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Reclaimed water in agriculture: A plot-scale study assessing crop uptake of emerging contaminants and pathogens

Adrià Sunyer-Caldú [a,1], Paola Sepúlveda-Ruiz [b,1], Miquel Salgot [b], Montserrat Folch-Sánchez [b], Damia Barcelo [a,b], M. Silvia Diaz-Cruz [a,*]

[a] Institute of Environmental Assessment and Water Research (IDAEA) Severo Ochoa Excellence Center, Spanish Council for Scientific Research (CSIC), Jordi Girona 18, 08034 Barcelona, Spain

[b] Biology, Sanitation and Environmental Department, University of Barcelona, Av. Joan XXIII, 08028 Barcelona, Spain

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ABSTRACT

Nowadays, water is a scarce resource, hence, water management is crucial as demand for agricultural, urban, and industrial purposes increases. The use of reclaimed water in agriculture can be a suitable solution. However, pathogens and chemical contaminants of emerging concern (CECs) present in reclaimed water can accumulate in the soil and ultimately, in the crop. To evaluate the potential transfer of biological and chemical pollutants from water to crop, two plots were designed for the cultivation of lettuce under field conditions. In this study, the influence of water quality, soil composition, and irrigation system on plant uptake of CECs and pathogens was assessed. The applied reclamation process reduced total suspended solids, *E. coli* (3–5 ulog), sulfite-reducing clostridia spores (1 ulog), Helminth eggs, and *Legionella spp* levels (complete removal) in water. Sodium adsorption ratio (SAR) and electric conductivity (EC) in the soils irrigated with reclaimed water were lower, and *E. Coli* was not detected. In lettuces, *E. coli* was only present in the crops irrigated with wastewater. Pharmaceuticals were the most frequently detected CECs in soils and waters, whereas UV filters achieved the highest concentrations. Diclofenac and salicylic acid were the most accumulated in soils, and diclofenac, ofloxacin, and benzophenone-4 were the most prevalent in the WWTP effluent. The irrigation water quality was the factor driving the transfer of CECs to the crops. Results show that the best combination to reduce pathogens and CECs was the use of reclaimed water, soils with high content of clay, and a sprinkling irrigation system.

1. Introduction

Fresh water is essential for human life, yet more than a billion people lack access to water, and by 2025, two-thirds of the world's population could suffer from a lack of water. When water is scarce, people cannot get enough to drink, wash or grow crops, leading to economic decline. Water scarcity is an issue that aggravates with population growth, increased food and energy demands, economic development, and environmental pollution [31]. Water is not only input for economic activities, it provides ecosystem services such as the maintenance of wetlands and river flows, and support for wildlife [53].

Climate change is one of the main causes of the shortage in water and one of the drivers of its changing demand [17]. Current data evidence the need to find alternatives to increase freshwater availability. A possible solution to this issue may be the integrated use of all available water resources, especially reclaimed water, that can be prioritized for purposes that do not require high quality, such as could be agriculture [30].

Wastewater use in agriculture has been a widely studied solution to deal with water shortages with promising benefits such as reduced fertilizer consumption [57]. Nevertheless, wastewater can contain a myriad of substances potentially toxic. Several pollutants ranging from heavy metals to organic chemical compounds - many of them constituting contaminants of emerging concern (CECs) including microplastics, pharmaceuticals, and personal care products (PPCPs) - can be found in wastewater [6], together with pathogens. Most of these chemical contaminants are released in large quantities to the sewerage systems and are only partially removed in wastewater treatment plants (WWTPs), reaching environmental compartments [22]. While waiting for more efficient and economic wastewater treatment technologies

* Corresponding author.
E-mail address: silvia.diaz@idaea.csic.es (M.S. Diaz-Cruz).
[1] These authors contributed equally to the research involved in this work.

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allowing the complete removal of pathogens and CECs in the WWTPs, their monitoring of the environment is essential. A pathogen is any microorganism that can cause disease once entering the human body, so its occurrence in any matrix (water, soil and especially crops) involved in the production of fresh food intended for human consumption must be avoided. Regarding CECs, many studies have reported adverse health effects on aquatic ecosystems and humans [26], as many of them display endocrine disruption [8] and antibiotic residues contribute to antibiotic resistance dissemination [45]. Therefore, if reclaimed water use is to be implemented, it is urgent to evaluate the occurrence of pathogens and CECs in the reclaimed water and to estimate their potential uptake by crops.

Regulation at the national (Ministerio de la Presidencia, 2007) or European level (The European Parliament and the Council, 2020) do not specify limitations about pathogen levels in crops. Only in some countries, pathogen and certain physicochemical parameters of reclaimed water aimed for agriculture irrigation are regulated for *E. coli*, total coliforms, turbidity, pH, and residual chlorine ([11,51]; the United States Environmental Protection Agency, 2022). Regarding CECs, they are mentioned as "additional requirements", but no limits of concentration in the irrigation water, soil or crop are specified. It is likely because most of the literature on the assessment of CECs' uptake by plants deals with case studies far from real scenarios [13,16,2,63]. At the global level, the World Health Organization's (WHO) latest guidelines were published in 1987 and followed the same pattern as the other regulations [60].

The scientific knowledge on plant joint exposure to pathogens and CECs under field conditions is yet limited because most studies focus solely on microbiological contamination or chemical contamination and were performed in hydroponic and greenhouse environments. So far, under field conditions, the effects of treated wastewater or reclaimed water use in terms of microbiological safety have only been evaluated in tomato, lettuces, chili, broccoli, and soil ([1,33,36,5]; A M [39]). Regarding CECs, their plant uptake from treated and reclaimed wastewater under real field conditions has been evaluated in carrots, potatoes, cabbage, spinach, radish, corn, rice, tomato, lettuce, cauliflower, broad and long bean, eggplant, cucumber, wheat, and maize [15,43,34,35,40,50,49].

All these studies proved the potential capacity of pathogens and CECs to reach the crops when irrigating with treated wastewater or reclaimed water, and even some of them show the relevance of the irrigation water quality in the final uptake of contaminants by the crop. Theoretically, other variables such as soil composition and irrigation system may also influence the contaminants' uptake. For example, Forslund et al., [19] and Palese et al., [39] stated that sprinkling irrigation or clayey soils enhance the survival of pathogens, thus increasing the possibility to be transferred to the crops. Drip irrigation has been commonly investigated ([15,1,33,35,36]; A. M. [39]), but there were also several studies using sprinkling irrigation systems [19], wetland columns [5], furrow [37,40] or even gravity irrigation [43,34]. The same applies to soil composition, since sandy soils were the most studied [35], but also combined with clay (from 3% to 40% of the total), loam, or silt ([15,43,1,33,34,37]; A. M. [39]). Due to this marked diversity of setups employed, comparison among results is impeded, and sound conclusions about the role that all these variables may have in the uptake of contaminants by crops cannot be drawn.

To the best of the authors' knowledge, none of the mentioned studies performed under real conditions have evaluated the joint influence of the selected variables. In this scenario, the present study aims to evaluate crop uptake of both microbiological and chemical organic pollutants from irrigation water, as well as the role of the soil, water quality, and the irrigation system in this process in real field conditions.

2. Materials and methods

2.1. Standards and reagents

All the standards used were > 98% of purity. Benzophenone-3 (BP3), benzophenone-1 (BP1), benzophenone-4 (BP4), 4HB, 4,4′-dihydroxybenzophenone (4DHB), avobenzone (AVO), UVP, 5,6-dimethyl-1 H-benzotriazole (DMBZT), nalidixic acid (NDX), oxolinic acid (OXL), tetracycline (TCY), succynil-sulfathiazole (S-STZ), sulfadiazine (SDZ), N4-acetylsulfadiazidine (acSDZ), sulfamerazine (SMR), N4-acetylsulfamerazine (acSMR), N4-acetylsulfamethazine (acSMZ), sulfamethoxazole (SMX), N4-acetylsulfamethoxazole (acSMX), sulfamethoxypyridazine (SMPZ), sulfapyridine (SPY), N4-acetylsulfapyridine (acSPY), sulfaquinoxaline (SQX), sulfathiazole (STZ), sulfisomidine (SMD), sulfadimethoxine (SDM), trimethoprim (TMP), acetaminophen, also known as paracetamol (APH), atenolol (ATL), gemfibrozil (GFZ), ketoprofen (KPF), mefenamic acid (MFA), carbamazepine (CBZ), norfluoxetine (norFXT), ofloxacin (OFX), ciprofloxacin (CFX), caffeine (CFF), ibuprofen (IBU), salicylic acid (SCY), diclofenac (DCF), diclofenac-13 C (DCF-13 C), methyl paraben (MePB), propyl paraben (PrPB), benzyl paraben (BePB), butyl paraben (BuPB) and benzophenone-(carbonyl-13 C) (BP-13 C) were purchased from Sigma Aldrich (Darmstadt, Germany). BP2, 2,2′-dihydroxy-4-methoxybenzophenone (DHMB), ethyl-4-(dimethyl-amino) benzoate (EtPABA), ethylhexyl methoxycinnamate (EHMC), and benzotriazoles (BZT) were obtained from Merck (Darmstadt, Germany). Enzacamen or 4-methyl benzylidene camphor (4MBC) was provided by Dr. Ehrenstorfer (Augsburg, Germany). 5-Methyl benzotriazole (MeBZT) was obtained from TCI (Zwijndrecht, Belgium). Flumequine (FLU), N-desmethylvenlafaxine (N-desVFX), diclofenac 4-hydroxy (DCF-OH), carbamazepine 10,11-epoxy (CBZ-E), and sulfamethazine-d4 (SMZ-d4) and acetaminophen-d4 (APH- d4) were purchased in Toronto Research Chemicals (Toronto, Canada). Oxytetracycline (O-TCY) and naproxen (NPX) were obtained from Honeywell Fluka (Wabash, United States). 2-hydroxy-4-methoxy-2′,3′,4′,5′,6′-d5 (BP3-d5), (±)− 3-(4-methylbenzylidene-d4) camphor (4MBC-d4), 1 H-benzotriazole-4,5,6,7-d4 (BZT-d4), flumequine-13C3 (FLU-13C3), trimethoprim-d3 (TMP-d3), carbamazepine-d10 (CBZ-d10), mefenamic acid-d3 (MFA-d3), caffeine-d3 (CFF-d3), ibuprofen-d3 (IBU- d3), salicylic acid-d6 (SCY-d6), diclofenac-d4 (phenyl-d4) (DCF-d4), benzyl paraben-d4 (BePB-d4) and 5-(2,5-dimethylphenoxy)− 2,2-bis(tri-deuteriomethyl)pentanoic acid (GMZ-d6) were from CDN isotopes (Quebec, Canada).

Information about solvents, stock solutions, extraction and analysis kits can be found in the Supporting Information (SI).

2.2. Field site

Palamós WWTP, the fifth-biggest plant in Catalonia (Spain), was selected to assemble the experimental plots. Detailed information about the facility can be found in the SI.

To improve the quality of the WWTP effluent to be used for agricultural irrigation, a Managed Aquifer Recharge pilot system with reactive barriers (rbMAR) was implemented as advanced tertiary treatment. The rbMAR system aims to improve biodegradation by generating different redox potential zones and enhancing microorganisms' CECs removal and pathogens retention. The pilot rbMAR described by Valhondo et al., [56,54] consists of five soil aquifer treatment systems implemented with a compost/wood ship reactive barrier, and a reference system without barrier; further, the infiltrated water flows along a 15 m simulated aquifer. Finally, the effluent is discharged and stored in a tank to homogenize waters for further use. The water used to feed the rbMAR systems is the WWTP effluent (previously homogenized in a tank to overcome differences in effluent water quality over time).

Two experimental agricultural plots, 4 m x 12 m, were constructed next to the rbMAR system (Fig. 1). Plot 1 was irrigated with rbMAR effluent (B water) and Plot 2 with WWTP effluent (W water). The plots

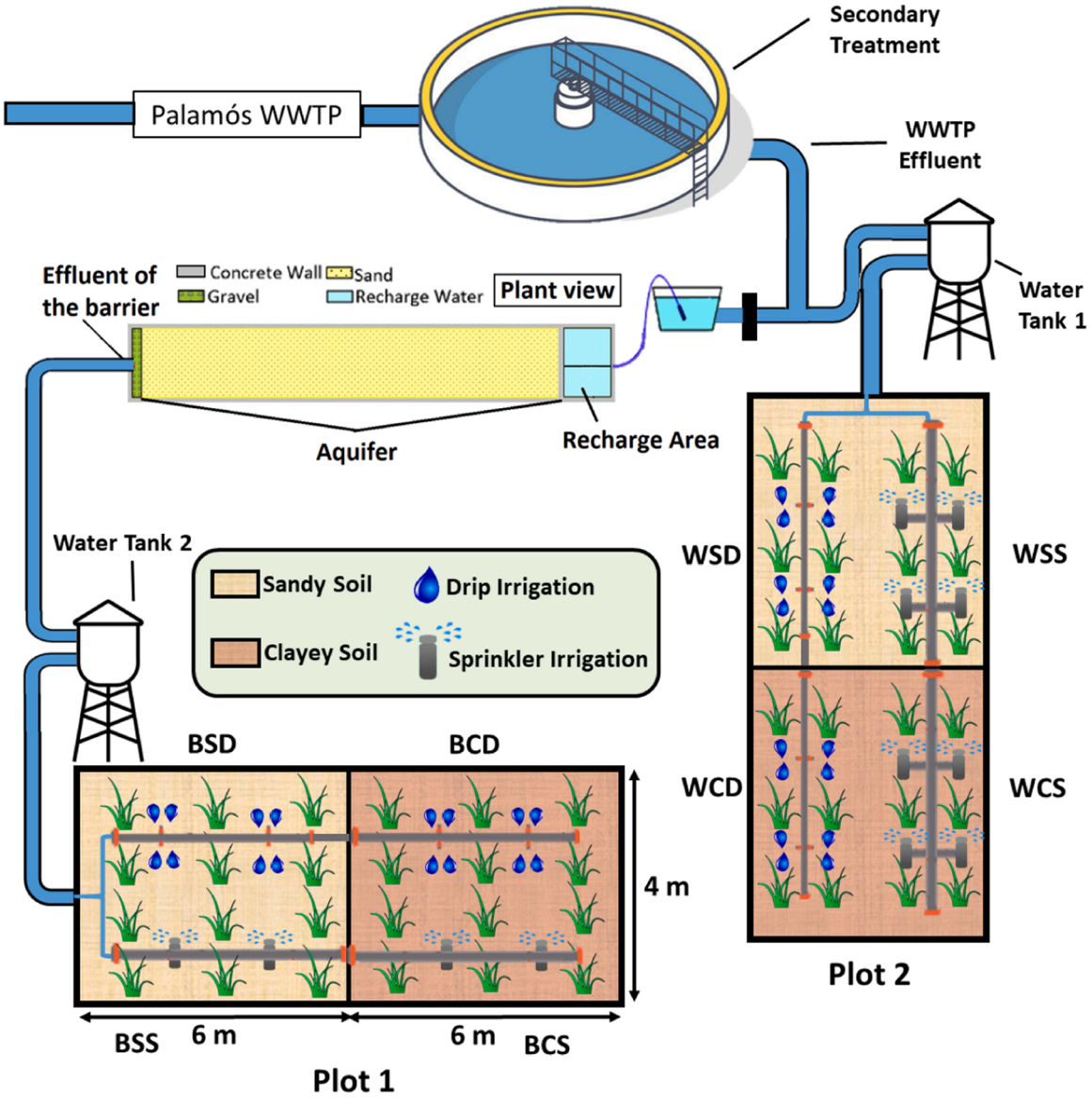

Fig. 1. Diagram of the experimental agricultural plots. BSD: Barriers irrigation, sandy soil, drip irrigation; BCD: Barriers irrigation, clayey soil, drip irrigation; BSS: Barriers irrigation, sandy soil, sprinkler irrigation; BCS: Barriers irrigation, clayey soil, sprinkler irrigation; WSD: Wastewater irrigation, sandy soil, drip irrigation; WCD: Wastewater irrigation, clayey soil, drip irrigation; WSS: Wastewater irrigation, sandy soil, sprinkler irrigation; Wastewater irrigation, clayey soil, sprinkler irrigation.

were made up of two types of soil: the first one was the soil from the WWTP terrain, mainly sand (S soil) and the second one was the same soil to which clay was added up to 10% content (C soil). The plots were implemented with two irrigation systems: drip and sprinkler. Different sensors were installed in the plots to control in real-time several parameters: two DataLogger Decagon Em50G to the system ECHO2 were installed for reading, storage, and data transmission via GPRS, and eight 5TE Decagon probes were installed to measure temperature, electrical conductivity, and soil moisture at six depths (10, 20, 30, 40, 50 and 60 cm). These probes were installed in the plots avoiding areas where water could have direct contact with the sensor. Two flowmeters Decagon were also installed for monitoring the flow rate of the irrigation waters. Real-time control of water availability allowed the automated irrigation system to only activate when the crops needed it i.e. after a storm event, the sensors detect large amounts of water at different depths and cancel the scheduled irrigation. On the contrary, in times of drought or hot summer, when water evaporates quickly, the sensors activate irrigation more frequently than the dulled.

2.3. Sampling

2.3.1. Water samples

For characterization, water samples were collected throughout each season of the year and additionally at harvest time. Regarding microbiological analysis and physicochemical characterization, W and B water samples were collected in sterilized 1 L glass bottles and transported in portable fridges to the laboratory. The analysis was performed within 24 h after sampling. For CECs analysis, water samples were collected in 1 L brown glass bottles, filled up to ¾ of capacity, and transported under cool conditions to the lab and then frozen.

2.3.2. Soil samples

For microbiological analysis, sterilized plastic bags and glass bottles were used. The microbiological analysis was performed within 24 h after the sampling. Soil samples were collected before sowing, every four months during one year, and at harvest time.

The samples were gathered with a small gardening shovel at the

surface, 10 and 20 cm depth around the crops, mixed by subplot and depth, and stored. For physicochemical analysis, soil samples were air-dried, then passed through a 2 mm sieve and stored until analysis. For CECs analysis, soils were freeze-dried and stored in aluminum foil at − 20 ºC until analysis.

2.3.3. Lettuces

Chicorium intybus lettuces variety (known as red oak leaf lettuce) was cultivated from October to December 2018. Approximately 50 lettuces per subplot were grown from a previously grown seedling. Once the lettuces were suitable for consumption (market size), 10 specimens per subplot were randomly collected, and further shaken to remove soil particles.

The samples were separated into two groups: one for microbiological analysis and the other for the analysis of CECs. Both were shipped to the laboratories under cool conditions. The roots of the lettuces were very short (few mg), so the whole lettuce had to be used and homogenized. The microbiological analysis was performed within the next 24 h. Upon arrival, the samples for CECs analysis were frozen. The next day, they were thawed, sliced, frozen again, lyophilized, and crushed. Finally, samples were frozen at − 20ºC until analysis.

2.4. Analytical methods

2.4.1. Microbiological and physicochemical analysis

Throughout the experiment, physicochemical parameters of water and soil were monitored and are listed in Table 1 [12,7]. For the irrigation water, physicochemical parameters were determined in the four seasons of the year considering the water's variation in quality and quantity. An initial characterization of the soil was carried out before the lettuces were planted.

Microbiological parameters and fecal indicators such as *E. coli* and spores of sulfite-reducing clostridia were analyzed. *Legionella* spp. was monitored because of the risk of transmission by aerosolization. As regards parasites, the presence of helminth eggs was monitored.

Concerning fecal contamination in water and soil, *E. coli* was selected as the indicator and the membrane filter method based on ISO 9308 (2014) [29] was performed. *E. coli* was determined in 30 g of soil and 270 ml of buffer solution, both homogenized for 2 min in a Stomacher to get 10^{-1} dilution [19]. The lettuces were analyzed according to the ISO 6887 (2017) [28]. To have a representative sample, leaves of different parts of the lettuces were cut and 25 g were taken under sterile conditions. Afterward, *E. coli* extraction was performed. Shredded lettuce was placed in sterile bags with 225 ml of peptone water. Subsequently, it was shredded in a Stomacher for 2 min to homogenize the sample and obtain a 10^{-1} dilution. Further, dilutions for the lettuce leaves and soil up to 10^{-4} were made and analyzed in the same way as for the water. The plates were incubated at 36 °C for 24 h and the colony-forming units (cfu) per g dry weight (dw) of soil or vegetable were counted.

Table 1
Methods applied for water and soil analysis (TSS: total suspended solids, COD: chemical oxygen demand, BOD_5: biological oxygen demand, TNK: total Kjeldahl nitrogen, OM: organic matter, EC: electrical conductivity, IC: Ion Chromatography, ICP-OES: Inductively coupled plasma-optical emission spectroscopy).

Parameter	Method for water analysis	Method for soil analysis
TSS	APHA[7], ref. 2540B	–
COD	APHA[7], ref. 5220 C	–
BOD_5	APHA[7], ref. 5220 B	–
TNK	APHA[7], ref. 4500.	ASA (1982)
N-NH_4	APHA[7]	ASA (1982)
NO_3	APHA[7], ref. 4110B. (IC)	–
Cl^-		
SO_4		
OM	–	Wet oxidation by Walkley and Black (1934)
Texture	–	Hydrometer method by Bouyoucos (1962)
pH	pH-meter (Crison GLP21)	ASA (1982) pH-measurement (Crison GLP21)
EC	Conductivity measurement (Crison GLP21)	ASA (1982) Conductivity measurement (Crison GLP21)
Mg^{+2}	APHA[7], ref. 3120B. (ICP-OES)	ASA (1982) (ICP-OES)
Ca^{+2}		
Na^+		

2.4.2. CECs analysis

The determination of CECs in the lettuces and soils was performed following the developed QuEChERS-based method by Sunyer-Caldú and Diaz-Cruz, [48]. Briefly, this method consists of a first extraction step of 1 g dw lettuce using QuEChERS kits (citrate and PSA-Kit-02 kits). Next, an aliquot of 5 ml was evaporated and reconstituted to 1 ml of the final extract. This extract was analyzed in a liquid chromatograph Symbiosis Pico from Spark Holland (Emmen, The Netherlands) using an LC-analytical column Hibar Purosher® STAR® HR R-18 (50 mm × 2.0 mm, 5 μm) coupled to a 4000 QTRAP mass spectrometer from Applied Biosystems-Sciex (Foster City, USA). Analyses were performed in selected reaction monitoring (SRM) mode using the two most intense transitions, in both positive and negative electrospray ionization (ESI+, ESI). The analytes were quantified by isotope dilution using 10 matrix-matched standard solutions to build the calibration curves. For the analysis of soils, the methodology was adapted from our previously developed methods and further validated at three concentrations (10, 50, and 100 ng/g dw). The method limits of detection (MLODs) ranged from 0.01 to 2.92 ng/g dw and the recovery rates were between 60% and 140% for 54 of the 56 determined compounds. Method validation parameters are compiled in the SI.

CECs in water samples were analyzed using on-line solid phase extraction and high-performance liquid chromatography-tandem mass spectrometry (on-line-SPE-HPLC-MS/MS) according to our methodology [21,23,54].

2.5. Quality assurance and quality control

In microbiological analysis, the quality control established by the ISO and Standards methods were followed.

In the CECs analysis, some measures are required to avoid contamination at trace levels. All the glass material was washed with MeOH and acetone and muffled at 400ºC for 4 h. Quality control and blank samples were introduced randomly in the sequence of analysis to evaluate the method's performance. The maximum tolerance permitted between chromatographic retention times (t_R) in the calibration curve and the samples was 2.5% and the maximum tolerance permitted for the relative ion intensities between the two selected SRM transitions was 15%. Following the EU normative (Commission Decision 2002/657/EC), all the compounds determined were identified with the t_R and the two selected SRM transitions.

2.6. Uptake factors

Measured CECs concentrations in lettuce, soil, and irrigation water were used to calculate the CECs uptake factors related to soil and water following Eqs. (1)–(3).

$$UF_{SOIL} = \frac{C_{CROP}}{C_{SOIL}} \quad (1)$$

$$UF_{WATER} = \frac{C_{CROP}}{C_{WATER}} \quad (2)$$

$$Kd = \frac{C_{SOIL}}{C_{WATER}} \quad (3)$$

where UF_{soil} is the soil-based uptake factor, UF_{water} is the water-based

uptake factor, C_{crop} is the contaminant concentration in the crop, C_{soil} is the contaminant concentration in the soil, C_{water} is the contaminant concentration found in the irrigation water, and Kd is the soil-water sorption coefficient for each contaminant. Uptake factors could only be calculated for contaminants present at least in two matrices. UF_{SOIL}, UF_{WATER} and Kd values are listed in Table S1.

2.7. Statistical methods

T-tests to evaluate individual correlations, principal component analysis (PCA) and partial least squares regression-discriminant analysis (PLS-DA) was performed with RStudio open software, v. 1.2.5001 (2019) RStudio, Inc. The results of the t-tests are included in Table S2.

2. Results and discussion

2.1. Physicochemical parameters

Table 2 lists the quality parameters of W and B waters used for irrigation. W water quality fluctuates notoriously throughout the year. However, the rbMAR system helps to reduce this variability. Beyond, the rbMAR system allows decreasing by 50% of the total suspended solids (TSS), achieving an average concentration of 9,2 mg/L, a COD reduction of 70%, and average removals of 43% of N-NTK and 37% of $N\text{-}NH_4$.

SAR was calculated in irrigation waters according to RD 1620/2007. This parameter informs about the relationship between exchangeable Na^+ and other exchangeable cations. When the concentration of Na^+ is high, it may replace Ca^{2+} and Mg^{+2} which influences soil structure aggregates stability and eventually may minimize soil permeability [59].

W and B waters showed similar SAR and EC, complying with the limits established by RD 1620/2007. Despite SAR values measured in the irrigation waters being high (> 4 meq/L), the also high EC (> 2,5 dS/m) helped to minimize the potential risk of decreasing the infiltration rate [9]. However, high salinity waters may reduce crop yields because of the accumulation of salts in the roots, decreasing water availability to the plant.

In soils, the organic matter content, total nitrogen, and pH after lettuce harvest did not show significant differences from the values determined before planting. However, soil organic matter content increased after one year of irrigation with both water types (Fig. S1). These results evidenced that B water may increase soil organic matter content minimizing any risk. Increased EC and SAR values of the plot irrigated with W water compared to those of the plot irrigated with B water were observed (Table S3). For the subplots having different sand-clay compositions, significant differences in these two parameters were not observed.

With a specific focus on the soils irrigated with B water, SAR was calculated in triplicate samples collected every four months over one year. As shown in Fig. 2, a significant increase in SAR was observed. Before irrigation, the SAR values in the soil samples were in the range 0,4 – 0,8 meq/L, increasing to 1,1 - 1,2 (150%). This behavior suggests that the use of B water in agriculture might increase soil sodicity and, consequently, alters soil structure and reduces crop production. An excess or deficiency of major plant nutrients, such as Ca^{2+}, can lead to disturbances in the availability, uptake, transport, or distribution of nutrients in the plant [59].

A t-test performed to evaluate if SAR values were influenced by the irrigation system and soil type indicated that both variables influenced SAR. These results agree with those reported by Stevens et al., [47], pointing out an increase in SAR in topsoil irrigated with reclaimed water compared to that in virgin soil. Similarly, Phogat et al., [41] simulated a long-term impact on soil irrigated with reclaimed water and reported a considerable increase in SAR after 7 years of irrigation.

Table 2
Quality parameters of W and B waters compared with RD 1620/2007 and Regulation EU 2020/741 standard limits. TSS: total suspended solids, COD: chemical oxygen demand, BOD_5: biological oxygen demand, EC: electrical conductivity, TNK: total Kjeldahl nitrogen, SAR: sodium adsorption ratio. n.d.: Not detected.

Parameter	W water	B water	RD 1620/2007 *	Regulation (EU) 2020/741 * *
TSS (mg/L)	20 ± 6.5	9.2 ± 4.4	20	10
COD (mg/L O_2)	99 ± 39	28 ± 19		
BOD5 (mg/L O_2)	17 ± 7.1	11 ± 2.4		10
pH	7.8 ± 0.20	7.7 ± 0.30		
EC (dS/m)	2.7 ± 0.80	2.6 ± 0.2	3	
TKN (mg/L)	69 ± 9.2	30 ± 13		
N-NH4$^+$ (mg/L)	60 ± 8.2	22 ± 14		
Cl$^-$ (mg/L)	430 ± 26	530 ± 29		
SO4^{2-} (mg/L)	< 0.10	0.60 ± 0.30		
Na$^+$ (ppm)	230 ± 13	211 ± 10		
Ca^{2+} (ppm)	123 ± 9.8	128 ± 8.5		
Mg^{+2} (ppm)	32 ± 4.4	30 ± 3.6		
SAR (meq/L)	4,79	4,34	6	
***E. coli* (cfu/100 ml)**	$4.4 \times 10^6 \pm 1 \times 10^7$	$6.6 \times 10^2 \pm 1 \times 10^3$	1×10^2	1×10^1
Helminth eggs (eggs/ 10 L)	1	n.d.	1	1
Spores of sulfite-reducing clostridia (cfu/100 ml)	$1.9 \times 10^4 \pm 7.0 \times 10^3$	$3.9 \times 10^2 \pm 1.4 \times 10^2$		
***Legionella* spp (cfu/L)**	50	n.d.	1000	1000

* Minimum reclaimed water quality class 2.1; ** Minimum reclaimed water quality class A.

2.2. Pathogen indicators analysis

Concerning microbiological parameters, *E. coli* is considered an indicator of fecal contamination and is regulated by RD 1620/2007 and EU Regulation 2020/741. B water showed reductions between 3 and 5 ulog of *E. coli* and a decrease of 1 ulog of sulfite-reducing clostridia spores compared to the levels measured in the W water. Helminth eggs (1 egg/10 L) and *Legionella spp* (50 cfu/L) were only detected in the W water.

Fig. 3 shows the annual fluctuation of fecal contamination in B water. During tourism's peak season, the *E. coli* rate was found over the limits. All the water samples from March, April, and August exceeded the limits, while 70% of July's samples had less than 1 log of *E. coli*. Nevertheless, in periods when the water input was lower and, consequently, the retention time increased (i.e., in winter), less than 1 log cfu/100 ml was detected, which meets the maximum level set up in the RD 1620/2007 (in case there is direct contact of reclaimed water with the edible parts for fresh human food) and with EU Regulation 2020/741.

Regarding fecal contamination in soil, at the time of lettuce harvest, it was observed that the soil irrigated with B water had no *E. coli* (Table 3). In contrast, the plot irrigated with W water by drip in C soil had a higher concentration of *E. coli,* $2{,}26 \times 10^4$ cfu/g. The S soil irrigated by drip presented a lower level of fecal contamination ($4{,}3 \times 10^2$ cfu/g) compared to $5{,}95 \times 10^3$ cfu/g when irrigated by the sprinkler system. Similar results were obtained by Forslund et al., [19]. This study showed that the highest values of *E. coli* were obtained in soil irrigated by a micro-sprinkler system using reclaimed water. However, soil properties and weather conditions may stimulate the persistence of *E. coli* in irrigated fields by reclaimed water. Fecal contamination in the soil also depends on the survival capacities of the pathogens [58]. In our study, soil samples were collected in autumn, which might have favored the persistence of the pathogens.

E. coli was not observed in lettuces irrigated with reclaimed water, as

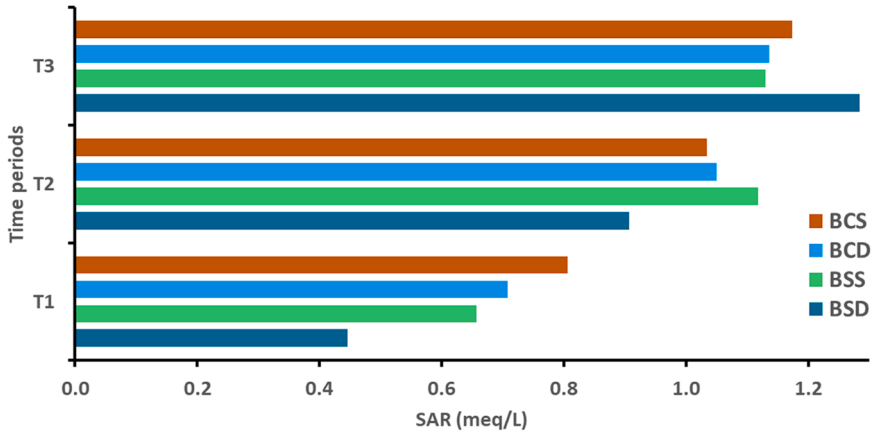

Fig. 2. SAR values in soil irrigated with B water at three time periods. T1: 4 months; T2: 8 months; T3: 12 months. BSD: S soil-drip irrigation; BSS: S soil-sprinkler irrigation; BCD: C soil-drip irrigation; BCS: C soil-sprinkler irrigation.

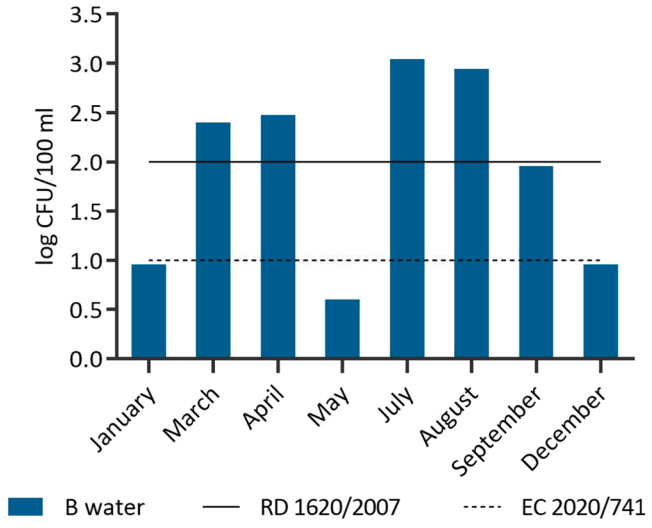

Fig. 3. Seasonal variability of *E. coli* (log cfu/100 ml) in B water compared with RD 1620/2007 and Regulation EU 2020/741 established limits.

Table 3
E. coli levels in soil and lettuces irrigated with W and B waters. S: soil composed of sand, C soil: S soil with 10% clay content.

Irrigation water	Type of soil	Irrigation system	*E. coli* in soil	*E. coli* in lettuce
(cfu/100 ml)			(cfu/g)	(cfu/g)
W	S	Drip	4.3×10^2	n.d.
		Sprinkler	5.9×10^3	9.7×10^3
(2.6×10^5)	C	Drip	2.3×10^4	5.7×10^3
		Sprinkler	1.0×10^3	1.1×10^4
B	S	Drip	n.d.	n.d.
		Sprinkler	n.d.	n.d.
(9.5)	C	Drip	n.d.	n.d.
		Sprinkler	n.d.	n.d.
n.d.: not detected				

expected since the B water had less than 10 cfu/100 ml (Table 3). However, its occurrence in W water was considerably higher ($2,6 \times 10^5$ cfu/100 ml), leading to values up to $1,15 \times 10^4$ cfu/g in the lettuces irrigated with this water. It is important to emphasize that both soils showed higher *E. coli* value when irrigated by sprinkling, while lettuces irrigated by dripping presented fecal contamination only when they were cultivated in the C soils. These results are in good agreement with those reported by Mañas et al. [36], who concluded that the use of drip irrigation for ready-to-eat vegetables could avoid microbial contamination. However, in one drip-irrigated lettuce sample of our study, *E. coli* was found, which could be attributed to the wind present in the zone. Nevertheless, the use of B water has not shown any risk of fecal contamination neither in soil nor in lettuce.

2.3. CECs analysis

The occurrence of PPCPs in the lettuces was discussed together with the description of the analytical method developed for their analysis in Sunyer-Caldú and Diaz-Cruz, [48]. In the present work, we focus on soil and water, however, to have the full picture, the concentrations of the PPCPs determined in the lettuces are listed in Table S4. The concentrations for each detected compound, MLODs, method limits of quantification (MLOQs), determination coefficients (r^2), and linear range for soil and water matrices are compiled in Tables S5 and S6, respectively.

The concentrations determined in the three types of samples are shown in Table 4. UVFs presented high bioaccumulation in lettuces and a notorious difference in the total load between the samples irrigated with W water and B water. In contrast, soils and waters had a low concentration of UVFs. As for PBs, the accumulated values were very low in all the matrices. Regarding the pharmaceuticals, the accumulation in the soils was very similar to that in the lettuces, suggesting a direct relationship between soil and plant. As expected, the concentration in waters was much lower, as soils-plants accumulate water-borne contaminants during all the months that the crops were being irrigated.

Of the 55 analyzed compounds, 10 were detected in soil (18%) and 35 were present in the irrigation waters (64%). The pharmaceuticals had the highest accumulated load (50–56 ng/g dw in soils and 3000–7500 ng/L in waters), while the UV filters (UVFs) presented the highest average value per compound (1.7 ng/g dw in soils and 180 ng/L in waters). In soils, PBs and CFF were not detected, despite being present at low concentrations in both types of water.

Table 4
Cumulative concentrations of PPCPs in water, soil, and lettuces. UVFs: UV filters, PBs: paraben preservatives, PhACs: pharmaceuticals, Others (caffeine, CFF)).

	∑UVFs	∑PBs	∑PhACs	∑Others
B water	1.21	n.d.	1.88	n.d.
W water	2.69	n.d.	4.93	n.d.
B soil	2.31	n.d.	50.9	n.d.
W soil	3.15	n.d.	54.6	n.d.
Lettuce Barriers	69.0	3.75	52.7	10.1
Lettuce WWTP	98.6	6.32	53.6	17.9

n.d.: not detected; Units (water): ng/L; Units (soil and lettuce): ng/g dw.

PPCPs concentrations found in S soil and C soil were very similar. In waters, however, there was a significant difference in the amount of PPCPs; the W water was much more contaminated than the B, as the accumulated values of UV filters and pharmaceuticals were two and three-fold higher, respectively.

In soils, DCF showed the highest concentration (37–43 ng/g dw) followed by SCY (7.4–10 ng/g dw). Regarding the other detected compounds, all were found at levels below 1.3 ng/g dw, showing a little affinity for soils. Similar concentrations of DCF and SCY in soil were reported in other studies, as in Carter et al. [14], where DCF was found at 50 and 70 ng/g dw, or Aznar et al. [10] where SCY was reported at 47 ng/g dw.

In W water, the compounds found at the highest concentrations were DCF (1390 ng/L), OFX (1240 ng/L), and BP4 (1030 ng/L), but other compounds were also measured at significant levels: GMZ (916 ng/L), BZT (623 ng/L), MeBZT (601 ng/L) and NPX (454 ng/L). In B water, the values were substantially lower, suggesting that the barriers boost degradation processes. These results are in agreement with reported concentrations in wastewater and reclaimed water of DCF [43,8], OFX [20,27], BP4 [44,8], GMZ [46], BZT [42,8], MeBZT [44] and NPX [46, 8].

In lettuces, in contrast to waters and soils, UVFs had the highest accumulated load (671 ng/g dw), showing that the accumulation patterns are not the same in the crops as in the waters and soils for all contaminants. This is also observed in many CECs (namely 4HB, AVO, UVP, SDZ, SMX, SMPZ, SMD, and desVFX) that were uptaken by the lettuces, but were detected neither in the irrigation waters nor in the soils. The compounds found at the highest concentrations in the lettuces were 4HB (61 ng/g dw), SCY (19 ng/g dw), CFF (16) ng/g dw, DCF (15 ng/g dw), and BP2 (13 ng/g dw). 4HB and BP2 are UVFs widely used, but also BP3 metabolites, so their presence in the crops could be due to the degradation of BP3, which was present in the irrigation waters, but absent in the lettuce samples. The presence of CFF and DCF can also be explained by the pretty high levels present in the irrigation waters, as DCF was the compound with the highest concentration, and CFF occurrence in WWTP effluents is also well documented [25,52].

2.4. Statistical analysis

To evaluate potential correlations between CECs concentrations and type of sample, PCA was performed, as shown in Fig. 4. The first two components (PC1 and PC2) explained 77.8% of the variance, being PC1 the one with the greatest contribution (52.7%). PC1 showed high positive loading values for CBZ (0.8) and BP4 (0.7) and high negative ones for BP3 (−0.9) and DCF (−0.9). PC2 had high positive loading values for GMF (0.8) and MFA (0.7) and high negative ones for AVO (−0.9), UVP (−0.9), and N-VFX (−0.9). The larger the value of the contribution, independently of it is positive or negative, the more the variable contributes to a component. Variables that are correlated with PC1 and PC2 are the most important in explaining the variability in the data set. As principal components are designed to explain the variance, the variables that are dimensionally close between them will have more correlation than the ones more separated. In our case, PC1 described the difference between the soil and the other matrices and additionally showed a slight difference between the two types of irrigation waters. PC2, however, described the difference between the lettuce and the B water not explained by PC1.

The three types of samples showed different CECs accumulation patterns, as shown by their separated location in the biplot (Fig. 4). Soils irrigated with B and W waters showed a similar CECs pattern, likewise lettuces. However, there is a higher separation between the two water types, indicating that the infiltration of the water through the barriers modified the CECs content. The different accumulation patterns among the matrices are in accordance with previous works, where the CECs load in the irrigation water was statistically different from that in the soils or the crops. For example, Christou et al., [15] reported that considerable concentrations of DCF, SMX, and TMP (50.6 ng/L, 41.3 ng/L, and 61.8 ng/L, respectively) in the irrigation wastewater did not display a cumulative or increasing pattern. Similarly, Liu et al., [34] found that the concentrations of SMX, SMZ, and TMP in reclaimed water (20 ng/L, 1 ng/L, and 3 ng/L, respectively) were higher than those of many other studied CECs and that their respective values in the irrigated soils and crops did not display a similar trend.

Three well-differentiated groups were observed among the CECs, one for each type of matrix. The largest group of compounds (green ellipse in Fig. 4) is related to the lettuces, the second group (blue ellipse) to the irrigation waters, and the third group (orange ellipse) to the soils. Some of the CECs investigated were not directly correlated with any of the matrices (they are halfway among them), showing similar contributions.

Regarding individual behaviors, the pharmaceutical CBZ is known to be present at high concentrations in wastewater, as we have observed, but its metabolite CBZ-E is mostly accumulated in the lettuces, probably because CBZ is metabolized when it is uptaken by the plant. Concerning UVFs, BZTs, the methylated derivatives are the major contributors to the W water. Conversely, BZT was associated with the lettuces irrigated with the same water. A possible explanation would be the existence of an

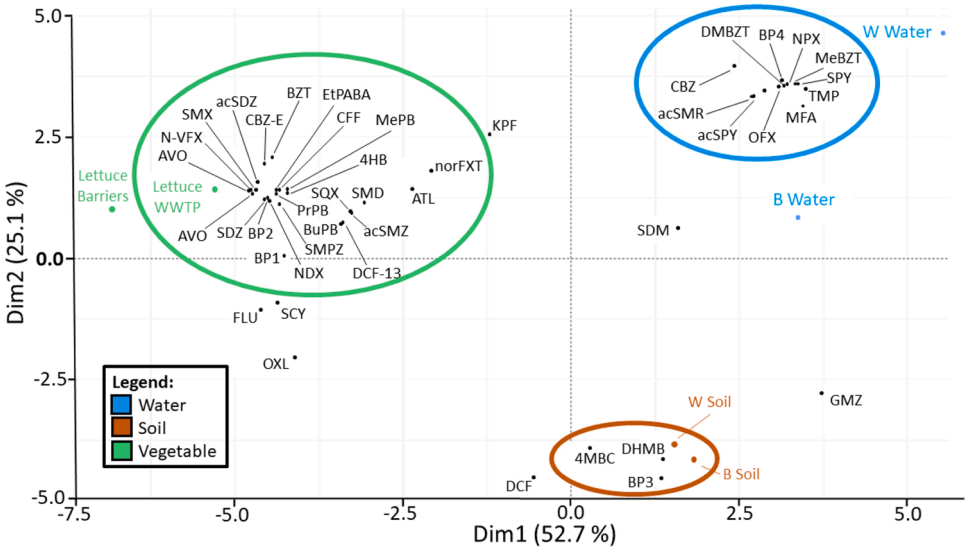

Fig. 4. Biplot of the three studied matrices (lettuce, soil, and water) showing the CEC's contributions.

enzymatic demethylation mechanism in plants. BP3, and some metabolites, are mostly found in soils, although they were also present in the waters, suggesting that it was accumulated thereafter irrigation. Then, soil microorganisms can degrade it, producing DHMB (BP8), and also impacting the soil matrix. Further, the DHMB formed is degraded during the uptaken process by the lettuces, as it was not detected in this matrix. When BP3 was metabolized in the plant, 4HB, BP1, and BP2 were generated, which would explain the high concentrations of these compounds accumulated in the lettuces. Regarding the paraben preservatives, all of them showed the same occurrence pattern in all matrixes, with the greatest correlations observed for the lettuces.

PCA and PLS-DA were also performed to evaluate potential correlations between measured physicochemical properties of the soils and CECs concentrations detected in them. The same analyses were applied to the irrigation waters; however, no correlation was observed in any case (Figs. S2 and S3).

2.5. Soil and lettuces uptake factors

The highest UF_{SOIL} value of 175, corresponded to 4HB, present at a low concentration in soil, but highly accumulated by the lettuces. However, we cannot rule out that part of the 4HB in the plant could be produced by the plant itself in the metabolization of the BP3 accumulated in addition to the 4HB directly transferred from the water to the soil [38]. The concentrations of BP3 measured in the irrigation waters and soils, and their absence in the lettuces would support this hypothesis. Like 4HB, BP1 is another major BP3 metabolite, and thus a similar explanation for its occurrence can be given.

Other compounds with high UF_{SOIL} values were SCY (2.7), FLU (2.4), OXL (1.8), and BP1 (1.3). The UF_{SOIL} values for the rest of the CECs were < 1. SCY is a plant hormone known for mediating host responses upon pathogen infection [32], so its UF_{SOIL} value probably is the result of the natural production of SCY by the lettuces. For the two fluoroquinolone antibiotics, our results are consistent with their reported uptake by lettuces. Tadić et al., [49] found TMP, OFL, and enrofloxacin between 1.9 and 37.8 ng/g dw in lettuces, while we have found FLU and OXL between 0.36 and 4.91 ng/g in this study.

Regarding UF_{WATER}, the compounds with the highest values, but still < 1 were BP2 (0.82) and BP3 (0.83); all other ratios were < 0.4. This could be explained by the relatively low lipophilicity (log Kow <4) of both compounds (log Kow 2.78 and 3.79), which make them preferably accumulate in organic matter components. The Kd values of all compounds were < 0.3, showing low sorption coefficients in soils after irrigation. However, it has to be considered that plant uptake can also contribute to the reduction of CECs in the soil, resulting in low Kd values.

2.6. Variables' role in contaminants' uptake

2.6.1. Irrigation waters

B water had lower levels of TSS, COD, *E. coli*, sulfite-reducing clostridia spores, Helminth eggs, *Legionella spp*, UVFs, and pharmaceuticals than W water. However, SAR, EC, CFF, and PBs were very similar in both. The differences observed evidence that the rbMAR system reduces the levels of pathogens and CECs [55,54]. As shown in Fig. 2, the fluctuation of pathogen indicators and contaminants' load in the influent water of the rbMAR system is an important factor that needs to be considered, especially if a high infiltration flow rate is to be applied, because the reduction of pathogens and CECs can only be effective with long residence times.

2.6.2. Soil

OM, N, and pH of soils were very similar regardless of the irrigation water used, showing that the nutrients present in the wastewater were not altered during infiltration through the reactive barriers and, therefore, B water provided similar nutrient levels to the soil as W water. However, other parameters such as *E. coli* had lower values in the soil irrigated with B water, in accordance with the values found in the irrigation waters. Overall, *E. coli* appears to have more affinity to soils with higher clay content. However, *E. coli* levels varied randomly between drip irrigated and sprinkle irrigated soils. Additionally, SAR and salinity values in the soils increased after a year of irrigation in all the plots, likely lowering the availability of nutrients as well as modifying the soil structure [59]. These negative impacts should be considered for long-term agricultural practices and guarantee further research. Regarding CECs, no significant differences were observed between C and S soils, and between soils irrigated with B water or W water. The poor correlation observed between the CECs occurrence in the water, soil, and crop is an issue reported in previous studies. de Santiago-Martín et al., [43] reported that the concentration of CECs measured in the irrigation water was not in agreement with the bioaccumulation pattern found for the fruit, as observed in both, the sediments and the soils. They showed as an example that APH, IBU, and CBZ (range 0.03–27.5 ng/g) were up taken by crops, but were in the lowest concentration ranges in the irrigation water (range <100–250 ng/L).

2.6.3. Lettuces

Following the same pattern observed in irrigation waters and soils, high values of *E. Coli* were found in lettuces irrigated with W water, but no fecal contamination was present in those irrigated with B water. Considering the difference in *E. coli* levels between W and B water (2.6×10^5 and 9.6 cfu/100 ml, respectively) and W and B soil (7.5×10^3 cfu/g and not detected, respectively), the accumulated levels in lettuces are consistent. Regarding soil composition and irrigation system, the *E. coli* levels found in lettuces (Table 3) suggest that C soil and sprinkling irrigation favor the accumulation of *E. coli* in the lettuces. According to the previous determination of PPCPs in lettuces [48], concentration values were much lower in crops irrigated with B water. But the other variables (soil composition and irrigation system) also appeared to impact the final concentrations of CECs in the plant, being C soils and sprinkling irrigation, the ones leading to a lower CECs' uptake by lettuces.

The irrigation with W water led to higher concentrations of PPCPs and fecal contamination (Fig. S4). However, the other variables seem to affect the levels of pathogen indicators and CECs in the lettuces, but with pretty different behavior. Dripping irrigation capacity to reduce the pathogens' transfer compared with that of sprinkling irrigation has been reported (Banach and Van der Fels-Klerx, 2020; [61]). Sprinkling likely contributes to higher levels of pathogens when they are scattered all over the lettuce surface, meanwhile by dripping the possibility to reach the crop is much lower. CECs, however, could not trespass the crop surface when sprinkled, so they are more uptaken when higher amounts reach the roots of the plant. Regarding soil composition, finer-textured (such as clay), which have better moisture and nutrient retention capacity, support superior pathogens' survival than sandy soils [18,3]. On the contrary, a higher dissipation of CECs in soils with higher clay content has been reported [24,62,64], which could explain the lower values found in the lettuces. However, degradation rates of CECs in soils are determined by different factors and processes and do not exclusively depend on a single factor [4].

Thus, contamination in the irrigation water is crucial to lowering pathogens and CECs levels in the crops. However, other factors such as soil composition and irrigation system have their own influence on the final outcome. According to the results, the best combination to lower the pathogen levels would be to irrigate with B water by drip in S soil, and the best combination to lower the CECs levels would be to irrigate with B water by a sprinkler system in C soil. Therefore, the most favorable conditions to lower pathogens and CECs levels would be the latest (B water, sprinkler, C soil), as pathogens are completely absent when irrigated with B water and the lower uptake of CECs occurs with sprinkler irrigation in C soil.

4. Conclusions

The present study showed that high concentrations of the pathogenic indicator *E. coli* and CECs in irrigation waters could lead to their accumulation in the soil and crops. The quality of the irrigation water turned out to be crucial in reducing the levels of pathogens and CECs in crops. However, other factors such as soil composition and irrigation system also influence the plant uptake of pathogens and CECs. Based on the results, it can be stated that the most favorable conditions to minimize the levels of pathogens and CECs transferred to crops would be the use of reclaimed water with sprinkler irrigation in soil rich in clay. However, the processes leading to this minor transfer of microbiological and chemical contamination were not fully understood. Further studies should be carried out on different types of crops (absorption processes in the crop may be different, as well as metabolism products), in all seasons of the year (fluctuation of contaminants in irrigation waters) and for longer periods (increased salinization) that can lead to a gradual decrease in the availability of nutrients and modification of the soil structure. Beyond the control of the chemical and microbiological quality of the irrigation water, to minimize the risk of fecal contamination in crops irrigated with reclaimed water, continuous monitoring of the irrigation water should be established in the storage tank usually used as a reserve for irrigation water, particularly concerning microbiological quality, since the regrowth of bacteria during water storage is a probable risk.

CRediT authorship contribution statement

Adrià Sunyer-Caldú: Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Paola Sepúlveda-Ruiz**: Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Miquel Salgot**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Montserrat Folch-Sánchez**: Term, Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Damià Barceló**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **M. Silvia Diaz-Cruz**: Term, Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. Adrià Sunyer-Caldú and Paola Sepúlveda-Ruiz contributed equally to this work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.108831.

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