uptake in crops irrigated with treated wastewater under uncontrolled environmental conditions. The results reported the presence of 7 pharmaceutical products at concentrations ranging from 25 to 96 μg/kg in the soil samples, and from 35 to125 μg/kg in the plants, whereas 7 pesticides were detected at levels between 25 and 366 μg/kg in the soil and between 35 and 5650 μg/kg in the vegetable samples. To date, most of this research has been carried out in the laboratory (unrealistic agricultural conditions), under uncontrolled environmental conditions or in field trials at concentration levels higher than those expected in reclaimed water (Wu et al., 2013; Malchi et al., 2014; Paz et al., 2016; Madikizela et al., 2018; Picó et al., 2018; Ju et al., 2019). Moreover, the mathematical models reported in the literature on plant uptake are limited to only a few substances or require compound-specific parameters to run (Collins and Finnegan, 2010; González García et al., 2019; Prosser et al., 2014).

Therefore, the general objective of this work is to measure the uptake of some of the most commonly detected CECs in treated water. The measurements were taken from the soil, plant tissues and fruit grown using reclaimed water under realistic agricultural conditions. This study determines the compounds that have a higher capacity in reaching the plant; these are then used as chemical markers to develop accumulation statistical models which allow us to estimate the levels of organic contaminants in the soil as well as in the different parts of the plants. Finally, the potential human risks from consuming the edible part of plants are assessed.

2. Materials and methods

2.1. Reagents and materials

A total of 30 CECs including 13 pesticides, 12 pharmaceutical products, and 5 transformation products were investigated. The target analytes' selection was based on their environmental relevance and the authors' previous experience (Martínez Bueno et al., 2012). The physicochemical properties of all the selected analytes have been included in Table 1. All reference standards were purchased from Sigma-Aldrich (Steinheim, Germany) at a high purity grade (>98%), except codeine which was obtained in pill form. Stock standard solutions were prepared individually in acetonitrile at concentrations ranging from 1000 to 2000 mg/L and stored in amber glass vials with screw caps in the dark at −40 °C.

Acetonitrile (AcN), methanol (MeOH), and formic acid were of LC-MS grade and supplied by Fluka Analytical (Steinheim, Germany), whereas the ultrapurewater was supplied by Fisher Scientific (Fair Lawn, NJ, USA). Anhydrous magnesium sulphate $(MgSO_4)_{anh}$, sodium chloride (NaCl), sodium hydrogenocitrate sesquihydrate ($Na_2HCitrate \cdot 1,5H_2O$), and sodium citrate tribasic dihydrate ($Na_3Citrate \cdot 2H_2O$) were obtained from Sigma–Aldrich (Steinheim, Germany). C-18 sorbent was purchased from Supelco (Bellefonte, PA, USA) and ChloroFiltr® dispersive centrifuge tubes containing 900 mg $MgSO_4$ and 150 mg ChloroFiltr® were acquired from United Chemical Technologies (UCT, Ref. ECMSGG15CT, Bristol).

2.2. Field experiments and sampling strategy

Tomatoes are commonly used in salads, and people often consume them raw. They are the second most important vegetable crop in the world after potatoes. The EU's production of tomatoes was 16.5 million tonnes in 2019, according to data released by the European Statistical Office (Eurostat). Almost two thirds of the EU-27s tomato production in 2019 came from Italy (5.3 million tonnes) and Spain (5.0 million tonnes) (Eurostat Statistics Explained, 2020). Based on these data, a tomato (*Solanum lycopersicum L.*) crop was grown in a greenhouse located in Almería (Spain) under controlled agronomic conditions and using reclaimed water applied by drip irrigation. The reclaimed water was spiked with a solution containing a mixture of the CECs selected for this study, each one at a concentration of approx. 1 μg/L, considering the worst-case scenario according to previous results from our research group (Martínez Bueno et al., 2012). In order to assess the degradation potential of carbamazepine and metamizole, none of their respective

Table 1
Physicochemical properties of all CECs selected in this study.

Compound	Family	Log*Kow*	pKa	Water solubility	Koc	DT_{50} soil	Soil/environmental fate
Ofloxacin	Antibiotic	−0.4	5.9	28,300	44	952–1820	Immobile in soil. Non-volatile.
Pymetrozine	Insecticide	−0.2	4.1	290	246–7875	707	Very Persistent. Low mobility in soil. Rapidly degraded in soil.
4-AAA	Analgesic	−0.1	12.4	40,226	240.7	n.d.	n.d.
Thiamethoxam	Insecticide	−0.1	0.4	4100	56	50	Moderately persistent. High mobile in soil. Photodegraded.
Caffeine	Stimulant	−0.1	14	21,700	741–7762	10–34	Low mobility in soil.
Hydrochlorothiazide	Diuretic	−0.1	8.0	722	12	9–11	High mobility in soil. Photodegraded. Non-biodegradable.
Atenolol	b-blocker	0.2	9.6	13,300	148.1	n.d.	High mobility in soil.
4-FAA	Analgesic	0.2	12.7	101,289	17	n.d.	n.d.
Ciprofloxacin	Antibiotic	0.3	6.1	30,000	61,000	1155–3466	Immobile in soil. Non-volatile.
Acetaminophen	Analgesic	0.5	9.4	14,000	20,844	30	High mobility in soil. Photodegraded and readily biodegradable.
4-AA	Analgesic	0.5	4.1	727,617	282.9	n.d.	n.d.
Imidacloprid	Insecticide	0.6	11.1	33	478	191	Persistent. Medium mobility in soil. Photodegraded.
4-MAA	Analgesic	0.6	n.d.	28,897	410.7	n.d.	n.d.
Acetamiprid	Insecticide	0.8	0.7	2950	200	1.6	Non-persistent. High mobility in soil. Biodegradable.
Codeine	Analgesic	1.2	8.2	<1	700	120	Low mobility in soil. Photodegraded.
Thiacloprid	Insecticide	1.3	1.6	184	1100	1–4	Non-persistent. Low mobility in soil. Biodegradable.
Carbendazim	Fungicide	1.5	4.2	8	122–2805	40	Moderately persistent. Medium mobility in soil.
Epoxide-CBZ	Antiepileptic	1.6	n.d.	1340	388.5	n.d.	Medium mobility in soil.
Furosemide	Diuretic	2.0	3.9	73	110	120	High mobility in soil. Photodegraded. Biodegradable.
Diuron	Herbicide	2.3	13.2	35	680	146	Persistent. Low mobility in soil. Non-biodegradable.
Thiabendazole	Fungicide	2.4	4.7	30	3983	500	Very persistent. Low mobility in soil.
Carbamazepine (CBZ)	Antiepileptic	2.4	13.9	18	510	462–533	Very persistent. Medium mobility in soil. Non-biodegradable.
Azoxystrobin	Fungicide	2.5	0.9	7	589	78	Moderately persistent. Medium mobility in soil.
Fluxapyroxad	Fungicide	3.1	12.6	3	1907	183	Persistent. Low mobility in soil.
Myclobutanil	Fungicide	3.2	2.3	132	950	560	Very persistent. Low mobility in soil.
Naproxen	Anti-inflammatory	3.2	4.1	16	330	17–69	Medium mobility in soil. Non-biodegradable.
Diazinon	Insecticide	3.4	2.6	60	609	9.1	Non-persistent. Low mobility in soil.
Penconazole	Fungicide	3.7	1.5	73	786–4120	117	Persistent. Low mobility in soil.
Diclofenac	Analgesic	4.0	4.1	2	245	3–20	Medium mobility in soil. Biodegradable.
Gemfibrozil	Lipid regulators	4.8	4.5	11	430	224–231	Medium mobility in soil. Non-biodegradable.

https://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm#; http://www.chemspider.com/; https://pubchem.ncbi.nlm.nih.gov/; K*ow*: octanol/water coefficient; pKa: negative log of the acid dissociation constant; Water solubility at 20 °C (mg/L); Koc: average coefficient of sorption (mL/g); DT_{50}: time required for the concentration to decline to half of the initial value (days); Kf: Freundlich constant; n.d.: Not data. Epoxide-CBZ (carbamazepine-10,11Epoxi); 4-MAA (4-methylamino-antipyrine); 4-AA (4-amino-antipyrine); 4-FAA (4-formylamino-antipyrine); 4-AAA (4-acetylamino-antipyrine).

metabolites were added to the irrigation water. The total amount of contaminated reclaimed water used was 2200 L. A detailed description of the field-plots location as well as the sampling strategy is given in the Supplementary material section.

2.3. Sample extraction

Vegetable and soil samples were extracted with a modified QuEChERS method, which was based on a method (with some small modifications) that our research group had recently published (García Valverde et al., 2021). Briefly, 10 g of plant tissue samples were weighed in a 50-mL PTFE centrifuge tube, and a surrogate standard mixture was added. Subsequently, the samples were shaken in an automatic axial extractor (AGYTAX®, Cirta Lab. S.L., Spain) for 4 min at 25 °C after the addition of 10 mL of acidified AcN (0.5% v/v, FA). For the soil samples, 5 mL of Milli-Q water was also added before the extraction solvent and left to stand for 5 min. Afterwards, 4 g MgSO4, 1 g NaCl, 1 g $Na_3Citrate \cdot 2H_2O$ and 0.5 g $Na_2HCitrate \cdot 1{,}5H_2O$ were added and centrifuged at 3500 rpm for 5 min. Then, 3 mL of the extract was transferred to a 15-mL PTFE centrifuge tube containing 750 mg of anhydrous $MgSO_4$ and 125 mg C-18, vortexed for 30 s and centrifuged at 3500 rpm for 5 min. In the case of the leaf/root samples, 900 mg $MgSO_4$ and 150 mg ChloroFiltr® were added to eliminate possible pigment interferences during the analysis. Finally, 100 μL of each extract was transferred to vials with screw caps, evaporated to dryness and reconstituted with 100 μL of AcN:water solution (1:9, v/v) containing dimethoate-d6. Fig. 1 shows a diagram of the extraction methods used for each of the studied matrices (soil, leaves/roots, fruit).

The water samples were filtered using a 0.45-mm PTFE syringe filter (Millipore, USA) to remove suspended solids and particulate matter, and then spiked with the selected labeled standard (dimethoate-d6) before analysis.

2.4. Sample analysis and quality control

The high-performance liquid chromatography analyses were performed in a Sciex Exion HPLC system connected to a Sciex 6500+ TripleQuad-LC-MS/MS. The chromatographic and acquisition parameters for the analyses are described elsewhere (García Valverde et al., 2021). The retention times, transitions and collision energies for the analyzed compounds are included as Supplementary material in Table S1. The data analysis was performed with the Sciex Analyst 1.7.1 software for the data acquisition/processing and MultiQuant 3.0.1 software for the data quantification. The criteria for the mass spectrometric confirmation and quantification of the target compounds were in line with current EU regulations (Commission Decision 2002/657/EC, 2002). The trueness, precision, selectivity, sensitivity, range, ruggedness, and limit of quantification of the developed analytical methodology were evaluated according to the EU quality control procedures (European Commission DG-SANTE, 2019).

To ensure the quality of the measurements, continuous monitoring of the analytical procedure was carried out. To check the correct performance of the analytical procedure, several labeled standards were used. Caffeine-13C, carbendazim-d3, dichlorvos-d6, and malathion-d10 were selected as surrogate standards to check the extraction efficiency. Dimethoate-d6 was used as the injection standard. A standard mixture (2 μg/L) containing all the targeted analytes was injected each day before the analysis in order to check the functioning of the analytical column and the mass spectrometer. Blank samples (solvent) were also included during the daily work sequence.

2.5. Bioconcentration factor (BCF) and human exposure

The bioconcentration factor (BCF) was employed to estimate the plant uptake of the selected CECs, similarly to previous published

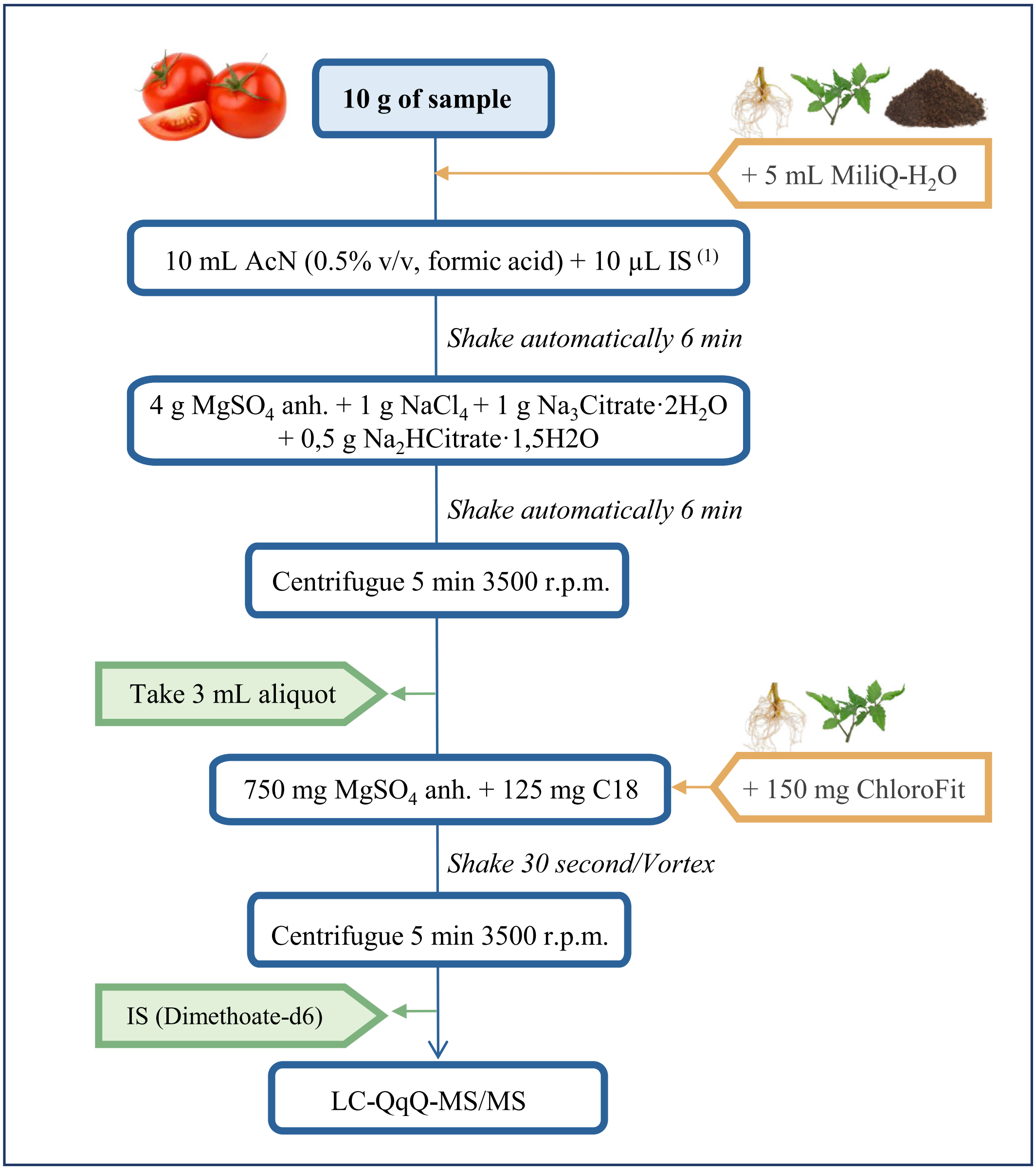

Fig. 1. Scheme of the extraction methods used to extract CECs in each of the matrices studied (fruit, leaf, root, and soil).

works (González García et al., 2019) It was calculated from the concentration of each individual contaminant measured in the tomato against the contaminant concentration applied to the crop via the irrigation water. That is because, the potential efficiency of drip irrigation systems is greater than 90% (Hedley et al., 2014). Thus, the difference between the amount of water that the plant absorbs and that we supply is less than 10%.

$$BCF\ (L/kg) = \frac{concentration\ in\ edible\ part\ of\ plant\ (\mu g/kg)}{concentration\ in\ irrigation\ water\ (\mu g/L)}$$

The daily human intake of each selected CEC was estimated by multiplying the concentration measured in the edible part of the crop (ng/g in f.w.) and the daily consumption per capita of fresh vegetables (g f.w./day). According to the latest reported data from Blázquez (2021), tomato was the most consumed vegetable in Spain in 2019. In fact, its consumption volume amounted to 613 million kilograms in that year, which is 13.3 kg per person/year (36.4 g/day).

3. Results

3.1. Validation of analytical methods

Table 2 summarizes the validation data obtained for the selected target compounds of each compound/matrix combination. The method sensitivity was calculated in terms of the limit of quantitation (LOQ). It was estimated as the lowest spiked level meeting the identification and method performance criteria for recovery and precision (European Commission DG-SANTE, 2019). The values were experimentally evaluated for each analyte/matrix combination. All the compounds showed LOQ values ranging from 0.05 to 0.1 ppb (µg/L or µg/kg) in all the matrices, except to ciprofloxacin and acetaminophen, which presented values of 0.5 ppb in the tomato matrix. Only 20% and 13% of the analytes showed LOQs higher than 0.1 ppb in the non-edible vegetable tissue and the soil matrix, respectively.

The linearity of the analytical response was evaluated based on the linear regression and squared correlation coefficient (r^2). Matrix-matched calibration curves were prepared by fortifying

Table 2
Validation data for target compounds in the reclaimed water, fruit, leaf, root, and soil matrices.

Compound	Water				Tomato					Leaf/root					Soil				
	LOQ	r2	ME	Inter/intraday	LOQ	r2	ME	Rec.	Inter/intraday	LOQ	r2	ME	Rec.	Inter/intraday	LOQ	r2	ME	Rec.	Inter/intraday
Ofloxacin	0.10	0.996	9	2/11	0.10	0.996	9	70	2/19	0.50	0.997	−78	52	3/20	0.10	0.999	−65	n.r	7/18
Pymetrozine	0.05	1.000	0	0/8	0.05	1.000	0	40	3/18	0.10	0.999	−15	47	1/19	0.05	1.000	−6	30	1/12
4-AAA	0.10	0.996	9	1/4	0.10	0.996	9	75	2/10	0.10	1.000	−15	83	2/15	0.10	0.994	−7	73	4/19
Thiamethoxam	0.05	1.000	2	1/7	0.05	1.000	2	97	2/11	0.10	1.000	−29	98	3/10	0.05	0.999	−9	87	1/6
Caffeine	0.05	1.000	2	1/4	0.05	1.000	2	90	3/12	0.05	0.996	−12	92	2/15	0.05	1.000	−6	72	1/16
Hydrochlorothiazide	0.05	1.000	−4	5/3	0.05	1.000	−4	94	3/20	0.10	1.000	17	86	2/9	0.05	1.000	1	85	5/18
Atenolol	0.05	1.000	13	1/5	0.05	1.000	13	116	1/15	0.10	0.998	−64	100	1/6	0.05	0.998	−25	73	314
4-FAA	0.10	0.999	4	2/6	0.10	0.999	4	96	0/6	0.10	1.000	−3	85	1/6	0.50	0.999	0	81	3/4
Ciprofloxacin	0.50	0.997	5	3/7	0.50	0.997	5	80	4/4	0.50	0.998	−68	64	11/13	0.50	0.999	−29	n.r	15/18
Acetaminophen	0.50	1.000	9	1/2	0.50	1.000	9	89	3/15	0.50	1.000	−31	92	3/16	0.50	0.999	−5	76	5/20
4-AA	0.05	0.999	0	1/3	0.05	0.999	0	50	3/20	0.10	0.998	5	74	1/5	0.05	0.999	−2	n.r	4/10
Imidacloprid	0.05	1.000	4	1/2	0.05	1.000	4	91	2/10	0.05	0.999	−40	98	1/11	0.05	1.000	−16	86	2/15
4-MAA	0.05	0.998	−2	1/3	0.05	0.998	−2	52	2/10	0.10	0.998	−19	51	2/13	0.05	1.000	−20	n.r	3/19
Acetamiprid	0.05	1.000	3	1/6	0.05	1.000	3	90	1/8	0.05	0.999	−40	97	1/6	0.05	0.998	−11	83	8/10
Codeine	0.05	0.991	13	2/7	0.05	0.991	13	82	6/7	0.10	1.000	−50	81	1/9	0.05	0.991	−7	50	2/5
Thiacloprid	0.05	0.999	0	3/3	0.05	0.999	0	90	1/9	0.05	1.000	−52	96	1/8	0.05	0.998	−20	82	3/6
Carbendazim	0.05	0.998	6	2/3	0.05	0.998	6	80	2/7	0.10	1.000	−45	99	1/6	0.05	0.998	−17	70	4/4
Epoxide-CBZ	0.05	0.998	5	1/5	0.05	0.998	5	86	5/12	0.05	0.998	−30	91	2/11	0.05	0.995	−13	68	3/10
Furosemide	0.10	1.000	6	4/6	0.10	1.000	6	94	2/9	0.50	1.000	−5	95	1/4	0.50	1.000	2	63	15/7
Diuron	0.05	1.000	1	2/6	0.05	1.000	1	80	2/6	0.10	1.000	−36	82	1/15	0.05	0.998	−11	79	2/6
Thiabendazole	0.05	0.998	8	1/1	0.05	0.998	8	82	1/6	0.05	0.999	−50	80	1/5	0.05	0.992	−15	62	7/19
CBZ	0.05	0.998	4	2/8	0.05	0.998	4	82	2/9	0.05	0.999	−31	89	1/12	0.05	1.000	−26	79	6/3
Azoxystrobin	0.05	0.997	3	1/4	0.05	0.997	3	90	1/9	0.05	0.995	−30	117	1/9	0.05	0.992	−33	71	5/6
Fluxapyroxad	0.05	1.000	−1	1/5	0.05	1.000	−1	83	2/11	0.05	0.998	−46	70	3/14	0.05	0.999	−21	73	4/3
Myclobutanil	0.05	1.000	−2	1/2	0.05	1.000	−2	87	1/10	0.05	0.997	−35	56	1/8	0.05	0.999	−17	81	15/15
Naproxen	0.10	1.000	7	1/9	0.10	1.000	7	86	1/12	0.50	0.999	−54	74	9/14	0.10	1.000	−17	82	3/9
Diazinon	0.05	0.998	1	1/1	0.05	0.998	1	84	0/16	0.05	1.000	−50	60	2/15	0.05	1.000	−34	84	4/6
Penconazole	0.05	1.000	−1	2/2	0.05	1.000	−1	84	1/18	0.10	0.997	−40	28	1/3	0.05	0.999	−30	73	7/19
Diclofenac	0.10	1.000	2	2/3	0.10	1.000	2	77	3/7	0.10	1.000	−20	10	2/5	0.10	0.997	−18	86	15/10
Gemfibrozil	0.05	1.000	0	1/8	0.10	1.000	0	87	3/19	0.50	0.992	−16	28	3/17	0.10	0.999	−33	76	9/13

LOQ: Limits of quantification (μg/L; μg/kg); Linearity expressed by the correlation coefficient (r2); ME: Matrix effect (%); Rec: Average recoveries (n = 5, %, 1 μg/kg to tomato, 10 μg/kg to leaf/soil); Inter/Intra repeatability expressed as relative standard deviation (RSD, %), n.r: Not recovered; Epoxide-CBZ (carbamazepine-10,11Epoxi); CBZ: Carbamazepine; 4-MAA (4-methylamino-antipyrine); 4-AA (4-amino-antipyrine); 4-FAA (4-formylamino-antipyrine); 4-AAA (4-4-acetylamino-antipyrine).

blank extracts of each matrix at five concentration levels (from 0.05 to 5 μg/L for the water and from 0.05 to 50 μg/kg for the soil and vegetable tissue). These were used to minimize matrix interference and to avoid any under/over estimation during the quantification step. All the selected compounds presented a very good response of three orders of magnitude, with correlation coefficients above 0.992 in all cases. Likewise, the matrix effects were studied comparing the calibration curve slopes in the matrix and the solvent. According to our results, no matrix effect (≤20%) was observed in the water or the tomato matrices (see Table 2). In the leaf matrix, only 5 of the 30 studied compounds presented a matrix effect over 50% (strong). In the case of the soil matrix, 70% of the targeted CECs presented no matrix effect, 27% showed an intermediate matrix effect (between 20 and 50%) and only one, ofloxacin, had a strong matrix effect (>50%).

The recovery studies evaluated per quintuplicate ($n = 5$) using spiked samples with each of the compounds selected in this study at 1 ng/g for the tomato, and 10 ng/g for the leaf and soil matrices. All the targeted CECs showed recovery values above 70% in the tomato matrix, except for two metabolites of the analgesic metamizole (4-AA and 4-MAA) and the insecticide pymetrozine (see Table 2). Regarding the leaf/root and soil matrices, 21 compounds were recovered above 70%. Only three compounds (penconazole, diclofenac and gemfibrozil) presented values below 30% in the non-edible tissue whereas four pharmaceuticals (4-AA, 4-MAA, ofloxacin and ciprofloxacin) were not recovered from the soil samples.

The repeatability and reproducibility expressed as the relative standard deviation (RSD, %) ranged between 0% and 20% in both cases. These results demonstrate the method's analytical precision and, therefore, its effectiveness for quantitative purposes. Finally, three blank samples of each matrix studied (water, fruit, leaf, and soil), extracted by the proposed method, were analyzed to assess the method's specificity and selectivity. At the specific retention times for the target compounds, no other significant peaks were detected.

3.2. Presence of CECs in the vegetable and environmental samples

Tomatoes grown in an experimental greenhouse with drip irrigation, consumed a total of 2200 L of contaminated reclaimed water during the three months of the crop cycle. The results obtained showed that the tomato crop in a greenhouse consumed a total of approx. 8 L of water by kg of plant, and therefore an average of 90 mL of water per day by plant. The data showed that 2 m^3 of water, that is to say, 2000 l, produced 150 kg of drip-irrigated greenhouse tomatoes. The analytical approach developed was applied to agricultural samples obtained from a pilot study under real-world field conditions. Three independent extractions of each sample type were analyzed. The internal standards (extraction and injection) were recovered between 70 and 120% in all cases. No targeted CEC residues were detected in the control samples (natural water, plant, and soil) at concentration levels higher than the LOQ values. The concentration ranges and average levels of the pesticides and pharmaceutical products found in each part of the crop irrigated with contaminated reclaimed water are summarized in Table 3. In accordance with the International System of Units, the data concentrations for the vegetable tissue were presented as fresh weight (f.w.) whereas for the soil, they were presented as dry weight (d.w.).

Table 3
Concentration levels of CECs detected in the plant and environmental samples ($n = 3$).

Compound	Reclaimed water[a]			Tomatoes			Leaf			Root					Soil
	Range μg/L	Average μg/L	μg	Range μg/kg	Average μg/kg	μg	Range μg/kg	Average μg/kg	μg	Range μg/kg	Average μg/kg	μg	Range μg/kg	Average μg/kg	μg
Pharmaceutical															
Ofloxacin	0.9–1.2	1.1	2395	–	–	–	–	–	–	–	–	–	–	–	–
4-AAA	n.a	n.a	n.a	–	–	–	–	–	–	–	–	–	–	–	–
Caffeine	0.7–0.9	0.8	1737	0.7–1.2	0.9	135	0.3–0.4	0.4	48	2.9–3.4	3.3	16	0.8–1.0	0.9	372
Hydrochlorotiazide	0.3–0.4	0.4	882	0.4–1.1	0.8	116	0.1	0.1	12	0.2–0.3	0.3	1	–	–	–
Atenolol	0.9–1.0	1.0	2243	–	–	–	–	–	–	2.0–2.5	2.4	12	0.1	0.1	28
4-FAA	n.a	n.a	n.a	1.5–2.3	2.1	308	–	–	–	–	–	–	–	–	–
Ciprofloxacin	0.7–1.2	0.9	1938	–	–	–	–	–	–	–	–	–	–	–	–
Acetaminophen	0.8–1.1	1.0	2205	–	–	–	–	–	–	–	–	–	–	–	–
4-AA	n.a	n.a	n.a	–	–	–	–	–	–	–	–	–	–	–	–
4-MAA	n.a	n.a	n.a	–	–	–	–	–	–	–	–	–	–	–	–
Codeine	0.7–1.0	0.9	1985	–	–	–	0.1	0.1	14	–	–	–	0.1–0.2	0.2	62
Epoxide-CBZ	n.a	n.a	n.a	0.1–0.2	0.2	23	0.4–0.6	0.5	63	–	–	–	–	–	–
Furosemide	0.1–0.3	0.2	441	0.1	0.1	21	–	–	–	–	–	–	–	–	–
Carbamazepine	0.8–1.0	0.9	1898	0.1	0.1	9	0.2–0.4	0.3	33	9.5–13.0	11.0	53	1.0–1.3	1.2	492
Naproxen	0.5–0.6	0.5	1103	≤LOQ	≤LOQ	≤LOQ	–	–	–	–	–	–	–	–	–
Diclofenac	1.0–1.2	1.1	2505	1.4–1.9	1.7	260	0.6–0.7	0.7	84	12.1–15.1	14.3	69	0.5–0.7	0.6	246
Gemfibrozil	0.6–0.7	0.6	1420	–	–	–	–	–	–	–	–	–	–	–	–
Total		9.4	20.751		5.8	871		2.1	254		31.3	150		3.0	1.200
Pesticide															
Pymetrozine	0.5–0.6	0.5	1078	0.1–0.2	0.2	26	–	–	–	2.0–2.7	2.5	12	1.0–1.1	1.0	410
Thiamethoxam	0.7–0.8	0.8	1764	1.5–1.9	1.8	267	0.3–0.4	0.4	47	1.8–2.1	2.0	10	0.8	0.8	328
Imidacloprid	0.5–0.7	0.6	1421	0.1–0.2	0.2	30	0.3–0.5	0.4	48	4.3–5.0	4.8	23	0.7–0.9	0.5	205
Acetamiprid	0.7–0.8	0.8	1807	0.1	0.1	15	0.1	0.1	10	2.8–3.6	3.5	17	0.1	0.1	34
Thiacloprid	0.9–1.0	1.0	2191	0.1	0.1	12	0.1–0.2	0.2	18	1.0–1.3	1.2	6	0.3–0.4	0.4	164
Carbendazim	0.8–1.0	0.9	2080	0.1	0.1	11	0.1	0.1	17	1.0–2.0	1.9	9	0.6	0.6	238
Diuron	0.7–0.9	0.8	1764	0.1	0.1	9	0.4–0.5	0.5	60	2.0–3.0	2.3	11	0.3–0.4	0.4	165
Thiabendazole	0.5–0.6	0.6	1260	0.1	0.1	12	0.1	0.1	11	6.8–8.6	8.0	38	0.7	0.7	300
Azoxystrobin	0.7–0.9	0.8	1804	0.1–0.2	0.2	32	0.1–0.2	0.2	25	9.0–11.2	10.0	48	1.0–1.3	1.2	492
Fluxapyroxad	0.7–0.8	0.8	1654	0.1	0.1	10	0.4–0.6	0.5	60	14.2–16.7	15.8	76	1.2–1.4	1.3	533
Myclobutanil	0.7–0.9	0.8	1684	0.1	0.1	9	0.5–0.6	0.6	72	15.2–16.8	16.0	77	2.0–2.2	2.0	820
Diazinon	0.3–0.4	0.4	794	0.1	0.1	20	0.1	0.1	7	3.0–4.5	4.0	20	0.3–0.4	0.4	164
Penconazole	0.5–0.7	0.6	1315	≤LOQ	≤LOQ	≤LOQ	0.1–0.2	0.2	20	16.0–18.0	17.3	83	1.5–3.0	2.0	820
Total		9.3	20.616		3.0	452		3.5	396		89.3	429		11.4	4.673

[a] Reclaimed water spiked at 1 μg/L of each selected CECs; LOQ: Limits of quantification; n.a: not add; R.S·D: Relative standard deviation (%).

3.2.1. CECs in the irrigation water

The total amount of CECs released over the three months of the crop cycle was 41,367 μg, which correspond to a total of 18.7 μg/L in the water. The analysis of the water samples showed that, generally, all the compounds were presented at levels between 0.9 μg/L and 1.1 μg/L, except for the insecticide diazinon (0.4 μg/L), and the diuretics furosemide (0.2 μg/L) and hydrochlorothiazide (0.4 μg/L). These lower concentrations (with respect to the added concentrations) may be due to degradation processes. Other authors have reported that these compounds are highly sensitive to UV light exposure (Mansour et al., 1997; Cies et al., 2015), with degradation rates of 30% and 50% for furosemide and diazinon, respectively. No degradation products of the anti-epileptic carbamazepine (carbamazepine-10,11-epoxide) or the analgesic metamizole (4-methylamino-antipyrine; 4-amino-antipyrine, 4-formylamino-antipyrine and 4-4-acetylamino-antipyrine) were detected in the irrigation water.

3.2.2. CECs in the tomatoes

Out of the 30 compounds selected in this study, 12 pesticide residues and 7 pharmaceutical products were identified in the tomato samples irrigated with contaminated reclaimed water (see Table 3). Of the pesticides, the insecticide thiamethoxam was found at the highest concentration level (1.8 μg/kg f.w.). The rest of the pesticides included in this study were also detected in the tomato at concentrations ranging from 0.1 to 0.2 μg/kg f.w. Only the fungicide penconazole was found at levels below its LOQ (<0.05 μg/kg). The analgesic diclofenac, the stimulant caffeine, and the diuretic hydrochlorothiazide were the pharmaceuticals detected at the highest levels - 1.7, 0.9 and 0.8 μg/kg f.w., respectively. The anti-epileptic carbamazepine (CBZ) and the diuretic furosemide were also found in the edible part of the plant, but at lower concentrations (up to 0.1 μg/kg f.w.). These results are in agreement with previous works. Wu et al. (2014) detected CBZ levels ranging from 0.19 ± 0.32 ng/g in tomatoes grown using fortified water in irrigated plots at approx. 300 ng/L. Neither of the antibiotics evaluated in this study (ofloxacin and ciprofloxacin) were detected. In contrast, two transformation products that had not initially been added to the irrigation water were determined in tomato samples: carbamazepine-10,11-epoxide (epoxy-CBZ) and 4-formylamino-antipyrine (4-FAA). The first of these, epoxy-CBZ, was measured at higher levels than the parent product (CBZ), 0.2 μg/kg f.w., while the second, the metabolite of the analgesic metamizole, was the drug found at the highest levels in the fruit, up to 2.1 μg/kg f.w. Previous works have reported the occurrence of transformation products in crops irrigated with treated municipal wastewater; however, all were taken up from the water by the plants (Margenat et al., 2018; Picó et al., 2019). For example, Margenat et al. (2018) reported similar levels of epoxy-CBZ in lettuce grown in a peri-urban area of northern Spain using furrow-irrigated water in open air channels from industrial, urban and agricultural activities. Nevertheless, the results from this study suggest that CBZ and metamizole can also be metabolized in the soil or synthesized in the plant since neither were detected in the irrigation water samples analyzed at the dripper outlets. This hypothesis is supported by other authors. Paz et al. (2016) suggest that CBZ is metabolized in the soil and, therefore, its main metabolite, 10,11-epoxy-CBZ, is available for uptake. In another work, Malchi et al. (2014) reported that the metabolite is synthesized in the plant through the metabolization of its precursor (CBZ) by CYP450 enzymes.

The presence of the pharmaceuticals (caffeine, hydrochlorothiazide, 4-FAA, and CBZ) and the pesticides (imidacloprid, diuron, and fluxapyroxad) can be explained based on their pKa values (>8.0, see Table 1). These non-ionic compounds can pass through the plants' cell membranes, entering via the roots and translocating to other parts of the plant through transpiration. Nonetheless, diclofenac, despite being an ionic compound (with a pKa of 4.1), was detected in the tomato samples. According to its properties, diclofenac has a negative charge in the soil environment (pH 7.7) so it should be repulsed by the cells in the roots – in our case, however, it was detected in the fruit. This is in line with the results reported by Christou et al. (2017), in which diclofenac was detected in tomato samples when the crop was irrigated for long periods with wastewater. The authors reported concentration levels ranging from 1.3 to 11.63 μg/kg f.w. in the tomatoes. Picó et al. (2019) explained the presence of ionic compounds in the plants as being due to differing pH levels, depending on the plant organs. Therefore, the pH could reach values as low as 4 in some tissues, meaning that these compounds would be primarily neutral. In fact, the pH value for a ripe tomato is about 4.6. This theory explains the high diclofenac levels found in the tomatoes in the present work.

In summary, the total amounts of pharmaceutical products and pesticide residues found in the tomato samples were 871 μg and 452 μg, respectively, which correspond to a total of 8.8 μg/kg f.w. The results suggest that all these CECs have the potential to accumulate in the fruits. However, the total load of CECs is not solely due to the contribution of contaminants present in the irrigation water, but the metabolization/degradation processes produced in the plant or in the soil are also another source of products, such as transformation products.

3.2.3. CECs in the leaves/roots

To determine the plant translocation rate, the roots and leaves (including stems) were separated and individually analyzed. In the case of the leaves, 6 pharmaceutical products and 12 pesticides residues were detected. Diclofenac and the CBZ metabolite (epoxide-CBZ) were the drugs measured at the highest concentrations, 0.7 and 0.5 μg/kg, respectively. The highest average concentration values were measured as follows: diclofenac (0.7 μg/kg f.w.), epoxide-CBZ (0.5 μg/kg f.w.), caffeine (0.4 μg/kg f.w.), and CBZ (0.3 μg/kg f.w.). In contrast, the lowest concentrations were found for hydrochlorothiazide and codeine (0.1 μg/kg f.w.). Regarding the pesticide residues, myclobutanil, diuron, fluxapyroxad, thiamethoxam and imidacloprid were the substances detected at the highest levels in the leaves, ranging from 0.5 to 0.7 μg/kg f.w. Similar results were observed in the roots, where 5 pharmaceutical products and 13 pesticides were found. CBZ and diclofenac were the drugs measured at higher concentrations, 11 and 14.3 μg/kg f.w., respectively. Thiabendazole, azoxystrobin, fluxapyroxad, myclobutanil and penconazole were the pesticides found in the roots at levels above 8 μg/kg. The other pesticides studied in this work were also detected at concentrations ranging from 0.1 to 0.2 μg/kg f.w. in the leaves and from 1.2 to 4.8 μg/kg f.w. in the roots (see Table 3). In general, the CEC concentrations detected in the roots were up to 10-times higher than those found in the leaves. These findings accord well with other studies, which suggest that CEC accumulation generally decreases in the order of root > leaf/stem. Wu et al. (2013) carried out a study to compare the translocation of pharmaceutical and personal care products (PPCPs) by common vegetables. The authors found higher concentrations of diclofenac in the roots than in the leaves in four different vegetable species (lettuce, spinach, cucumber and pepper). Ju et al. (2019) suggested that hydrophobic compounds with low water solubility accumulate more in the roots than in the leaves because the translocation is restricted. Curiously, the CECs detected at higher levels in the roots have moderate hydrophobicity (a logK_{ow} between 2.5 and 3.7) and low water solubility (<132 mg/L), which coincides with that discussed above.

Overall, the total amounts of pharmaceutical products and pesticide residues found in the leaves were 254 μg and 396 μg, respectively, whereas in the roots, the amounts were 150 μg and 429 μg, respectively. These measurements correspond to a total load of 5.6 μg/kg f.w. in the leaves and 120.6 μg/kg f.w. in the roots. The main CBZ metabolite was only detected in the leaves, thus supporting the hypothesis that it is synthesized in the plant.

3.2.4. CECs in the soil

All the targeted pesticides (13) and pharmaceuticals (5) were detected at concentrations above their LOQs in the agricultural soil samples irrigated with contaminated reclaimed water. The CECs total concentration was measured by kg of soil dry weight. The pesticide residues were found at levels between 0.1 and 2.0 μg/kg d.w. The fungicides myclobutanil and penconazole were the compounds found at the highest concentrations (2.0 μg/kg d.w.) whereas the insecticide acetamiprid was detected at the lowest level (0.1 μg/kg d.w). The physicochemical properties of these compounds support these data. As we can see in Table 1, in general, persistent pesticides (DT_{50} ≥ 75 days) were detected at higher concentrations than those with high mobility and low persistence in the soil. Similar results have recently been reported in different types of agricultural soil samples for penconazole (3.9 μg/kg d.w.) and myclobutanil (2.4 μg/kg d.w.) (Acosta-Dacal et al., 2021). Concerning the pharmaceutical products, the average concentrations ranged from 0.1 to 1.2 μg/kg d.w. Carbamazepine was the drug measured at the highest levels in the soil samples analyzed, followed by caffeine, with average concentrations of 1.2 and 0.9 μg/kg d.w., respectively. Conversely, codeine and atenolol were quantified at the lowest concentrations, 0.2 and 0.1 μg/kg d.w., respectively. Caffeine was the second pharmaceutical detected at high levels, with an average concentration of 0.9 μg/kg d.w. Previous studies agree with our result. Beltrán et al. (2020) found CBZ concentrations higher than atenolol in three different crops. Conversely, caffeine levels 10-times higher than those found in this study were reported in soil samples irrigated with reclaimed water in Saudi Arabia (Picó et al., 2019). Despite their high persistence (DT_{50} ≥ 1000 days) and low mobility in soil, neither of the antibiotics selected in this study (ofloxacin and ciprofloxacin) were detected in any of the soil samples analyzed. This can be explained because these compounds were not recovered from the soil samples using the developed extraction method. Two diuretics (furosemide and hydrochlorothiazide), and the analgesic acetaminophen, were not detected in any of the soil samples analyzed in this study. This can be explained because all are bio/photodegradable compounds, which is in line with the bibliographic data (see Table 1).

In summary, the total amounts of pesticides and pharmaceuticals measured in the agricultural soil samples were 4673 μg and 1200 μg, respectively, which corresponds to a total of 14.4 μg/L d.w. None of the selected degradation products were detected in the soil samples analyzed.

3.3. Accumulation and modeling of the CECs in a tomato crop

The accumulation rates were calculated from the concentrations of individual contaminants measured in each part of the crop against the contaminant concentration applied to the crop via the irrigation water. The results from the pilot study conducted in a greenhouse under real field conditions showed that the average total load measurements were as follows: 41368 μg in the irrigation water, 5873 μg in the soil, 1323 μg in the tomatoes, 650 μg in the leaves, and 579 μg in the roots. Considering these values, the accumulation rates in each part of the crop were: 14% in the soil, 3% in the edible part (fruit), 2% in the leaves, and 1% in the roots (see Fig. 2).

3.3.1. Accumulation in the fruit

The CECs' accumulation percentages in the tomatoes ranged from 0.5% to 15%, both for the pesticides and for the pharmaceutical products. Thiamethoxam (15%), 4-FAA (15%), hydrochlorothiazide (13%), diclofenac (10%), and caffeine (8%) were the compounds detected at the highest accumulation rates. All these substances have a high polarity (−0.1 ≤ log*Kow* ≤ 0.2), expect diclofenac, which has a log*Kow* = 4. The concentrations of the detected compounds were compared with the log*Kow* values. As can be seen in Fig. 3, a trend in the outcomes was observed. The experimental data obtained under agronomic field conditions were used to determine a mathematical equation. Most of the compounds studied fitted well to a second-order quadratic equation ($y = 0.0005x^2 - 0.0167x + 0.1705$), the correlation coefficient (r^2) being acceptable at 0.8857. Subsequently, the theoretical accumulation values for each compound were calculated using the equation obtained

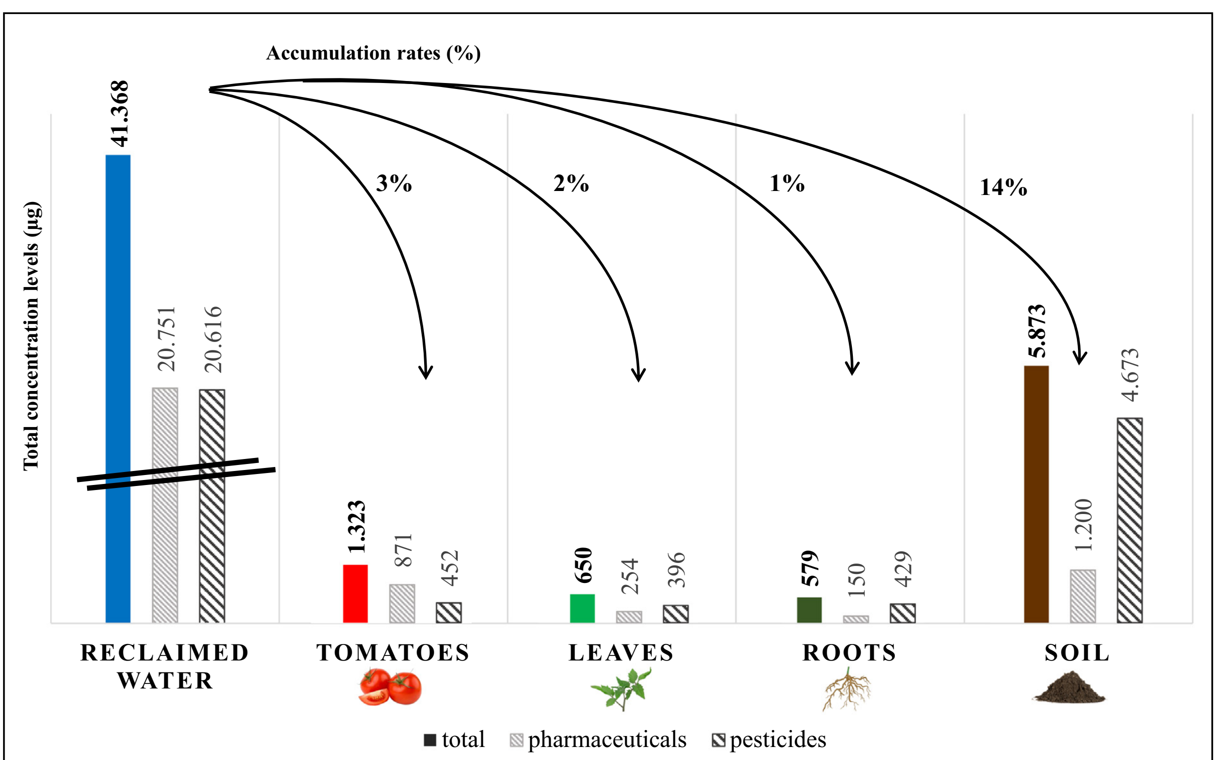

Fig. 2. Distribution of the total concentration levels (μg) and the accumulation rates (%) of the pharmaceuticals and pesticides detected in each part of the crop (reclaimed water, fruit, leaf, root, and soil).

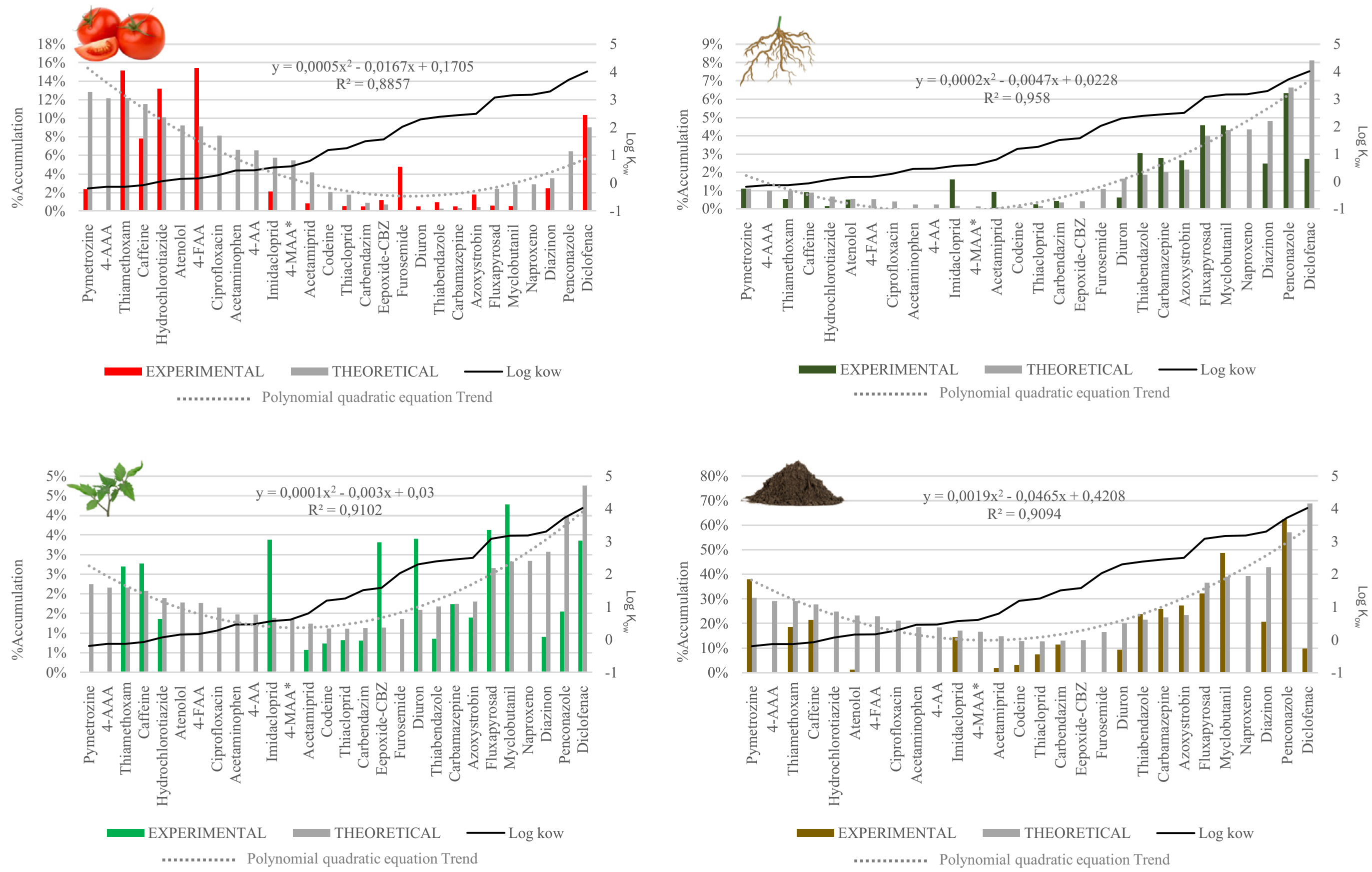

Fig. 3. CEC accumulation (%) detected in each part of the crop (tomato, leaf, root, and soil).

from the experimental data. As can be seen in Fig. 3, the detected compounds generally presented experimental values higher than the theoretical values. The variations between the experimental and theoretical results were lower than 70% in all the detected cases, except for pymetrozine, acetamiprid, furosemide, azoxystrobin, fluxapyroxad and myclobutanil. These data are supported by the low half-life values for acetamiprid (DT_{50} = 1.6 days), or the low mobility in the soil of pymetrozine, fluxapyroxad and myclobutanil. Acetaminophen and penconazole were not detected in any of the tomato samples analyzed in this study. This can be explained because acetaminophen is a photodegraded compound whereas penconazole is a persistent compound in the soil (see Table 1).

3.3.2. Accumulation in leaves/roots

The accumulation percentages of the pesticides and the pharmaceutical products in the non-edible parts of the plants were similar. In the leaves, they ranged from 0.9% to 4% for the pesticides and from 1% to 3% for the drugs, whereas in the roots, they ranged from 0.3% to 6% and from 0.2% to 3%, respectively. The highest accumulation rates in the leaves were presented by the fungicides fluxapyroxad and myclobutanil (4%) followed by thiamethoxam, caffeine, imidacloprid, epoxy-CBZ, diuron, and diclofenac (3%). In the roots, the fungicide penconazole presented the most elevated accumulation rates (6%) followed by the fungicides fluxapyroxad and myclobutanil (5%), and by the CECs azoxystrobin, CBZ, thiabendazole, and diclofenac (3%). Again, a correlation between the log*Kow* and the CECs' accumulation in the plant tissue (the non-edible parts) was observed. Two different polynomial equations having adequate r^2 were obtained both for the leaves and for the roots. Fig. 3 summarizes the variations between the experimental and the theoretical results. For the leaves, the following mathematical equation, $y = 0.0001x^2 - 0.003x + 0.03$, was obtained with an $r^2 = 0.912$. The variations between the experimental and the theoretical results were below 70% for all the detected compounds, except for imidacloprid, epoxide-CBZ, and diuron. Most of them presented experimental values lower than the theoretical values. With respect to the roots, another polynomial quadratic equation was obtained ($y = 0.0002x^2 - 0.0047x + 0.0228$) with a satisfactory coefficient ($r^2 = 0.958$). As can be seen in Fig. 3, the experimental results were similar to the theoretical results. Only three compounds (hydrochlorothiazide, imidacloprid, and acetamiprid) presented differences higher than 70%.

3.3.3. Accumulation in the soil

The accumulation percentages of the pesticides and the pharmaceutical products in the soil ranged from 2% to 62% and from 1% to 26%, respectively. The pesticides found at the highest accumulation rates were penconazole (62%), myclobutanil (49%), pymetrozine (38%), and fluxapyroxad (32%). Thiabendazole, azoxystrobin and diazinon were the other pesticides detected at high percentages, between 21% and 27%. Acetamiprid was the pesticide measured at the lowest accumulation percentage (2%) in the agricultural soil samples. The biodegradation data supported this low value (DT_{50} in soil = 1.6 days). The pharmaceuticals CBZ (26%), caffeine (21%), and diclofenac (10%) were the compounds with the highest accumulation percentages in the agricultural soil. Atenolol and codeine were the pharmaceuticals found at the lowest accumulation percentages in the agricultural soil samples, at 1% and 3%, respectively. Looking at the physicochemical properties (see Table 1), both compounds are highly hydrophilic in character with a log*Kow* ≤ 1.2, implying a light binding to the soil; however, codeine is also a photodegradable compound whereas atenolol has a high mobility in soil (solubility in water = 13,300 mg/L). Hence, the experimental results under controlled conditions suggested that the CECs' accumulation in the soil was strongly influenced by the lipophilic

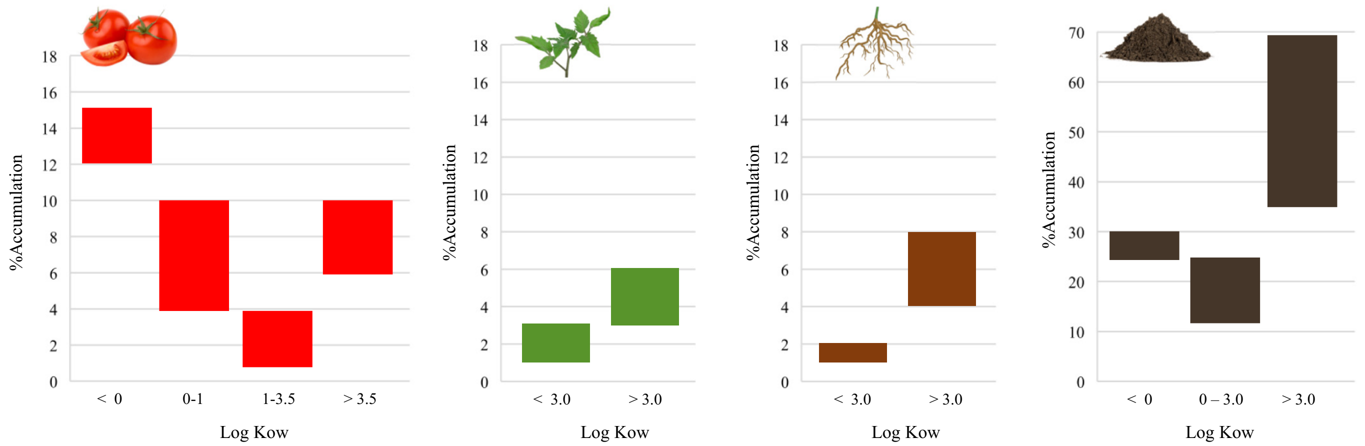

Fig. 4. CEC uptake model in each part of the crop based on the log*Kow* parameter.

character of the compounds. Overall, CECs with moderate and high polarities (log*Kow* ≥ 2.5) presented greater accumulation rates in the soil (see Fig. 3). Pymetrozine and caffeine also presented high accumulation rates, 38% and 21%, respectively. Despite their low log*Kow* values (−0.2 and −0.1, respectively), both compounds have high sorption coefficients in soil (Koc between 246 and 7875 mL/g and between 741 and 7762 mL/g, respectively), which explains how they can be firmly fixed to the organic matter in the soil and accumulate in it. A linear curve derived from plotting the concentration of each of the contaminants measured in the agricultural soil against their log*Kow* values was obtained. Again, the majority of the detected compounds fitted well to a second-order quadratic equation ($y = 0.0019x^2 - 0.0465x + 0.4208$), with a suitable correlation coefficient ($r^2 = 0.9094$). The differences between the experimental/theoretical results observed in the soil samples ranged from 9% to 53%, the exceptions being atenolol (95%), imidacloprid (87%), codeine (76%), and diclofenac (86%). Diclofenac presented an accumulation rate of 10%. However, given its high log*Kow* value (4.0) and its low solubility in water (2 mg/L), higher percentages should be found. As reported in previous works, diclofenac is easily degraded in the environment (Carter et al., 2014), which support this low result.

Overall, some small differences between the experimental/theoretical accumulation data were observed in all the matrices analyzed. In general, the mathematical equations found in this study allowed a suitable estimation of the accumulation percentages in each part of the tomato crop (the soil, leaves, roots and fruit) for many different classes of CECs, based on the hydrophobic/lipophilic character of the compounds. However, the accumulation rates were also influenced by other parameters such as persistence, mobility, and solubility.

Finally, the data obtained were used to establish an uptake model of the contaminants in each part of the crop, based on the log*Kow* parameter (see Table 1). It is expected that compounds with a log*Kow* below 0 will present accumulation rates between 12% and 15%, while compounds with a log*Kow* greater than 3.5 will have an uptake between 6% and 10% in the tomatoes. In the soil, CECs with a log*Kow* greater than 3 will present an accumulation rate between 40% and 70%, while those with a log*Kow* lower than 3 will accumulate at between 10% and 30%. In the leaves and roots, only compounds with a log*Kow* greater than 3 will reach accumulation levels between 4% and 8%.

3.4. Human exposure

This work has shown that tomato plants, when irrigated with reclaimed water containing CECs, are capable of selectively accumulating contaminants in their edible parts. In all cases, the values for the detected CECs complied with the levels established for tomato by the latest Regulation, (EC) No 155/2021, applicable from 02/09/2021, concerning maximum residue levels (MRLs) for pesticides in food (European Commission, 2021). As can be seen in Table 4, these were between 100 and 10,000-times lower than the levels established by the European Commission. According to our experimental data, the pesticides detected in the fruit posed no risk to human health.

Three pharmaceutical products and one pesticide were the compounds with the highest bioconcentration factor values (BCF). Caffeine, diclofenac, and hydrochlorothiazide had BCF values of 1.1, 2.0 and 1.5 (L/kg), respectively, indicating that they tend to accumulate a lot in the fruit. Thiamethoxam was the only pesticide with a high BCF value (2.3 L/kg); of all the selected CECs, this compound had the greatest tendency to accumulate in the fruit.

Consumption of these contaminated tomatoes could potentially pose a risk to humans via their dietary intake. Some scientific papers have reported on studies regarding the human health risks that arise from consuming vegetables irrigated with treated water containing contaminants, especially pharmaceutical and personal care products (PPCPs). Most of these works have been carried out under hydroponic conditions (Wu et al., 2013), under unrealistic agricultural conditions (Malchi et al., 2014), or in field trials at concentration levels higher than those expected in reclaimed water (González García et al., 2019). In our study, we estimated the daily human intake values for each detected CEC (pesticides and pharmaceutical products) based on experimental data obtained from the pilot study carried out under agronomic conditions and considering the latest data reported on per capita consumption of fresh tomato (Blázquez, 2021). As can be seen in Table 4, the highest daily human exposure from consuming contaminated tomatoes in a conventional diet came from the metamizole metabolite (4-FAA, 0.075 μg/day), followed by diclofenac (0.062 μg/day), caffeine (0.033 μg/day), and hydrochlorotiazide (0.029 μg/day), while thiamethoxam was the only pesticide with a high daily exposure value (0.066 μg/day). On the other hand, it has been estimated that these values will be as much as 3-times higher in a vegetarian diet than in a conventional diet, ranging from 0.225 μg/day (4-FAA) to 0.09 μg/day (hydrochlorotiazide). These results are in agreement with another work, in which Wu et al. (2014) reported annual CBZ exposure levels (0.08 μg/year) in tomato grown using treated water fortified at 0.2 μg/L (0.9 μg/L in our case). The total daily values for exposure to pesticides and pharmaceutical products were 0.11 μg/day and 0.21 μg/day, respectively, in a conventional diet; whereas the values rose to 0.33 μg/day and 0.64 μg/day, respectively, in a vegetarian diet. These amounts were more than 3 orders of magnitude less than are present in a single medical dose of these pharmaceutical products (typically between 10 and 200 mg), both for the conventional and vegetarian diets.

The definition of ADI (the acceptable daily intake) is established as "an estimate of the amount of a residue, expressed on a body-weight basis, that can be ingested daily over a lifetime without appreciable

Table 4
Estimated per capita daily exposure values to CECs (μg) from the intake of vegetables.[a]

Compound	Water	Tomato			Daily human intake (μg/day)		
	Average concentration (μg/kg)	Average concentration (μg/kg)	MRL (μg/kg)	BCF (L/kg)	Conventional diet	Vegetarian diet	ADI (mg/day)
Pharmaceutical							
Caffeine	0.8	0.9	–	1.1	0.033	0.10	1000
Hydrochlorotiazide	0.4	0.8	–	2.0	0.029	0.09	3500
4-FAA	Not add	2.1	–	–	0.075	0.22	–
Epoxide-CBZ	Not add	0.2	–	–	0.005	0.02	–
Furosemide	0.2	0.1	–	0.7	0.005	0.02	1500
CBZ	0.9	0.1	–	0.1	0.004	0.01	1200
Diclofenac	1.1	1.7	–	1.5	0.062	0.19	150
Pesticide							
Pymetrozine	0.5	0.2	20	0.4	0.007	0.02	2.1
Thiamethoxam	0.8	1.8	200	2.3	0.066	0.20	1.8
Imidacloprid	0.6	0.2	500	0.3	0.007	0.02	4.2
Acetamiprid	0.8	0.1	500	0.1	0.004	0.01	1.7
Thiacloprid	1.0	0.1	500	0.1	0.003	0.01	0.7
Carbendazim	0.9	0.1	300	0.1	0.003	0.01	1.4
Diuron	0.8	0.1	10	0.1	0.002	0.01	0.5
Thiabendazole	0.6	0.1	10	0.1	0.003	0.01	70
Azoxystrobin	0.8	0.2	3000	0.2	0.007	0.02	14
Fluxapyroxad	0.8	0.1	600	0.1	0.002	0.01	1.4
Myclobutanil	0.8	0.1	600	0.1	0.002	0.01	1.7
Diazinon	0.4	0.1	10	0.4	0.005	0.01	0.01
Total					0.323	0.968	

[a] Data calculated from a tomato crop irrigated with contaminated reclaimed water at 1 μg/L under controlled field conditions; MRL: maximum residue levels of pesticides in tomato (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN); BCF: bioconcentration factor; Consumption data: 13.3 kg per person/year in a conventional diet (https://www.statista.com/statistics/745474/fresh-vegetables-consumption-per-person-in-spain-2015-by-product/#statisticContainer), in a vegetarian diet is estimated 3 times more (approx. 40 kg per person/year); ADI: Maximum acceptable daily intake without appreciable health risk, for pesticides estimated values considering an average weight of 70 kg per individual (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN; https://www.vademecum.es).

health risk" (World Health Organization, 1987). These values are summarized in Table 4, taking 70 kg as the average weight of an individual. In all cases, the estimated daily human intake values were more than 3 orders of magnitude less than the acceptable limits, except in the case of diazinon. This pesticide was found at similar levels to the maximum daily intake value for a vegetarian diet (0.01 μg/day) at which there is no health risk. However, given that fortified irrigation water (in the worst-case scenario, this would be about 1 μg/L) was used to obtain these preliminary experimental data, the human exposure from consuming vegetables irrigated with reclaimed water is expected to be even lower than the above results. A more realistic view of the risk to human health that the consumption of these products entails would be the evaluation of the synergistic effect of exposure to the mixture of detected CECs in them. However, according to the best of authors' knowledge, the current models to consider a mix of effects are not established.

4. Conclusion

From the total amount of CECs released during the crop irrigation (41×10^3 μg), the soil samples exhibited the highest CEC content (5873 μg), followed by the fruit (1323 μg), leaves (650 μg), and roots (579 μg). These values represent accumulation rates from the total load of 14%, 3%, 2%, and 1%, respectively. Overall, the edible part of the plant presented a total CEC amount between 40 and 50% more than the non-edible parts. Some CECs (e.g., ofloxacin, ciprofloxacin, acetaminophen, and gemfibrozil) were not detected in any plant tissue, indicating that these compounds have a limited potential for plant uptake under the field conditions studied here. The results obtained in our study have highlighted that the physicochemical properties of the contaminants (mainly the pKa, log*Kow*, and log*Koc*) play a crucial role in the uptake and translocation through the plants. A negative correlation was observed between the environmental fate with the distribution of CECs within the plants and the log*Kow*. As was expected, hydrophilic compounds tended to translocate from roots to leaves and finally to the fruit, whereas hydrophobic contaminants tended to remain in the roots and soil. Overall, it was noted that the CEC accumulation percentages in the soil were approx. 5-times higher than those found in the plant.

Further studies on different crops are needed to acquire better theoretical accumulation models to allow us to estimate the CEC levels in relation to different physicochemical properties in the soil as well as in the different parts of plants that are irrigated with reclaimed water over a long period. It is strongly recommended that future works also monitor the three macrolide antibiotics included in Commission Implementing Decision (EU) 2018/840 (azithromycin, used in the treatment of Covid, clarithromycin, and erythromycin), as well as other ubiquitous and highly concentrated compounds, such as venlafaxine, due to their incessant discharge into WWTPs. Additionally, the analgesic diclofenac presented the highest bioconcentration factor values, indicating that this compound tends to be highly accumulated in the fruit, while also showing high accumulation rates in the soil. Although this compound has been removed by the new EU 2018/840 legislation and has not been included in the latest European Commission watch list decision, the results obtained in this study highlight the importance of its continued monitoring.

Regarding the human risk derived from consuming tomatoes that are permanently irrigated with contaminated reclaimed water, the obtained results suggest that an adult would need to consume a few hundred kilograms of contaminated tomatoes daily to reach the acceptable intake limit. However, even though the present study might be considered a worst-case scenario, it only encompasses 30 substances in a tomato crop. Therefore, the total daily exposure values may be higher when using more generalized reclaimed water and when screening for other chemicals. It is also recommended that specific soil cleaning treatments are undertaken between crops to facilitate the soil's continuous reuse.

CRediT authorship contribution statement

1. Guarantor of integrity of the entire study: MMG; ARF.
2. Study concepts and design: MJM; MMG; JASA; DB; ARF.
3. Literature research: MJM; MGV.
4. Laboratory work: MGV; MJM; MMG.

5. Data analysis: MGV; MJM; JASA; DB; ARF.
6. Statistical analysis: MJM; ARF.
7. Manuscript preparation: MJM; MGV; ARF.
8. Manuscript editing: MJM; DB; ARF.

Declaration of competing interest

The authors declare no conflict of interest. This is an independent research. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150909.

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