Deliverable Report D1.5 CECs in soil including D1.6 CECs in plants

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1 Introduction to the project SERPIC

The project *Sustainable Electrochemical Reduction of contaminants of emerging concern and Pathogens in WWTP effluent for Irrigation of Crops – SERPIC* developed an integral technology, based on a multi-barrier approach, to treat the effluents of wastewater treatment plants (WWTPs) to maximise the reduction of contaminants of emerging concern (CECs). The eight partners of the SERPIC consortium were funded by the European Commission and by six national funding agencies from Norway, Germany, Italy, Spain, Portugal and South Africa. The official starting date of the SERPIC project was 1. September 2021. The project had a duration of 40 months and ended 31. December 2024.

The overall aim of the SERPIC project was to investigate and minimise the spread of CECs and antimicrobial resistant bacteria/antibiotic resistance genes (ARB/ARG) within the water cycle from households and industries to WWTPs effluents, and afterwards via irrigation into the food chain, into soil and groundwater and into river basins, estuaries, coastal areas, and oceans with a focus on additional water sources for food production.

A membrane nanofiltration (NF) technology was applied to reduce CECs in its permeate stream by at least 90 % while retaining the nutrients. A residual disinfection using chlorine dioxide produced electrochemically was added to the stream used for crops irrigation (Route A). The CECs in the polluted concentrate (retentate) stream were reduced by at least 80 % by light driven electro-chemical oxidation. When discharged into the aquatic system (route B), it will contribute to the quality improvement of the surface water body.

A prototype treatment plant was set-up and evaluated for irrigation in long-term tests with the help of agricultural test pots. A review investigation of CECs spread was performed at four regional showcases in Europe and Africa. It included a detailed assessment of the individual situation and surrounding condition. Transfer concepts was developed to transfer the results of the treatment technology to other regions, especially in low- and middle-income countries.

2 Report summary

The aim of the deliverables D1.5 and D1.6 was to determine the fate of the CECs in soil and plant uptake, after long-term crop irrigation with the effluent of SERPIC technology Route A. The field test was conducted by growing carrots and potatoes in soil, irrigated with three different water qualities: municipal tap water, water from a WWTP secondary effluent and the effluent from the SERPIC technology Route A. Soil and crop samples were collected at UCLM in July 2024, and transported to a laboratory at Stellenbosch University, South Africa, where it was analysed for the presence of CEC's. Low or undetectable levels of sulfamethoxazole, diclofenac and iopromide were detected in soil and crops irrigated with secondary effluent from both the WWTP and Route A. Venlafaxine could be detected in low concentrations in soil samples and the leaves of potatoes and carrots irrigated with both secondary WWTP and Route A effluent. Results include LC-MS analysis of soil and crops, sampled after a long-term field study involving irrigation with Route A effluent in comparison to secondary WWTP and tap water.

3 Deliverable description as stated in the Project Description

The deliverable contains the main results from **T1.3** concerning the behaviour of the target CECs once reached the soil (D1.5) and the plants (D1.6) according to route A of the SERPIC schematics and an analysis of the main factors affecting them.

4 Introduction

Increasing water scarcity is a significant global challenge, driven by factors such as climate change, urbanization, and desertification. This scarcity impacts both human health, through the provision of safe drinking water and sanitation, and agricultural productivity, which is crucial for food security. After use, water is often discharged into the environment, carrying anthropogenic pollutants that can affect aquatic ecosystems.

The reuse of treated wastewater for irrigation offers benefits such as reduced reliance on freshwater and decreased fertilizer use due to nutrient-rich effluent. However, it also presents challenges related to bioaccumulation in soil and the potential uptake of CECs by crops. These contaminants can enter the food chain, posing health risks to humans and other organisms.

The SERPIC technology consists of nanofiltration and subsequent electro-chemical oxidation of WWTP effluent, with potential application in crop irrigation (Route A). The project aims therefore to reduce CECs in the effluent, ensuring a safe and sustainable water source for agricultural irrigation. By minimizing the spread of CECs and antibiotic-resistant bacteria (ARB) and genes (ARG), the project seeks to protect soil and crop health while supporting food production. A long-term field test was planned by growing carrots and potatoes in soil, irrigated with three different water qualities: tap water, secondary effluent of the WWTP, and effluent treated by the SERPIC prototype plant. At the beginning and end of each test, a soil sample was collected. During the approximately six days of testing, liquid samples were collected from the irrigation streams, and finally roots, leaves and vegetables were collected and kept frozen until further processing and CEC analysis.

5 Results

5.1 Methodology

5.1.1 Experimental field description and design

The experimental crops growth period lasted from August to December 2023 (3 months). For this purpose, a soil reclamation facility with a 48 m³ plot, available at the University of Castilla-La Mancha (Ciudad Real, Spain), was used (Figure 1). The installation was divided into six independent sections, each with a capacity of 8 m³. The soil composition consisted of a gravel layer at the bottom, followed by a sand layer, silty loam, and a top layer of vegetable soil. The experimental design was based on the use of three different qualities of water for irrigation: wastewater treatment plant (WWTP) secondary effluent, SERPIC technology Route A effluent (Route A) and municipal tap water. The secondary effluent was collected from the conventional activated sludge process of a municipal WWTP in Ciudad Real (Spain) on 31 August 2023 and stored in a 10 m³ tank. Additionally, throughout the study, secondary effluent was continuously supplied to the SERPIC prototype, where it was treated using electrochemical technologies to produce SERPIC effluent (Route A). Meanwhile, tap water, free from microbial and organic contamination, was provided through the municipal drinking water supply for irrigating the control plots. Carrots and potatoes were grown during the experimental period. Carrots were grown in pots from seeds while potatoes were grown using seed potatoes (bulbs). To ensure uniform irrigation and to minimise evaporation, irrigation drippers were installed. Three irrigation rows were implemented, each with three drippers per row and plot. The experiment was carried out in an open field without any protection against rain.



Figure 1: Experimental field layout and irrigation system with different water qualities.

5.1.2 Collection, storage and selected of samples

Samples (soils from 0-10 cm and crops) were collected on 5 December 2023. Each of the six study plots was divided into nine sub-plots, as shown in Figure 2. Soil and crop (potatoes and carrots) samples were taken at all sampling points. Samples were placed in sterile 1 L plastic bags and transported to the laboratory. Soil samples were stored in a dark environment at room temperature, while culture samples were washed with milli-Q water and stored at -20°C until further processing, extraction and analysis.



Figure 2: Mapping of soil and crop sampling irrigated with SERPIC Route A effluent (SERPIC effluent), Tap water and secondary WWTP effluent.

A total of 15 samples were randomly selected for analysis from all the soil samples collected. Of these, 12 samples (six from pots irrigated with secondary effluent and six from pots irrigated with SERPIC effluent) were used for quantification of organic CECs. The remaining three samples, obtained from soil irrigated with tap water, were used for recovery studies. Table 1 presents the subplot numbers selected for each case. For the crop samples, a total of 18 samples were analysed for quantification of organic CECs, considering roots, vegetable and leaves separately. As crops did not grow in all plots irrigated with Route A effluent and tap water, it was necessary to combine three sub-plots from each plot irrigated with different water qualities to obtain sufficient root, vegetable and leaf material (5 g per sample). To maintain consistency in methodology, three sub-plots were also combined for pots irrigated with secondary effluent (Table 2). Specifically, one sample of roots, vegetables and leaves was analysed for each plot irrigated with secondary effluent (Table 2). The remaining 12 samples, obtained from plots irrigated with tap water, were used for recovery studies.

Soils (0-10 cm)							
Water quality	Type of crop	Sub-plots	Number of samples analysed	Use			
Route A	Carrots	1B, 2B, 3C	3 (one for each selected plot zone)				
effluent	Potatoes	1A, 1C, 3C	3 (one for each selected plot zone)	For quantification			
Secondary	Carrots	1B, 2B, 3C	3 (one for each selected plot zone)	of organic CECs			
effluent	Potatoes	1A, 1C, 3C	3 (one for each selected plot zone)				
Tap water	Carrots	1B	3	For recovery studies (blank, 50 ppb and 500 ppb)			

 Table 1:
 Number of soil samples analysed in each plot.

Table 2:	Number of crop	samples anal	ysed in each plot.
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Crops							
Water quality	Type of crop	Sub-plots	Part of crop	Number of samples analysed	Use		
			Leaves	1			
Route A effluent	Carrots	1B, 2B, 3C*	Vegetables	1			
			Roots	1			
			Leaves	1			
	Potatoes	1A, 1C, 3C*	Vegetables	1	For quantification of organic CECs		
			Roots	1			
	Carrots		Leaves	1			
		1B, 2B 3C*	Vegetables	1			
Secondary			Roots	1			
effluent	Potatoes		Leaves	1			
		1A, 1C, 3C*	Vegetables	1			
			Roots	1			
			Leaves	3			
	Carrots	2C	Vegetables	3	For room/on/		
Tap water			Roots	3	studies (blank, 50		
iap water			Leaves	3			
	Potatoes	1A,1C*	Vegetables	3			
			Roots	3			

* Crops from the selected sub-plots are combined to obtain a homogeneous sample.

5.1.3 Sample preparation

Soil samples were collected during December 2023 and stored at room temperature. Prior to analysis, all soil samples were taken from storage and spread out on a flat surface at room temperature to dry overnight. To ensure complete drying, samples were then spread on trays and dried in an oven at 35°C until all moisture had been removed. Soil samples were then crushed using a pestle and mortar, where after the crushed soil was sieved through a 2 mm, followed by a 1 mm stainless steel sieve into a tray. Soil particles too large to pass through the sieves were crushed again and sieved. Particles such as small pebbles that were too large to pass through the sieves during the final round of sieving were discarded. Soil samples were considered homogenous following the crushing and sieving steps. Five grams of homogenized soil was

weighed out per sample into a 50 mL Falcon tube. Preparation of crop samples during July 2024 did not include a drying step but was analysed as wet mass. Prior to analysis, samples were removed from the freezer to thaw overnight. For both the carrot and potato samples, the respective roots, vegetable and leaves sections were homogenized in a blender, where after 5 g of sample was weighed out into a 50 mL Falcon tube.

5.1.4 Recovery studies

CEC recovery from each of the matrices used during this study was measured. This included the roots, vegetables and leaves for both potatoes and carrots, respectively. CEC recovery was also measured for water and soil. Relatively high (500 ppb) and low (50 ppb) concentrations of an analyte stock were included as well as an unspiked blank. For the solid matrices (crops and soil), 5 g of material for each respective section (e.g. roots, vegetables, leaves) was weighed out three times to be subjected to the respective 500 ppb, 50 ppb and blank spikes. Samples subjected to the 500 ppb spike were spiked with 50 μ L of a 10 ppm analyte mix containing sulfamethoxazole, venlafaxine, diclofenac and iopromide. Samples subjected to the 50 ppb spike were spiked with 50 μ L of a 1 ppm analytes. All recovery samples were spiked with 50 μ L of a 1 ppm internal standard mixture containing sulfamethoxazole-d4, diclofenac-13C6, venlafaxine-d6 and iopromide-d3. After spiking, all samples were stored at 4°C for 30 min to allow contact time between the chemicals and the sample material.

Ten millilitres of a 50:50 (methanol:ultrapure water) mixture with pH 3 was added to each sample, where after samples were vortexed for 10 min and placed on a rotator for 30 min to facilitate mixing of the sample with the methanol/water mixture. Samples were then subjected to sonication in an ultrasonication water bath for 60 min. All samples were then centrifuged for 15 min. Following centrifugation, the supernatant was collected, and the process was repeated twice more. 320 mL ultrapure water (MilliQ) was then added to the total 30 mL collected supernatant to reduce the methanol concentration in the sample to below 5%. Samples were then filtered through 0.7 μ m glass fibre filters to remove solid particulates prior to solid phase extraction (SPE). For water samples, 100 mL of ultrapure water was spiked with the respective concentrations, left for 30 minutes at 4°C and filtered through 0.7 μ m glass fibre filtered through 0.7

For SPE, 3cc HLB cartridges (Waters) were preconditioned with 2 mL HPLC grade methanol, followed by 2 mL of ultrapure water under gravity. Samples were then extracted under vacuum at an approximate flow rate of 5 mL/min using a vacuum manifold (Supelco, VISIPREP). After extraction, the extraction lines were flushed with 2 mL of ultrapure water, where after samples were dried under vacuum for 30 min, and stored at -20°C until further processing. Samples were transported under cold storage conditions to the laboratory at Stellenbosch University, South Africa, where it was thawed for 30 min under vacuum, followed by sample elution using 4 mL of MeOH under gravity. Samples were dried under nitrogen gas, after which it was reconstituted in 500 μ L of MeOH and filtered through a 0.22 μ m PTFE hydrophobic syringe filter. Reconstituted samples were then transferred to glass mass spectrometry (MS) vials and stored at -20°C until chemical analysis was performed.

Quantitative chemical analysis was performed using liquid chromatography coupled with quadruple mass spectrometry (TQS-Micro UPLC MS/MS; Waters). The LC-MS method for the targeted list of chemicals (sulfamethoxazole, venlafaxine, diclofenac and iopromide) was developed at Stellenbosch University. Final integration of the detected chemicals were performed using TargetLynx (V4.2;Waters,UK). The limit of quantification, including the method detection limit (MDL) and method quantification limit (MQL) for each chemical of interest was determined using the European Commission Council Directive 2002/657/EC (European Commission, 2002)

for quantification of organic analytes using LC-MS. Integration of a standard curve for each CEC of interest was prepared in a range of 0.5 ppb to 500 ppb. The MQL of each respective chemical was as follows: sulfamethoxazole – 1 ppb, venlafaxine – 0.5 ppb, diclofenac – 5ppb and iopromide – 10 ppb. It should however be noted that these concentrations were quantified in a standard curve and that concentrations lower than these MQLs can be quantified when concentration factors are used. Method detection limits are dependent on each sample as it is an indication of a signal/noise ratio between 3 and 10.

5.1.5 Quantification analysis of CECs in soil and crops

The potential CEC uptake path was studied by analysing the water used for irrigation, the soil and the respective sections of the crops (roots, vegetables and leaves). In total, twelve soil samples were analysed. Six soil samples were taken from each of the plots irrigated with treated ozonated water and untreated WWTP effluent water, respectively. From the six samples, three samples were taken from the areas planted with carrots, while the other three samples were taken from areas planted with potatoes. Processing of all samples occurred in a similar manner to that described in section 5.1.3. Samples for quantification were only spiked with 50 μ L of the 1 ppm internal standard mixture.

5.2 Results and Discussion

5.2.1 Recovery studies

Recovery studies were performed on the various solid matrices to determine how effectively each respective CEC could be recovered during sample processing. Typically, the recovery percentage of the 50 ppb and 500 ppb spiked samples are calculated for each CEC by subtracting its concentration in the blank from the measured concentrations in the 50 ppb and 500 ppb samples. However, in this study the concentration of the four target chemicals in the blank were always less than 5% of the recovery percentage in the 50 ppb and 500 ppb samples and therefore were not subtracted. Good recovery was seen for sulfamethoxazole, venlafaxine and diclofenac in the 50 ppb spiked soil sample (Table 3), with all three chemicals displaying 100% recovery. lopromide, however, showed poor recovery and could not be detected. All four chemicals showed at least 100% recovery in the 500 ppb spiked sample. Recoveries for all four CECs were higher than 80% in both the 50 ppb and 500 ppb spiked water samples (Table 4), with no detectable concentrations present in the blank sample.

In the recovery analysis for the potato samples (Table 5 – 7), sulfamethoxazole, venlafaxine and diclofenac showed at least 100% recovery for both the 50 ppb and 500 ppb spiked root samples, indicating that these chemicals can be effectively recovered from this matrix. Iopromide, however, could not be quantified in the 50 ppb sample and showed over-recovery in the 500 ppb sample with a recovery percentage of 607.5%, indicating that this matrix influences the measurement accuracy. In both the potato vegetable and leaves samples, recoveries were high overall, with only sulfamethoxazole showing < MQL concentration in the 50 ppb spiked vegetable sample, and iopromide showing poor recovery in the 50 ppb spiked vegetable sample and both spiked leaves samples. Carrot root, vegetable and leaves samples showed good recoveries overall for sulfamethoxazole, venlafaxine and diclofenac, with these chemicals typically showing recovery percentages between 80 – 100% in both the 50 ppb and 500 ppb spiked samples (Tables 6 – 8). Iopromide showed less optimal recovery, falling below the detection and quantification limits respectively for the 50 ppb and 500 ppb spiked leaves samples. Iopromide could also not be quantified in the 50 ppb carrot vegetable sample and showed over recovery of 1807.5% in the 500 ppb spiked sample.

From the recovery studies carried out on the respective sections of each matrix, it was concluded that all four CECs could effectively be recovered from water, but for soil and the respective carrot and potato matrices, iopromide showed poor recovery overall and could not be recovered as effectively as sulfamethoxazole, venlafaxine and diclofenac.

Chemical	Soil WWTP Carrots 1b	Recovery Soil WWTP Carrot 1b	Recovery %	Recovery Soil WWTP Carrot 1b 500 ppb	Recovery %
		50 ppb			
Sulfamethoxazole	<mql< th=""><th>54,9</th><th>109,8</th><th>585,7</th><th>117,14</th></mql<>	54,9	109,8	585,7	117,14
Venlafaxine	0,25	59,3	118,6	581	116,2
Diclofenac	<mdl< th=""><th>65,3</th><th>130,6</th><th>619,8</th><th>123,96</th></mdl<>	65,3	130,6	619,8	123,96
lopromide	<mdl< th=""><th><mql< th=""><th><mql< th=""><th>789,3</th><th>157,86</th></mql<></th></mql<></th></mdl<>	<mql< th=""><th><mql< th=""><th>789,3</th><th>157,86</th></mql<></th></mql<>	<mql< th=""><th>789,3</th><th>157,86</th></mql<>	789,3	157,86

 Table 3:
 Recovery studies performed on soil irrigated with WWTP effluent.

 Table 4:
 Recovery studies performed on ultra-pure water

Chemical	Recovery Water	Recovery Water	Recovery %	Recovery Water	Recovery %
	Blank	50 ppb		500 ppb	
Sulfamethoxazole	<mdl< th=""><th>43,1</th><th>86,2</th><th>473,5</th><th>94,7</th></mdl<>	43,1	86,2	473,5	94,7
Venlafaxine	<mql< th=""><th>49</th><th>98</th><th>477,4</th><th>95,48</th></mql<>	49	98	477,4	95,48
Diclofenac	<mdl< th=""><th>41,8</th><th>83,6</th><th>442,4</th><th>88,48</th></mdl<>	41,8	83,6	442,4	88,48
lopromide	<mdl< th=""><th>59,3</th><th>118,6</th><th>450,7</th><th>90,14</th></mdl<>	59,3	118,6	450,7	90,14

Table 5:	Recovery	studies	performed	on	potato	roots
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Chemical	Recovery Potato	Recovery Potato	Recovery	Recovery Potato	Recovery %
	Roots Blank	Roots 50 ppb	%	Roots 500 ppb	
Sulfamethoxazole	<mdl< th=""><th>55,1</th><th>110,2</th><th>546,1</th><th>109,22</th></mdl<>	55,1	110,2	546,1	109,22
Venlafaxine	0,25	61	122	567,1	113,42
Diclofenac	<mdl< th=""><th>120</th><th>240</th><th>1063,4</th><th>212,68</th></mdl<>	120	240	1063,4	212,68
lopromide	<mdl< th=""><th><mql< th=""><th><mql< th=""><th>3037,6</th><th>607,52</th></mql<></th></mql<></th></mdl<>	<mql< th=""><th><mql< th=""><th>3037,6</th><th>607,52</th></mql<></th></mql<>	<mql< th=""><th>3037,6</th><th>607,52</th></mql<>	3037,6	607,52

Table 6:
 Recovery studies performed on potato vegetables

Chemical	Recovery Potato Vegetable blank	Recovery Potato Vegetable 50 ppb	Recovery %	Recovery Potato Vegetable 500 ppb	Recovery %
Sulfamethoxazole	<mdl< th=""><th><mql< th=""><th><mql< th=""><th>444,8</th><th>88,96</th></mql<></th></mql<></th></mdl<>	<mql< th=""><th><mql< th=""><th>444,8</th><th>88,96</th></mql<></th></mql<>	<mql< th=""><th>444,8</th><th>88,96</th></mql<>	444,8	88,96
Venlafaxine	0,14	60,4	120,8	588,2	117,64
Diclofenac	<mdl< th=""><th>63,5</th><th>127</th><th>754,3</th><th>150,86</th></mdl<>	63,5	127	754,3	150,86
lopromide	<mdl< th=""><th>22</th><th>44</th><th>647,5</th><th>129,5</th></mdl<>	22	44	647,5	129,5

Table 7:	Recovery	studies	performed	on po	otato	leaves
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Chemical	Recovery Potato Leaves Blank	Recovery Potato Leaves 50 ppb	Recovery %	Recovery Potato Leaves 500 ppb	Recovery %
Sulfamethoxazole	<mdl< th=""><th>49,7</th><th>99,4</th><th>542,4</th><th>108,48</th></mdl<>	49,7	99,4	542,4	108,48
Venlafaxine	0,1	62,6	125,2	557,7	111,54
Diclofenac	<mdl< th=""><th>102</th><th>204</th><th>1058,3</th><th>211,66</th></mdl<>	102	204	1058,3	211,66
lopromide	<mdl< th=""><th><mdl< th=""><th><mdl< th=""><th><mql< th=""><th><mql< th=""></mql<></th></mql<></th></mdl<></th></mdl<></th></mdl<>	<mdl< th=""><th><mdl< th=""><th><mql< th=""><th><mql< th=""></mql<></th></mql<></th></mdl<></th></mdl<>	<mdl< th=""><th><mql< th=""><th><mql< th=""></mql<></th></mql<></th></mdl<>	<mql< th=""><th><mql< th=""></mql<></th></mql<>	<mql< th=""></mql<>

 Table 8:
 Recovery studies performed on carrot roots.

Chemical	Recovery Carrot Root Blank	Recovery Carrot Root 50 ppb	Recovery %	Recovery Carrot Root 500 ppb	Recovery %
Sulfamethoxazole	<mdl< th=""><th>44,9</th><th>89,8</th><th>497,8</th><th>99,56</th></mdl<>	44,9	89,8	497,8	99,56
Venlafaxine	0,6	54,7	109,4	529,3	105,86
Diclofenac	<mql< th=""><th>47,9</th><th>95,8</th><th>526,4</th><th>105,28</th></mql<>	47,9	95,8	526,4	105,28
lopromide	<mdl< th=""><th>36,8</th><th>73,6</th><th>740,7</th><th>148,14</th></mdl<>	36,8	73,6	740,7	148,14

 Table 9:
 Recovery studies performed on carrot vegetables

Chemical	Recovery Carrot Vegetable Blank	Recovery Carrot Vegetable 50 ppb	Recovery %	Recovery Carrot Vegetable 500 ppb	Recovery %
Sulfamethoxazole	<mdl< th=""><th>44,6</th><th>89,2</th><th>518,3</th><th>103,66</th></mdl<>	44,6	89,2	518,3	103,66
Venlafaxine	0,13	54,2	108,4	512,4	102,48
Diclofenac	<mql< th=""><th>54,7</th><th>109,4</th><th>511</th><th>102,2</th></mql<>	54,7	109,4	511	102,2
lopromide	<mdl< th=""><th><mql< th=""><th><mql< th=""><th>9037,7</th><th>1807,54</th></mql<></th></mql<></th></mdl<>	<mql< th=""><th><mql< th=""><th>9037,7</th><th>1807,54</th></mql<></th></mql<>	<mql< th=""><th>9037,7</th><th>1807,54</th></mql<>	9037,7	1807,54

 Table 10:
 Recovery studies performed on carrot leaves

Chemical	Recovery Carrot Leaves Blank	Recovery Carrot Leaves 50 ppb	Recovery %	Recovery Carrot Leaves 500 ppb	Recovery %
Sulfamethoxazole	<mdl< th=""><th>42,1</th><th>84,2</th><th>497,9</th><th>99,58</th></mdl<>	42,1	84,2	497,9	99,58
Venlafaxine	0,07	54,1	108,2	532,8	106,56
Diclofenac	<mql< th=""><th>46,5</th><th>93</th><th>511,6</th><th>102,32</th></mql<>	46,5	93	511,6	102,32
lopromide	<mdl< th=""><th><mdl< th=""><th><mdl< th=""><th><mql< th=""><th><mql< th=""></mql<></th></mql<></th></mdl<></th></mdl<></th></mdl<>	<mdl< th=""><th><mdl< th=""><th><mql< th=""><th><mql< th=""></mql<></th></mql<></th></mdl<></th></mdl<>	<mdl< th=""><th><mql< th=""><th><mql< th=""></mql<></th></mql<></th></mdl<>	<mql< th=""><th><mql< th=""></mql<></th></mql<>	<mql< th=""></mql<>

5.2.2 Quantification analysis of CECs in soil and crops

As indicated by Figure 3, sulfamethoxazole, diclofenac and iopromide were not found in detectable levels in soil samples irrigated with Route A effluent. In the soil sample of potato plot 5C, irrigated with WWTP effluent, 0.27 μ g/kg sulfamethoxazole and 0.09 μ g/kg diclofenac were detected. In the soil samples of potato plots 1C and 3C and carrot plot 2b, irrigated with Route A effluent, venlafaxine was detected at 0.17 μ g/kg; 0.68 μ g/kg and 0.13 μ g/kg respectively. Venlafaxine was also detected in soil irrigated with WWTP effluent in the potato plots 1a, 1c and 5c at 0.6 μ g/kg, 0.63 μ g/kg;,1.61 μ g/kg respectively and in carrot plots 1b, 2b, 3c at 0.25 μ g/kg, 3.19 μ g/kg and1.66 μ g/kg respectively.

The presence of detectable concentrations of diclofenac in so few of the soil samples irrigated with WWTP effluent is however unexpected, especially considering diclofenac's high log K_{ow} value of 4.51, rendering it more prone to bind to particles around it rather than moving with the water through the soil (Pilon-Smits, 2005; Zhang et al., 2016).



Figure 3: CECs concentrations in soil samples irrigated with SERPIC Route A effluent (Route A) and WWTP secondary effluent (WWTP), respectively.

As displayed in Figure 4, analysis of crop samples indicated that no sulfamethoxazole or iopromide were present in any of the roots, vegetables or leaves of the potatoes and carrots planted in soil irrigated with either WWTP effluent or Route A effluent. This corresponds to the results of the analysis of the soil samples.

Venlafaxine, however showed different results and a clear uptake pattern was visible. Venlafaxine could be detected in the leaves of the carrots (0.03 μ g/kg and 0.05 μ g/kg) and potatoes (0.10 μ g/kg and 0.15 μ g/kg) in plots irrigated with WWTP and Route A effluent, respectively. It was also detected in carrot and potato roots, irrigated with WWTP effluent at 0.14 μ g/kg and 0.27 μ g/kg, respectively. Considering the natural uptake path, higher concentrations of venlafaxine were irrigated onto the plots receiving the WWTP effluent. As venlafaxine is a cationic compound, its behavior is related to its log D_{ow} value rather than log K_{ow} , where its pH dependence in an aqueous solution is also considered. Basic compounds like venlafaxine are therefore expected to translocate in plants, rather than accumulate in the roots (Verlicchi et al., 2023). However, at the higher concentrations present in the soil irrigated with secondary WWTP effluent, a portion of venlafaxine most likely also accumulates around/in the roots as part of it translocates through the plants.

Diclofenac was detected at 0.15 μ g/kg, only in the roots of carrots grown in plots irrigated with Route A effluent. This does not correspond to the data from the soil analysis but points to some accumulation of diclofenac in this plot. This uptake pattern is to be expected considering diclofenac's high log K_{ow} value of 4.51, render it more prone to bind to the roots rather than moving up into the plant in the direction of the transpiration stream.

According to the previous results of Delivery Report 1.4 *CECs in product water of v1 prototype*, the SERPIC goals of 90% and higher removal rates of CEC's were attained. However, it is evident and confirmed that low concentrations of sulfamethoxazole, diclofenac and venlafaxine were present in Route A effluent, that was applied in the irrigation of the crops during the field study.



Figure 4: CEC concentrations in respective roots, vegetables and leaves of potatoes and carrots grown in plots irrigated with Route A effluent (Route A), and crops grown in plots irrigated with WWTP secondary effluent (WWTP).

5.3 Conclusion

Applying secondary effluent (corresponding to the effluent of a conventional activated sludge process, the most common treatment adopted for urban wastewater) for irrigation of crops (potatoes and carrots) could lead to uptake of certain CECs at the various stages along the plant uptake pathway. The fate of the CECs in soil and plant uptake clearly indicated that irrigation of crops with SERPIC Route A effluent introduces lower concentrations of these CECs into the potential uptake pathway and is therefore a potential technology to treat secondary effluent for irrigation applications to ensure safe food production.

6 Publications and other dissemination activities

Verlicchi, P., Grillini, V., Lacasa, E., Archer, E., Krzeminski, P., Gomes, A.I., Vilar, V.J.P., Rodrigo, M.A., G\u00e4bler, J., Sch\u00e4fer, L., 2023. Selection of indicator contaminants of emerging concern when reusing reclaimed water for irrigation – A proposed methodology. STOTEN. 873. http://dx.doi.org/10.1016/j.scitotenv.2023.162359

7 Literature

Pilon-Smits, E., 2005. Phytoremediation. Annu. Rev. Plant Biol. 56, 15 – 39. https://doi.org/10.1146/annurev.arplant.56.032604.144214

Zhang, Y., Lv, T., Carvalho, P.N., Arias, C.A., Chen, Z., Brix, H., 2016. Removal of the pharmaceuticals ibuprofen and iohexol by four wetland plant species in hydroponic culture: plant uptake and microbial degradation. Environ. Sci. Pollut. Res. 23, 2890 – 2898. https://doi.org/10.1007/s11356-015-5552-x