Sustainable Electrochemical Reduction of contaminants of emerging concern and Pathogens in WWTP effluent for Irrigation of Crops – SERPIC

Report
to the Water Research Commission

by

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EXECUTIVE SUMMARY

The objective of the SERPIC project was to develop a new technology to remove contaminants of emerging concern (CEC) like residues from pharmaceuticals and pesticides, and antimicrobial resistant (AMR) bacteria from the effluent of wastewater treatment plants (WWTP). Those compounds and pathogens are dangerous for the environment and human health, but are mostly not removed by the existing treatment steps in municipal WWTPs. This creates risks for aquatic life as the treated water from WWTPs is usually discharged into surface waters. Furthermore, it hinders the reuse of the treated water, e.g. for the agricultural irrigation of crops, because the compounds might be accumulated in the soil and in the plants.

Becoming effective 1.1.2025, the European Commission has set a regulation for CEC limits that WWTPs must meet for the discharge of treated water. Ozonation and activated carbon adsorption are technologies already used or tested in a few WWTPs in Europe. However, these technologies have some disadvantages like high energy demand, huge need for consumables and only selected efficiency to remove a huge spectrum of CECs. This creates the need to research other technologies to overcome these barriers.

The concept of the SERPIC technology is designed not only to remove the compounds from the water but to destroy them by oxidation so that they are removed from the environment and the water cycle. The first process step of the SERPIC technology is nanofiltration. The membrane separation allows for the production of a permeate of high quality. To create a multi-barrier approach, a subsequent in-situ disinfection via electrogenerated ozone gas was implemented, also allowing the removal of some CECs not rejected by the NF membrane. The project results showed that all six of the selected target compounds or pathogens are reduced via this two-step treatment by more than 90 % of the inlet contents. The concept of SERPIC is to reuse this stream for the irrigation of crops (Route A).

The nanofiltration concentrate stream collects most of the compounds of the inlet wastewater. This stream is treated via electrogenerated persulfate activated by UV-C irradiation using a membrane photoreactor. The combined action of UV-C light and persulfate generates highly reactive species that destroy both CECs and bacteria. The results showed that the compounds could be reduced by 80 – 99 % of the concentrate stream content. Only the removal of the antidepressant venlafaxine was limited to 70 %. The treated concentrate complies with the requirements for discharges from WWTPs (Route B) as defined by the EU Urban Wastewater Treatment Directive, including COD, BOD_5, total suspended solids, etc.

A long-term field test by irrigating carrots and potatoes with route A stream was performed to control the fate of the few remaining CECs and AMR bacteria into soil and plants. SERPIC treatment removed sulfamethoxazole, venlafaxine and diclofenac from WWTP effluent to almost undetectable levels. Therefore, low or undetectable levels of these CECs were detected in soil and crops irrigated with SERPIC-treated water. The recalcitrant organic compound Venlafaxine could be detected in the leaves of potatoes and carrots irrigated with SERPIC-treated water, but in lower concentrations in comparison to the crops irrigated with WWTP effluent.

A further development of the SERPIC technology and finally the application in WWTPs will create water with strongly reduced contents of CECs and AMR bacteria. This will create a positive impact by improving the health of the aquatic environment. Furthermore, it will enable the use of the treated water, e.g. for irrigation in water-scarce regions. This will help to mitigate the effects of man-made climate change and will create a positive impact by ensuring sufficient and safe food for humans.

Positive economic impacts will be generated for manufacturers of water treatment equipment by selling treatment solutions based on the SERPIC technology to WWTPs. This will safeguard jobs, create added value and contribute to the well-being of our society.

BACKGROUND

Increasing water scarcity is one of the biggest challenges worldwide. The provision of safe water in sufficient amounts is essential for both human health (drinking water and sanitation) and – via irrigation of crops – for a sufficient human nutrition. SERPIC connects the urban water cycle with the water cycle in agriculture and the natural water cycle. The reuse of the effluents of wastewater treatment plants (WWTPs) constitutes a significant and constantly available water resource that can be safely used for irrigation. This was the motivation for the SERPIC project to develop technologies to reduce CECs from conventional WWTP effluent.

SERPIC PROJECT TEAM

Project lead: Fraunhofer-Gesellschaft e.V. (Fraunhofer IST)
Partners: Fraunhofer ISE (Germany); SolarSpring GmbH (Germany); Universidad de Castilla-La Mancha (Spain); Università degli Studi di Ferrara (Italy); Universidade do Porto (Portugal); AdP VALOR, Serviços Ambientais (Portugal); Norwegian Institute for Water Research (Norway); Stellenbosch University (South Africa).

The official starting date of the SERPIC project was 1 September 2021 to 31 December 2024.

SERPIC PROJECT AIMS

The following were the aims of the project:

1. Minimise the spread and transformation of CECs, including ARB and 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;
2. Reduce CECs from WWTP effluent by developing an innovative treatment technology, based on membrane filtration and light-driven electrochemical processes. Reduction targets: Route A > 90 %, Route B > 80 %
3. Elaborate methodologies and tools for monitoring, health and environmental risk assessment and for the implementation of new reuse concepts

METHODOLOGY

With the SERPIC technological multibarrier approach, secondary effluent of a municipal wastewater plant underwent a sequence of reclaiming treatments: A nanofiltration separated the effluent into a high-quality permeate stream (Route A) and a more polluted concentrate stream (Route B). Route A stream was disinfected by ozone gas, electrochemically generated using diamond electrodes, for safe irrigation of crops. The CECs in the Route B stream were degraded by persulfate, electrochemically generated using diamond electrodes, for safe discharge into the aquatic environment. A prototype plant was set up and tested in a long-term field test by irrigating crops with the treated water.

RESULTS AND DISCUSSION

A methodology was developed to select relevant target CECs for the analysis to prove the performance of the technology. Process components were developed and built. A prototype plant was commissioned and put into operation. It could process 34 L/h of water with an energy consumption of 630 W. The CECs in the Route A water for irrigation were reduced by 90-99 %. The CECs in the Route B water for discharge were reduced by 70-99 %. LCA and LCC were done, as well as a risk analysis of the plant operation. An exploitation of the results was planned and discussed with stakeholders.

GENERAL

In terms of the SERPIC project, the following aims were attained:

Aim 1 was achieved since SERPIC successfully developed a CEC removal technology up to prototype level (TRL5), offering a knowledge base for further development until real application in WWTPs (TRL9). When this is reached, CECs, including ARB and ARGs, will be removed, resulting in a minimisation of their spread in both urban and natural water cycles.

Aim 2 was to reduce CECs from WWTP effluent by developing an innovative treatment technology, based on membrane filtration and light-driven electrochemical processes. For the two application routes, different reduction targets were followed. For Route A, SERPIC achieved reduction rates for the six target CECs between 89.9 % and 99.4 %, i.e. a full achievement. For Route B, SERPIC achieved reduction rates between 96.1 % and 99.6 % for five of the six target compounds, and 70.3 % for Venlafaxine.

Thus, the SERPIC consortium achieved 11 of the 12 reduction targets, i.e. 92 % of objective 2.
For aim 3, SERPIC elaborated a methodology to select relevant target CECs based on four criteria: occurrence, persistence, bioaccumulation and toxicity. This method can be used not only for investigations in treatment technologies, but also for many other purposes, like monitoring and risk assessment.

The contribution of SU in this project assisted the SERPIC team in attaining these aims.

CONCLUSIONS

The technology has proven that the CEC removal rates are sufficient and promising. The most important step for further development is to scale up the technology and show that it is working well, also with higher volume flows. The SERPIC prototype plant had a volume flow of 34 litres per hour. A medium-sized WWTP has a volume flow in the range of 1000 cubic metres per hour. That means that for application in WWTPs, the technology must be scaled up by five orders of magnitude. This can only be implemented in several steps. The first step would be to scale it up to demonstrator level, with a volume flow of app. one cubic metre per hour (1000 litres). This affords another funded project and the participation of engineering equipment manufacturers, like membrane manufacturers, diamond electrode manufacturers or manufacturers of electrochemical cells. Funding programmes like LIFE+, Horizon Europe or Water4All might be suitable for this step. Such a project affords, amongst others, quite a lot of hardware budget for the bigger scale. Therefore, ERA-NETs will most likely not be suitable, as they usually do not provide large hardware budgets. If the technology proves to be successful in reducing CECs on this volume flow level, more reliable estimations of energy demand and investment costs can be made.

RECOMMENDATIONS

Disinfection by electrogenerated ozone gas is regarded as a promising alternative to gas ozonation with ozone produced from liquid oxygen.

The results offer new opportunities for nanofiltration as a central CEC separation method. Nanofiltration also enables a very clean permeate that could be used for irrigation, industry and drinking water.

Water treatment with electrochemically generated persulfate offers a low-energy alternative with much higher CEC degradation rates compared to activated carbon adsorption and ozonation.

A special advantage of SERPIC is offered for applications requiring the treatment of smaller flow rates, e.g. decentralised wastewater treatments, like remote places or areas without a sewer system.

See APPENDIX B for the Serpic project summary published in a booklet.

ACKNOWLEDGEMENTS

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Research manager	
Dr Eunice Ubombo-Jaswa	WRC
Post-doc students	
Dr Alno Carstens	SU
Dr Christoff Truter	SU
Dr Edward Archer	SU

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ACRONYMS & ABBREVIATIONS

AdP	AdP VALOR, Serviços Ambientais
ARB	Antimicrobial resistant bacteria
ARG	Antimicrobial resistant gene
BOD	Biological oxygen demand
CEC's	Contaminants of emerging concern
COD	Chemical oxygen demand
DIC	Diclofenac
IOP	Iopromide
ISE	Fraunhofer-Gesellschaft e.V. ISE
IST	Fraunhofer-Gesellschaft e.V. IST
NIVA	Norwegian Institute for Water Research
SMX	Sulfamethoxazole
SU	Stellenbosch University
UNIFE	Università degli Studi di Ferrara
UP	Universidade do Porto
UV-C	Ultra-violet C
WWTP	Wastewater treatment plant
VNLX	Venlafaxine

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Increasing water scarcity is one of the biggest challenges worldwide. Climate change, urbanisation and desertification are the main reasons for it. The provision of safe water in sufficient amounts is essential for both human health (drinking water and sanitation) and – via irrigation of crops – for sufficient human nutrition. After human use, the water is discharged, and the added anthropogenic pollutants can affect the aquatic environment.

SERPIC connects the urban water cycle with the water cycle in agriculture and the natural water cycle. Countries that suffer from water scarcity or imbalances, such as those of the Mediterranean and African regions, rely on exploiting alternative water sources, in addition to natural surface and groundwater sources. The reuse of the effluents of wastewater treatment plants (WWTPs) constitutes a significant and constantly available water resource that can be safely used for irrigation.

However, municipal and industrial WWTPs face an increasing number of emerging pollutants (e.g. prescription and over-the-counter drugs, personal care products, and chemicals used in agriculture and industry), pathogens, antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), collectively called contaminants of emerging concern (CECs) in the following. CECs carry a remarkably diverse threat to human health, with the risk of many diseases, including cancer. A special risk is connected to antimicrobial resistance (AMR), as it poses the danger that antibiotics might not work against bacterial infections. Corresponding regulatory limits for the most critical CECs for WWTP effluents have recently been established in the European Union in the case of a release into the environment. The revised Directive concerning urban wastewater treatment EU 2024/3019 (UWWTD), in force starting from January 1st 2025, introduces the need for an additional treatment to reduce the content of organic micropollutants in the final effluent of large WWTPs (> 150 000 people equivalent, p.e.) to be released into the environment, as well as for small WWTPs (> 10 000 p.e.) placed in an area at risk of accumulation/pollution due to CECs.

With proper treatment technologies, a high reduction of CECs would be achievable, and treated effluent would represent a flux of always available safe water for agricultural irrigation. This is the motivation for the SERPIC project to develop technologies to reduce CECs from conventional WWTP effluent.

The overall aim of the SERPIC project was to investigate and minimise the spread of CECs and ARB/ARG with a focus on additional water sources for food production.

1.2 PROJECT AIMS

The following were the aims of the SERPIC project:

1. To minimize the spread and transformation of CECs, including ARB and 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
2. To reduce CECs from WWTP effluent by developing an innovative treatment technology, based on membrane filtration and light-driven electrochemical processes.
3. To elaborate methodologies and tools for monitoring, health and environmental risk assessment and for the implementation of new reuse concepts, including new treatment technologies, as a basis for better policy and decision-making, for regulatory issues and new standards.

1.3 SCOPE OF STELLENBOSCH UNIVERSITY

The work programme was organised in five work packages:

1 Sources, spread and transformation
2 Treatment technology and prototype
3 Transfer strategies
4 Education, communication, exploitation
5 Management

Stellenbosch University was responsible for specific tasks within the different work packages as stipulated and reported below.

Work package 1
Task: Selecting target CECs
Six CECs were identified to monitor during the project investigations at bench scale and with the prototypes. The selection of the six target compounds, one ARB, one ARG and four organic compounds (CECs in general), was be based on their occurrence in the water cycle (wastewater, treated effluent and surface water), persistence during treatment, bioaccumulation and toxicity to health and environment of the four showcases; Italy, Spain, Portugal and South Africa.

Report: Published via deliverable D1.1 on serpic-project.eu and via this open access paper: Verlicchi, P.; Grillini, V.; Lacasa, E.; Archer, E.; Krzeminski, P.; Gomes, A. I. et al. (2023): Selection of indicator contaminants of emerging concern when reusing reclaimed water for irrigation - A proposed methodology. In *The Science of the total environment* 873, p. 162359. DOI: 10.1016/j.scitotenv.2023.162359.

Task: Analysis of bench-scale processes
Analytical methods were developed, validated and tested for the detection of the selected CECs in the different matrices: water (raw municipal wastewater and treated effluent), soil and crops in collaboration with the partners and the secondments of researchers from UNIFE at UCLM.
Report: Chapter 2

Task: Analysis of compounds in value chain
Effect-based screening (toxicity assays) of water sampled from the pilot plant at UCLM were carried out. These assays included the Yeast estrogen- and androgen screen (YES/YAS) and aryl hydrocarbon receptor assay.
Report: Chapter 3

Work package 2

Task: Assisting in the interpretation of CEC results
Assisting in the interpretation of CEC results to evaluate the performance of the prototypes
Report: Booklet in Appendix B

Work package 3

Task: Transfer concepts
Development of transfer concepts with the focus on Southern Africa (South Africa) and West Africa (Nigeria).
Report: Chapter 4

Work package 4

Task: Mobility:

Exchanges of human resources within the consortium to bring together all necessary expertise at the right places to fulfil the project tasks and to facilitate knowledge transfer.
Report: One post-doc student from SU travelled to Spain in July 2024 to conduct solid-phase extraction of the water, plant and soil material samples.

Task: Communication
Presentation of project results at workshops
Report: The SERPIC project was presented by SU at the following events:

The **SERPIC 3rd general assembly (GA3)** was hosted by SU at Swadini Forever Resort on 3 to 4 November 2022. The workshop was attended by colleagues from University of Pretoria (Hans Hoheisen Wildlife station), Fraunhofer institutes (IST, ISE), local farmers and local industry partners.

The **SERPIC project** was virtually presented by SU at the World Water Week hosted by the Stockholm International Water Institute, on 28 August 2024.

The HORIZON-CL6-2022-ZEROPOLLUTION-01 project entitled 'Preventing groundwater contamination related to global and climate change through a holistic approach on managed aquifer recharge (MAR2PROTECT)' started in December 2023 and will end in December 2027. The 2nd MAR2PROTECT Cape Flats Aquifer LivingLab was hosted by Stellenbosch University Water on 28 January 2025. The workshop aimed to demonstrate technology and to co-design a societal engagement event. The **SERPIC project** was also presented at this event. The workshop was attended by 35 people from industry (Umvoto, Resalt); government (Department of Water and Sanitation; Department of Environmental Affairs and Development Planning, City of Cape Town (Bulk Water); Drakenstein municipality); academia (SU; Academy for Environmental Leadership SA) and the Stellenbosch Farmer Support Unit.

CHAPTER 2: ANALYSIS OF BENCH-SCALE PROCESSES

2.1 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 electrochemical 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, water from the secondary effluent of the WWTP and effluent treated by the SERPIC prototype plant. At the beginning and end of each test, a soil sample will be collected. During the approximately six days of testing, liquid samples will be collected from the irrigation streams, and finally roots, leaves and vegetables will be collected and kept frozen until further processing and CEC analysis.

2.2 METHODOLOGY

2.2.1 SERPIC Prototype

The approach of SERPIC was to test an additional treatment step after the primary (sedimentation) and secondary (biological) treatment, usually implemented in wastewater treatment plants (WWTP). With the SERPIC technological multibarrier approach, the secondary treated effluents of the municipal WWTP underwent a sequence of reclaiming treatments that started with nanofiltration, which separated the raw effluent into a high-quality permeate stream, so-called Route A, and a more polluted concentrate stream, so-called Route B. Both streams were further processed: the first, Route A, for safe irrigation of crops, and the second, Route B, for safe discharge into the environment. See Figure 2.1.

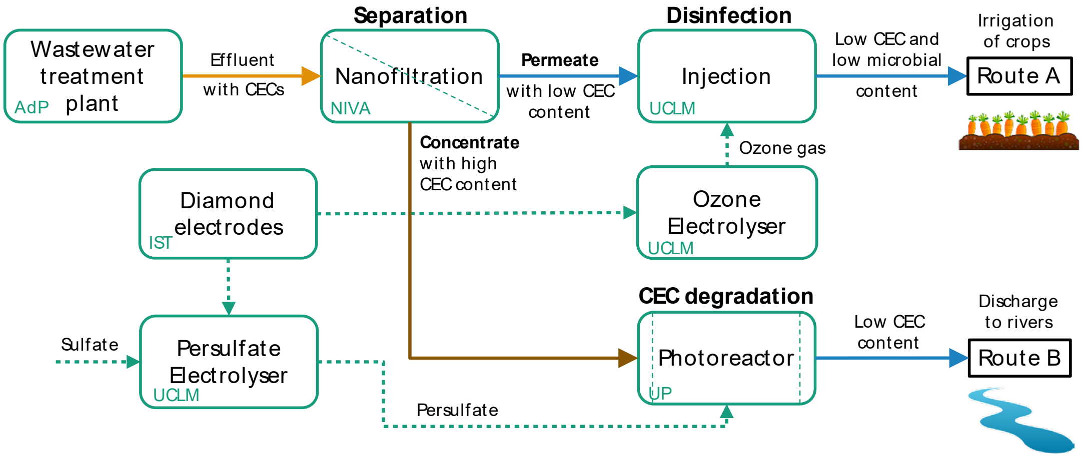

Figure 2.1 Process chain of the SERPIC water treatment

To make sure that irrigation is safe, the stream of Route A underwent disinfection by ozonation produced in an electrolyser equipped with a proton exchange membrane (PEM) and diamond electrodes using a novel cell, adapted from a previous project. This warrants complete disinfection and removal of CECs that could be produced by a potential inefficient performance of the nanofiltration.

Regarding the concentrate stream of Route B, it underwent an advanced oxidation process consisting of the addition and activation of persulfates in a novel photoreactor. These oxidants were also produced in a specially designed electrochemical cell, manufactured by 3-D printing. The photoreactor was specifically designed to activate persulfates and warrants an outstanding efficiency in the removal of CECs because it assures a good fluid-dynamic pattern, which is a key input for the removal of species contained at rather low concentrations.

2.1.2 Experimental field description and design

The experimental crop's 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 2.2). 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 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.

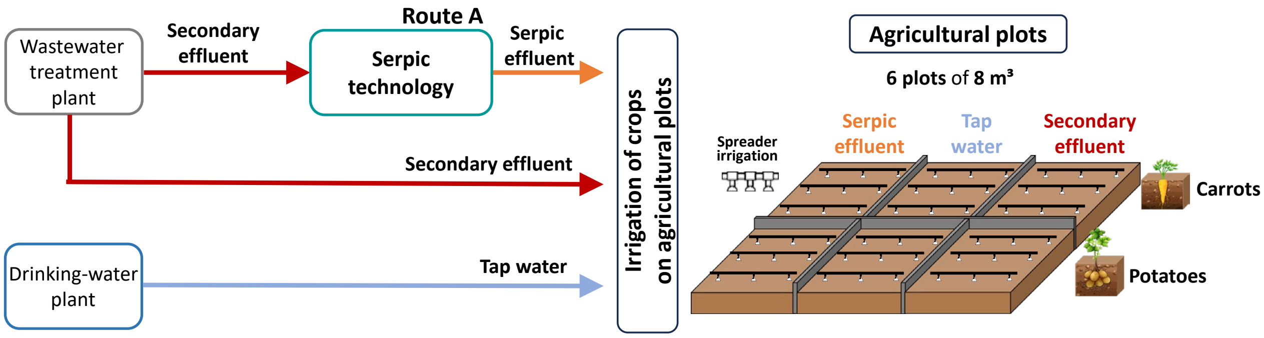

Figure 2.2 Experimental field layout and irrigation system with different water qualities

2.1.3 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 2.3. 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.

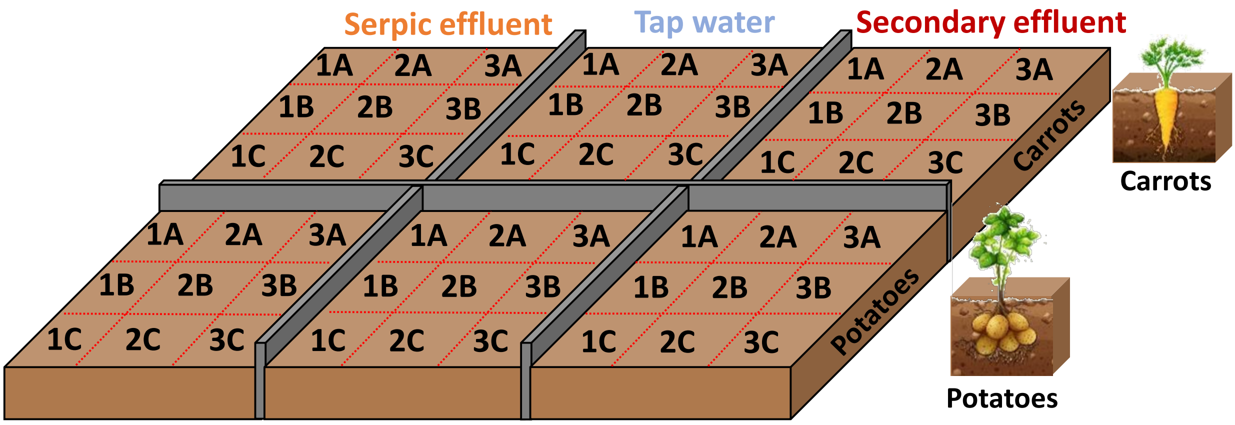

Figure 2.3 Mapping of soil and crop sampling irrigated with SERPIC Route A effluent (Serpic effluent), tap water and secondary WWTP effluent

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 2.1 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, vegetables 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 2.2). Specifically, one sample of roots, vegetables and leaves was analysed for each plot irrigated with secondary effluent and SERPIC effluent (6 samples in total for potatoes and carrots). The remaining 12 samples, obtained from plots irrigated with tap water, were used for recovery studies.

Table 2.1 Number of soil samples analysed in each plot

Soils (0-10 cm)				
Water quality	**Type of crop**	**Sub-plots**	**Number of samples analysed**	**Use**
Route A effluent	Carrots	1B, 2B, 3C	3 (one for each selected plot zone)	For quantification of organic CECs
	Potatoes	1A, 1C, 3C	3 (one for each selected plot zone)	
Secondary effluent	Carrots	1B, 2B, 3C	3 (one for each selected plot zone)	
	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 2.2 Number of crop samples analysed in each plot

Crops					
Water quality	**Type of crop**	**Sub-plots**	**Part of crop**	**Number of samples analysed**	**Use**
Route A effluent	Carrots	1B, 2B, 3C*	Leaves	1	For quantification of organic CECs
			Vegetables	1	
			Roots	1	
	Potatoes	1A, 1C, 3C*	Leaves	1	
			Vegetables	1	
			Roots	1	
Secondary effluent	Carrots	1B, 2B 3C*	Leaves	1	
			Vegetables	1	
			Roots	1	
	Potatoes	1A, 1C, 3C*	Leaves	1	
			Vegetables	1	
			Roots	1	
Tap water	Carrots	2C	Leaves	3	For recovery studies (blank, 50 ppb and 500 ppb)
			Vegetables	3	
			Roots	3	
	Potatoes	1A,1C*	Leaves	3	
			Vegetables	3	
			Roots	3	

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

2.1.4 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 analyzed 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.

2.1.5 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 µL of a 10 ppm analyte mix containing sulfamethoxazole, venlafaxine, diclofenac and iopromide. Samples subjected to the 50 ppb spike were spiked with 50 µL of a 1 ppm analyte mix containing the same analytes. Blank recovery samples were not spiked with analytes. All recovery samples were spiked with 50 µL 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 milliliters of a 50:50 (methanol: ultrapure water) mixture with pH 3 was added to each sample, whereafter 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 µm glass fiber 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 µm glass fiber filters.

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, whereafter 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 µL of MeOH and filtered through a 0.22 µm 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 was 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.

2.1.6 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 analyzed. 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 µL of the 1 ppm internal standard mixture.

2.2 RESULTS AND DISCUSSION

2.2.1 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. Iopromide, 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.

2.2.2 Quantification analysis of CECs in soil and crops

As indicated by Figure 2.4, 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 3C, irrigated with WWTP effluent, 0.27 µg/kg sulfamethoxazole and 0.09 µg/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 µg/kg; 0.68 µg/kg and 0.13 µg/kg respectively. Venlafaxine was also detected in soil irrigated with WWTP effluent in the potato plots 1a, 1c and 5c at 0.6 µg/kg, 0.63 µg/kg,1.61 µg/kg respectively and in carrot plots 1b, 2b, 3c at 0.25 µg/kg, 3.19 µg/kg and1.66 µg/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).

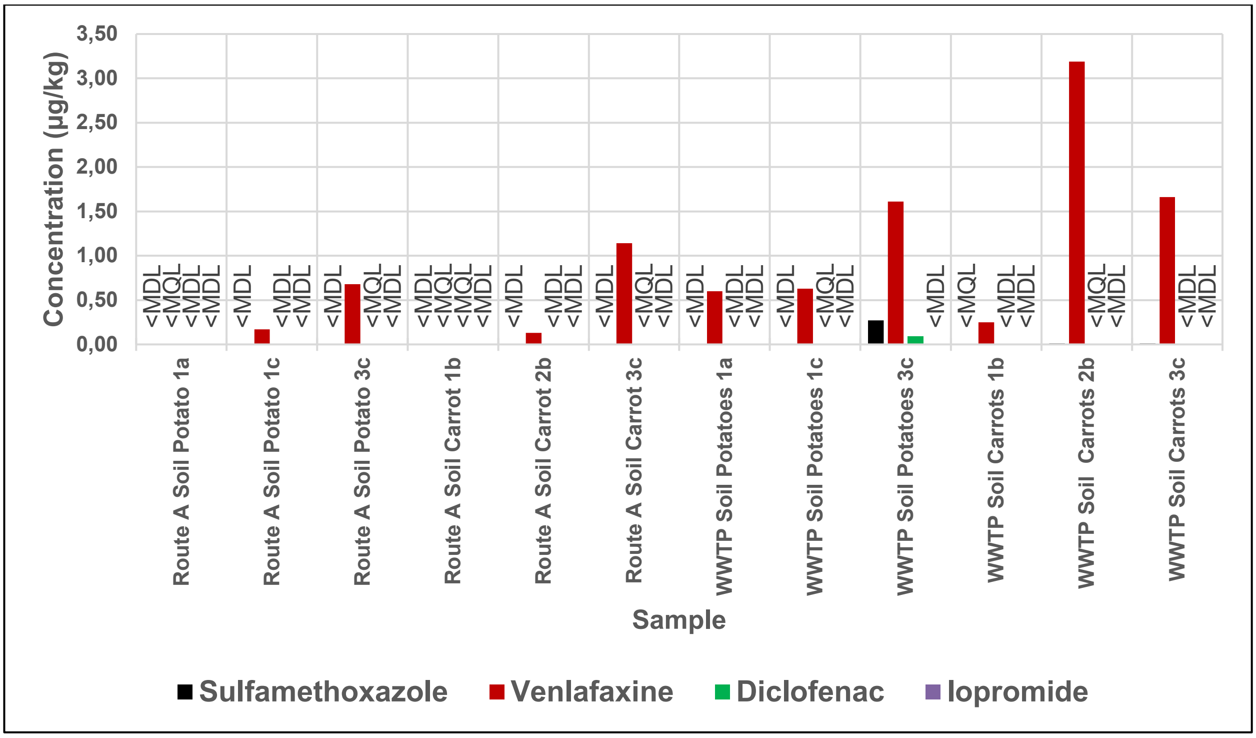

Figure 2.4 CECs concentrations in soil samples irrigated with SERPIC Route A effluent (Route A) and WWTP secondary effluent (WWTP), respectively

As displayed in Figure 2.5, 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 µg/kg and 0.05 µg/kg) and potatoes (0.10 µg/kg and 0.15 µg/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 µg/kg and 0.27 µg/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 µg/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 (See Appendix B). 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.

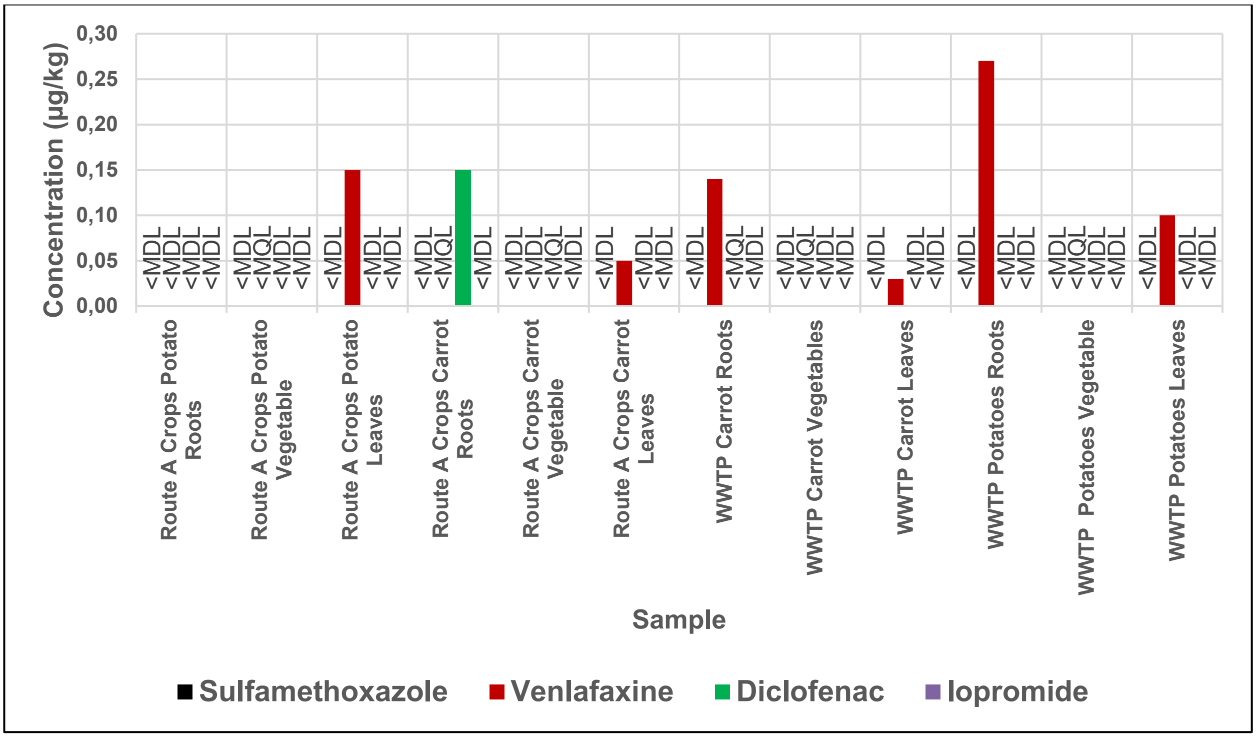

Figure 2.5 CEC concentrations in respective roots, vegetables and leaves of potatoes and carrots grown in plots irrigated with Route A effluent (Route A), and crops grown in plots irrigated with WWTP secondary effluent (WWTP)

2.3 CONCLUSION

Applying secondary effluent (corresponding to the effluent of a conventional activated sludge process, the most common treatment adopted for urban wastewater) for the 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.

CHAPTER 3: ANALYSIS OF COMPOUNDS IN VALUE CHAIN

3.1 SUMMARY

This chapter reports on bioassay analyses of water samples using effect-based methods (EBMs). The panel included screens for estrogenicity, anti-androgenicity and arylhydrocarbon receptor (AhR) activation. The aim was to assess the removal efficiency of bioactive chemicals from treated wastewater treatment plant (WWTP) effluent by the SERPIC technology. Estrogenicity was only observed in the WWTP effluent, and at a low potency of 0.44ng/ℓ estradiol equivalents (EEQs). Anti-androgenic activity was present in all the samples tested, with the highest potency being observed in the "permeate" samples (271.98μg/ℓ flutamide equivalent [FEQ]), followed by the WWTP effluent (125.99 μg/ℓ FEQ) and ozonation (37.80 μg/ℓ FEQ) groups, respectively. Arylhydrocarbon receptor activation was observed in the WWTP effluent (988.5 ng/ℓ β-naphthoflavone equivalent [βNF-EQ]) and post SERPIC treatment (579.83ng/ℓ βNFEQ, but not in the post ozonation samples. The data suggests that the SERPIC treatment has the capacity to remove estrogenic chemicals present at concentrations in the parts per trillion range but cannot completely remove anti-androgenic and AhR active chemicals. The increased anti-androgenic activity observed in the permeate relative to the WWTP effluent may be due to processes such as transformation (producing bioactive metabolites) or deconjugation or occurring during the treatment. Further research is needed to determine which chemicals in the samples were responsible for the anti-androgenic and AhR activation activity observed.

3.2 INTRODUCTION

Effect-based methods (EBTs) are currently being promoted for water quality screening and routine monitoring due to the advantages over conventional chemical analysis, including broader contaminant cover, representation of mixture effects, and sensitivity (Brack et al., 2019; Neale et al., 2023). The use of EBTs can provide data on both the potential health risks associated with exposure to a particular water source, and the presence of classes of contaminants as an indicator of water quality.
One of the best-known biomarkers for EBTs is agonism of the estrogen receptor (i.e., estrogenicity), an important endocrine-disrupting chemical mode of action. The screening of estrogenic activity in water samples can be performed using in vitro or in vivo reporter gene assays. Genetically engineered yeast represents a validated approach to the screening of estrogenicity, and various assays for the purpose have been developed to date (Bovee et al., 2004; Pham et al., 2012; Routledge and Sumpter, 1996). Various environmental chemicals are known to be estrogenic, including natural and synthetic hormones, certain pharmaceuticals, plasticisers and other industrial chemicals and pesticides (Pamplona-Silva et al., 2018). The most recent proposed amendment to the EU Water Framework Directive, the groundwater directive and the directive on environmental quality standards includes the screening for estrogenic chemicals (Backhaus, 2023). Although the proposed amendment is yet to be adopted, the move to include estrogenic chemicals screening in member states show the relevance of EBMs for future water quality monitoring.

A further important mode of action representing environmental micropollutants is anti-androgenicity. As is the case with estrogenicity, screening for anti-androgenicity can also be performed using yeast engineered to represent human hormone signalling (Sohoni and Sumpter, 1998). Some of the best-known sources of anti-androgenic chemicals in the environment are agriculture (Archer and Van Wyk, 2015; Orton et al., 2011), wastewater treatment works (Chen et al., 2007; Jobling et al., 2009).

Aryl hydrocarbon receptor (AhR) activation is a biomarker for xenobiotic activity. This transcription factor is known to regulate various enzymes involved with xenobiotic metabolism, including certain cytochrome P450s and evidence exists linking AhR action and various cancers, autoimmune disorders, inflammatory bowel diseases, multiple sclerosis and arthritis (Granados et al., 2022; Lin et al., 2022). The ligands of AhR include hydrocarbons, brominated compounds, phytochemicals and certain pharmaceuticals, among others (Lin et al., 2022), and are known to be present in environmental waterbodies (Gouukon et al., 2020). The Global Water Research Coalition included AhR activation in the framework for drinking water quality testing (GWRC, 2023).

AhR activation was one of the biomarkers identified as a priority by the European Commission-funded SOLUTIONS project (Brack et al., 2019). Currently, the only jurisdiction enforcing ER and AhR screening as part of regulation is the state of California as part of recycled water quality control. Nonetheless, the EU, Australia and other developed countries will likely follow suit due to the obvious advantages of Effect-based Monitoring (EBM) over analytical chemistry for hazard assessment.

3.3 METHODOLOGY

The aim of the investigation was to assess the contaminant loads in selected water samples using EBTs representing three key modes of action (i.e., estrogenicity, anti-androgenicity, and aryl hydrocarbon receptor binding) by applying in vitro bioassays, including the Yeast Estrogen Screen (YES), Yeast Anti-Androgen Screen (YAAS), and Arxula Yeast Dioxin Screen.

3.3.1 Sample collection

Water samples were collected in methanol-rinsed glass and stored at 4°C. The sources included (1) wastewater treatment effluent, (2) SERPIC nanofiltration (Route A) effluent and (3) post ozonation. Three independent samples were collected per source. An ultrapure water field blank/control was also included in the experiment. The sample processing, including solid phase extraction, was performed within 48h of collection.

3.3.2 Solid phase extraction

One litre of water from each replicate container was passed through a 1.2 m glass fibre filters to remove particulate matter. The filtered samples were subsequently passed through 200 mg, 6cc hydrophilic-lipophilic balanced (HLB) columns (Oasis, Waters, USA) at a flow rate of approximately ±5ml/min using a vacuum manifold. The columns were pre-conditioned using 6 ml of methanol and 6 ml of ultrapure water. The columns were subsequently dried under vacuum and sealed for shipping to Stellenbosch University. The columns were stored at -20°C prior to elution. Extracts were eluted from the columns using 6 ml of methanol, subsequently evaporated to dryness using a gentle stream of nitrogen, and reconstituted in 1 mℓ of ethanol (1000x concentrated state).

3.3.3 Yeast Estrogen Screen

Estrogenicity and anti-androgenicity were screened for using the YES and YAAS recombinant yeast assays as previously described (Routledge and Sumpter, 1996; Truter et al., 2017a). The yeast strains constitutively express human hormone receptors (ERα and AR) and furthermore contain response elements upstream of a β galactosidase reporter gene functioning as biomarker for estrogenic or anti-androgenic compounds. Sample extracts were tested in duplicate as a dilution series (i.e, 25x, 12.5x and 6.25x relative to environmental concentration) to increase the assay sensitivity and to pre-empt toxicity seeing since poor quality samples are known to be cytotoxic to the yeast. Each assay plate included a 10-point two-fold serial dilution of 17-β-estradiol for YES (2.76 μg/ℓ to 53.95 ng/ℓ) and flutamide for YAAS (2762.1 to 53.95 μg/ℓ), functioning as reference standards. An ultrapure H2O method blank representing the filtration and SPE workflows was also tested to correct for possible background activity. Assay plates were incubated for 48 h at 30°C and for a further 12h to 48h at room temperature. Absorbance was then measured at 570 nm and 625 nm, respectively as indicators of reporter gene expression levels and cell density (iMark, Biorad, USA). Turbidity-normalized absorbance 570nm values were used to calculate β-estradiol equivalents (EEQs) and flutamide equivalents (FEQ) using linear regression equations of the reference standard curves.

3.3.4 Aryl hydrocarbon receptor activation screen

A commercially available yeast assay was applied to test extracts for AhR agonists (A-YDS, New Diagnostics, DE). The kits feature engineered yeast expressing the human AhR protein as well as an alkaline phosphatase reporter gene induced through AhR binding to xenobiotic response elements (XREs). The kit was used according to the manufacturer's instructions. In short, lyophilized yeast was reactivated and samples were exposed at a 10x enrichment factor to increase the sensitivity of the assay. A seven-point standard curve of the AhR agonist β-naphthoflavone (βNF) was included as reference standard (20 to 0.73μg/ℓ a serial dilution).

An ultrapure H2O method blank representing the filtration and SPE workflows was also tested to correct for possible background activity. All samples and standards were tested in duplicate. Samples were exposed to activated yeast cells for 20 h at 30°C and 700 rpm after which cells were pelleted out through centrifugation, supernatants removed and supplemented with a developer solution and incubated for a further 1 h. Reactions were then stopped using 5M NaOH and absorbance was measured at 415 nm (iMark, Biorad, USA). Yeast pellets were resuspended, and absorbance was measured at 625 nm as an indicator of turbidity and therefore cell numbers. Turbidity-normalized absorbance 415nm values were used to calculate βNF equivalents (βNF-EQ) using a linear regression equation of the βNF standard curve.

3.4 RESULTS AND DISCUSSION

Of the three sources tested, only the WWTP effluent exhibited estrogenic activity, and at a low potency of 0.44 ng/ℓ EEQ (Figure 3.1, Table 1). Conversely, all three the sources tested were anti-androgenic, with activity ranging between 37.80 and 271.98 μg/l FEQ (Figure 3.1, Table 3.1). The anti-androgenic activity observed varied significantly among sources ($F = 24.77$, $P = 0.001$,1-ANOVA), being higher in the nanofiltration permeate samples than in the WWTP Effluent and ozonation effluent samples (Figure 3.2). Aryl hydrocarbon receptor (AhR) activation was observed in the WWTP effluent and nanofiltration permeate, but not in the ozonation effluent (Figure 3.3, Table 3.1). The AhR activity was significantly lower in the nanofiltration permeate than in the WWTP effluent (Figure 3).

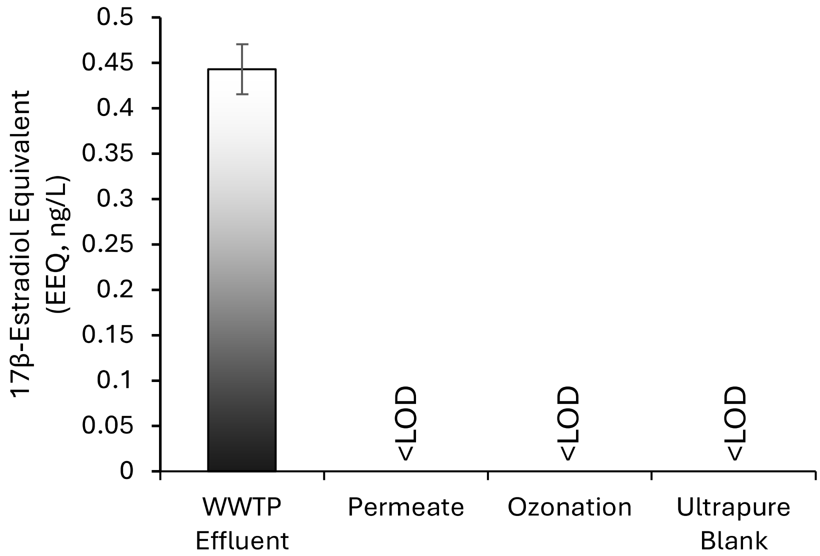

Figure 3.1 Mean ± standard deviation of estradiol equivalents (EEQ, ng/ℓ) observed in WWTP effluent, SERPIC nanofiltration permeate, ozone-treated permeate and an ultrapure water blank.

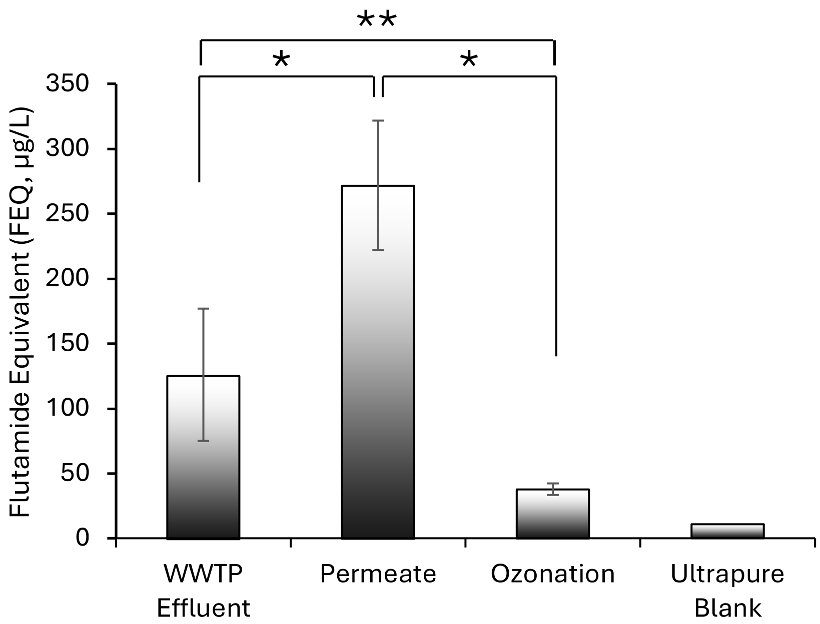

Figure 3.2 Mean ± standard deviation of flutamide equivalents (FEQ, µg/ℓ) observed in WWTP effluent, SERPIC nanofiltration permeate, ozonation permeate and an ultrapure water blank. Asterisks indicate significant differences - *P<0.05, **P<0.005.

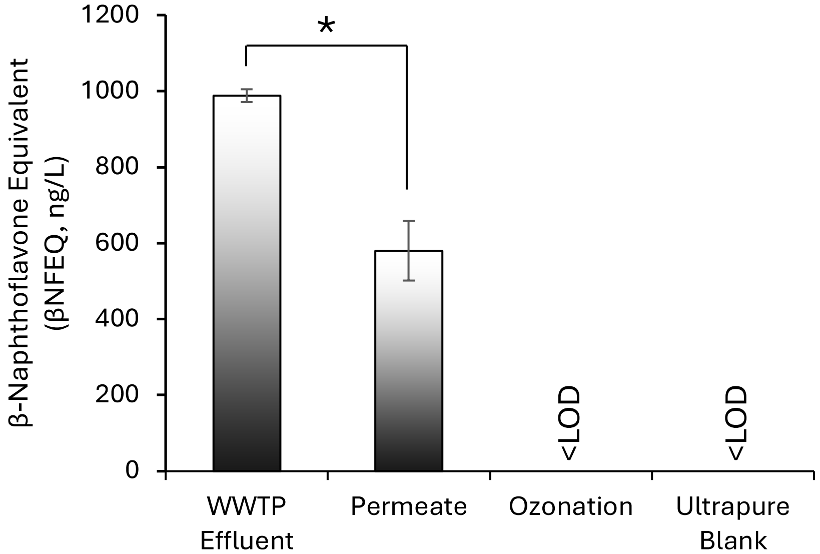

Figure 3.3 Mean ± standard deviation of β-naphthoflavone equivalents (βNFEQ, ng/ℓ) observed in WWTP effluent, SERPIC nanofiltration permeate, ozonation permeate and an ultrapure water blank. Asterisks indicate significant differences, *P<0.05, **P<0.005.

Table 3.1 Mean ± standard deviation of estradiol equivalents (E-EQ), flutamide equivalents (F-EQ) and β-naphthoflavone equivalents (βNFEQ) detected in water samples from different sources.

Source	EEQ (ng/ℓ)	FEQ (µg/ℓ)	βNFEQ (ng/ℓ)
WWTP Effluent	**0.44 ± 0.03**	**125.99 ± 50.91**	**988.95 ± 16.94**
Permeate	**<LOD**	**271.98 ± 49.71**	**579.83 ± 78.84**
Ozonation	**<LOD**	**37.80 ± 4.41**	**<LOD**
Ultrapure Blank	**<LOD**	**11.06**	**<LOD**

The present data confirm estrogenicity in a WWTP effluent, indicating the presence of estrogen receptor agonists. The data further shows that the SERPIC nanofiltration treatment has the capacity to remove estrogenic chemicals present in the parts per trillion range from water. California State, USA, is the only territory globally that currently requires monitoring for estrogenicity in water, and particularly in recycled water (California EPA, 2018). The ER activity currently observed did not exceed the California EPA reporting and trigger limits of 0.5 ng/ℓ and 3.5 ng/ℓ EEQ.

Anti-androgenicity was observed in all the sources tested, with activity increasing post SEPRIC nanofiltration treatment, suggesting the process causes an increased concentration of androgen receptor antagonists. Possible explanations for increased activity post-treatment include the formation of more bioactive metabolites (transformation products) or higher bioavailability due to de-conjugation. Several environmental chemicals are known to be androgen receptor antagonists, including certain phthalates, parabens, and phytoestrogens, nonylphenol, triclosan, crude oil and petrochemicals, among others (Houtman et al., 2021; Lange et al., 2015; Sohoni and Sumpter, 1998; Truter et al., 2017b; Zhou et al., 2022).

No guidelines for anti-andogenic activity in environmental water or effluents currently exist. Effect-based trigger (EBT) values have however been described for anti-androgenicity ranging between 3.28 and 25 μg/l FEQ (Neale et al., 2023). These EBTs are, however, assay specific but these values are assay specific and to date published values only represent mammalian cell culture assays and not yeast as assays as the one used in the present investigation. Nonetheless, the FEQs observed in the current study in both the WWTP effluent and nanofiltration permeate were considerably higher than the published EBTs suggesting health risks and high concentrations of anti-androgenic chemicals present.

The WWTP effluent and nanofiltration permeate sources exhibited AhR activity corresponding relatively to a the data of a study using a similar bioassay to assess sewage treatment plant water (Stalter et al., 2011). In particular, Stalter et al. (2011) reported FEQs of 387 - 741 ng/ℓ in a WWTP after secondary clarifier and before advanced treatment. The conventional reference standard for AhR activation is the dioxin TCDD due to the high potency and identity as toxic AhR agonist. The less toxic yet carcinogenic benzo[a]pyrene has also been used to derive AhR equivalents, and EBTs for risk assessment purposes have been described for the said substance (Escher et al., 2018; Neale et al., 2020, 2023). β-naphthoflavone used in New Diagnostics A-YDS kits (used in the present investigation) can also function as a reference standard for AhR activation, but effect-based trigger values for the substance is yet to be described. Trigger values for β-NFEQs are likely to be published soon considering the increased application of EBMs for water quality monitoring and the relatively recent release of the A-YDS assay kit by New_Diagnostics (DE).

To better understand the sources of anti-androgenicity and AhR activation in the samples high-performance thin layer chromatography reporter gene assays could be performed to identify bioactive groups of compounds of defined polarity (Schoenborn et al., 2017). Non-targeted chemical analysis of fractionated samples can also be applied to further assess what the source of the observed bioactivity is (See Houtman et al., 2021)

CHAPTER 4: TRANSFER STRATEGIES

4.1 SUMMARY

The SERPIC technology presents both significant advantages and some notable challenges for water re-use applications. By leveraging its strengths while addressing weaknesses through strategic planning and community engagement, there is potential to enhance water reuse prac-tices significantly within Africa, Europe and beyond. Continued alignment with regulatory frame-works and stakeholder interests will be essential for successful implementation and sustainability of the technology in addressing water scarcity challenges faced by agriculture sector. Evidence from both planned and existing initiatives indicates that the implementation pace within the water services sector has been hindered by socio-political, technical, and economic challenges. Nevertheless, water supply authorities are increasingly recognizing that water reuse should no longer be viewed merely as an emergency measure; instead, water reclamation and reuse is becoming an alternative water source and it must be integrated into long-term water supply strategies within resource planning.

4.2 INTRODUCTION

Climate change, increasing demand for freshwater from various users, population growth, rapid urbanization, and deteriorating water quality have significantly strained freshwater supplies worldwide, especially in third world countries. As a result, there is a growing emphasis on exploring alternative water sources. Among these alternatives, water reclamation and reuse have emerged as strategic options that could help countries achieve their development objectives. The SERPIC technology therefore serves as a potential solution to be especially implemented in low-and middle-income (LMI) countries where water scarcity is a concern as well as the occurrence of CECs in wastewater discharge in the river systems and subsequently in crop irrigation. To improve the scientific quality and societal relevance of the project results in different socio-economic settings, the development of transfer concepts to these regions, provides a rich understanding of the technology and solution impacts in different geographies and socio-economic contexts. Efficient implementation of the SERPIC technology in LMI countries, requires a strategic approach that leverages its strengths, addresses weaknesses, capitalizes on opportunities, and mitigates threats. The aim of this study was to develop transfer concepts with the focus on Southern Africa (South Africa) and West Africa (Nigeria). For the Southern Africa, concepts are focused on centralized approach whereas for West Africa a more decentralized approach, with on-site treatment, was considered.

4.2.1 Background information on South Africa and Nigeria as case studies

4.2.1.1 South Africa (Southern Africa)

South Africa primarily relies on surface and groundwater resources to satisfy its household, municipal, industrial, and agricultural water needs. With current water demands, the country is projected to face a water deficit of 17 % by 2030. Globally, water scarcity has emerged as a significant driver for the adoption of water reuse practices. Consequently, several water reuse initiatives have already been implemented, with more planned for the future (DWA, 2013). As South Africa grapples with these challenges, diversifying its water sources through innovative methods such as water reclamation and reuse will be crucial for ensuring a sustainable water supply for its growing population. While South Africa is looking into desalination as one of the alternative water sources, it has been demonstrated that water reclamation and reuse is a more cost- and energy-efficient option without a need to address the desalination brine management challenge.

By 2040, South Africa aims to increase its reliance on water reuse from 14% to 18%, aligning with national priorities outlined in the National Water and Sanitation Master Plan (NW&SMP) to expand the number of water reuse schemes and enhance capacity across the country (DWS, 2018). While several additional municipal water reuse projects are currently in the planning stages, there is no guarantee that these initiatives will proceed as intended or that the targets will be met.

Despite the existing operational capacity of wastewater treatment plants and planned reuse schemes, wastewater remains an underutilized resource. South Africa has approximately 824 wastewater collection and treatment facilities with a combined hydraulic design capacity of 6,509 ML/day. Among these, 59 are classified as macro wastewater treatment works (with capacities exceeding 25 ML/day) and account for 65% of the nation's total wastewater capacity. Collectively, these facilities have a hydraulic capacity of around 4,000 ML/day and an estimated potential for water reuse of about 2,500 ML/day. Notably, coastal cities contribute approximately 1,100 ML/day to this capacity, while inland cities account for the remaining 1,400 ML/day (DWS, 2018).

The slow pace of implementing water reuse initiatives in South Africa can be attributed to a complex interplay of technological, economic, and socio-political factors. Research has shown that socio-political issues play a more significant role in hindering widespread adoption of planned water reuse projects compared to technical and economic challenges (DWA, 2013; van Niekerk and Schneider, 2013; Swartz et al., 2015; Muanda et al., 2017; Slabbert and Green, 2019).

To overcome these barriers and enhance water reuse strategies, a coordinated effort involving improved governance, public engagement, and investment in technology is essential. By leveraging these insights and addressing the outlined challenges, South Africa can enhance its agricultural resilience through effective implementation of water reuse practices.

4.2.1.2. Nigeria (West Africa)

Nigeria faces significant challenges in water management, despite its abundant water resources. The country is classified as economically water-scarce, with an estimated total annual water demand of approximately 5.93 billion cubic meters in 2021, projected to rise to 16.58 billion cubic meters by 2030. The primary sources of freshwater demand include agriculture, municipal use, and industry, with over half of the abstractions coming from groundwater (Ior & Leo, 2023). Oyetoro (2022) identified several key constraints affecting the transfer of agricultural technologies in Nigeria:

Complexity of Technology:
The complexity inherent in agricultural technologies poses significant challenges for uneducated farmers. Many technologies fail not due to their inherent quality but because they are too complicated for farmers to implement effectively. The adoption rate of these innovations is heavily influenced by their characteristics, with many being prohibitively expensive and difficult to access. Often, these technologies are developed in controlled environments tailored to specific conditions, and the necessary operational details are frequently not communicated to farmers. When information is provided, it often does not align with local needs, leading to technical failures that exacerbate existing issues.

Issues with the Technology Transfer System:
The procedures involved in technology transfer are predominantly top-down, which complicates adaptation for smallholder farmers. Most technological advancements do not include farmers in the development process, resulting in a disconnect. Additionally, these technologies often arrive too late in the production cycle for farmers to benefit from them. These again underlines the importance of crucial stakeholder’s involvement at the early stages of technology transfer, necessity of adjusting to the local conditions, and bottom-up approaches to increase the uptake of the novel solutions.

Socio-Economic Attributes of Farmers:
The personal characteristics and circumstances of farmers significantly influence their willingness to adopt new technologies. Key factors include:

- Education: Literacy levels among farmers play a crucial role in technology adoption. Educated farmers are more likely to understand and utilize complex innovations effectively. Kafando et al. 2022 emphasize that educational initiatives must be reassessed to better meet farmers' needs.
- Age: Younger farmers tend to be more open to adopting new technologies compared to older farmers, although some technologies may favor older demographics (Ior & Leo, 2023).
- Experience: Experienced farmers are generally better equipped to manage risks associated with new technologies than their less experienced counterparts (Donkoh et al. 2019).
- Income and Social Status: A farmer's financial situation can greatly affect their ability to adopt new technologies, as those with better financial resources can more readily absorb associated risks.
- Culture: Introducing technologies that conflict with traditional societal norms can be counterproductive. For instance, altering gender roles within agricultural tasks may face significant resistance (Ior et al. 2023).
- Group Membership: Farmers who belong to various social organizations are more likely to adopt new technologies due to shared experiences and collective decision-making processes. Encouraging the formation of cooperatives can enhance technological adoption rates.

John et al. 2022 also noted that factors such as age, household size, education level, farm size, access to credit, and visits from extension agents significantly impact the transfer of improved technologies. Younger farmers are particularly more inclined to embrace new innovations. Providing adequate educational resources is essential for increasing adoption rates, while access to credit is crucial for enabling farmers to take on new technological risks.

Local Peculiarities and Differences:
Rural smallholder farmers often resist abandoning established practices for new technologies unless these innovations have been proven advantageous and compatible with local customs and conditions (Ior et al. 2023).

The effectiveness of technology development and transfer strategies in Nigeria largely hinges on the critical roles played by technology developers, disseminators, and users throughout the value chain. Currently, the potential for technology advancement and transfer in Nigeria is supported by emerging technologies such as precision agriculture, weather tracking systems, satellite imaging, agricultural robotics, and radio frequency identification (Ior et al. 2023). To enhance these efforts, a more pragmatic and results-oriented approach is necessary. This should involve deploying selected experts to collaborate closely with farmers in the field to advice on best practices and address specific challenges over defined periods. Additionally, there should be a push for increased private sector involvement in the multiplication and dissemination of affordable production technologies that meet farmers' needs. Moreover, a pluralistic funding model for agricultural extension activities is essential. This model should involve contributions from federal, state, and local governments, each with clearly defined roles and proportional funding commitments to ensure effective implementation.

4.3 METHODOLOGY

The following strategies were developed considering the results of the SWOT analysis conducted by UNIFE (Table 1) as well as relevant literature.

Table 4.1. Results from the SWOT analysis of the SERPIC technology.

	Factors	Main reasons for the factor selection
Strengths	Water quality improvement	The SERPIC technology is able to remove most of the residuals of conventional pollutants and a wide spectrum of micropollutants (CECs) not only organic compounds but also microbials (including ARB and ARGs). It provides stable, high quality irrigation water. Route A final effluent can thus be directly reused for agricultural needs.
	Easy to control	The SERPIC technology includes different quality controlling devices (among them: flowrate measurement, pH, and conductivity probes) and it can be automated to be controlled remotely. It also allows for easy integration of additional control systems, e.g., various water quality parameters, if necessary.
	Dedicated treatment for NF concentrate	The SERPIC technology (Route B) includes a photoreactor to remove the CECs in the NF membrane concentrate.
	No chemicals addition	The oxidants necessary in the SERPIC technology (ozone and persulfates) are produced electrochemically on site.
	No waste production	The SERPIC technology does not produce waste streams (for instance NF concentrate) to be disposed of.
Weaknesses	Variability of the SERPIC technology performance	The SERPIC technology performance varies according to different parameters related to the feeding characteristics and the operational conditions
	Safety concerns	Ozone is potentially corrosive, and it enhances fire hazards. For workers, it is irritating and toxic, and may cause respiratory problems at 0.1 ppm peak concentration in 15 minutes
	Operational problems	The prototype requires specific operational procedures (i) periodic cleaning and eventually replacement of NF membrane (due to membrane fouling), (ii) regular monitoring of ozone equipment and (iii) control of the toxicity in the ozonated effluent due to the potential formation of unknown transformation ozonated products
	Complex construction and equipment	SERPIC technology requires specific equipment to generate the oxidants and to promote CEC degradation (photoreactor) and to monitor the processes
	High energy demand	The SERPIC technology has a high energy consumption compared to the common quaternary treatments (based on ozonation and activated carbon).
Opportunities	Customer request (of a promising and valuable low cost (green) technology to be included in a dedicated treatment)	Increased customer interest in integrating conventional treatments with polishing ones able to remove CECs
	European rules encourage water reuse	The reuse practice is in agreement with the European Union (EU) Water Framework Directive (EU Directive, 2000/60/EC) and the recent European minimum requirements for water reuse (EU Regulation, 2020/741).
	European rules require CECs removal during urban wastewater treatment	The revised Directive concerning urban wastewater treatment EU 2024/3019 (UWWTD), in force starting from January 1st 2025, introduces the need for a quaternary treatment to reduce the content of ~~organic~~ micropollutants in the final effluent to be released in the environment as well as for small wastewater treatment plants placed in an area at risk of accumulation/pollution due to CECs.
	European Green Deal initiatives	The initiatives promote green technologies and solutions with zero pollution discharges and limiting CO_2 emissions. In addition, the new UWWTD, EU 2024/3019, requires actions towards the energy neutralization, asking for the adoption of renewable energy sources (solar/wind/hydraulic) in the wastewater treatment sector.

	Factors	Main reasons for the factor selection
	National policies (Implementation of national policies to reduce CECs in WWTP effluent)	In some countries, national regulations are in force for CEC removal from WWTPs effluents (for instance in Switzerland, Germany, etc.)
	Public (farmers) interest in water reuse	Normally people are aware of environmental issues so they may be willing to contribute to the reuse of treated wastewater as it is a promising solution to address the (fresh) water scarcity and drought events, which are increasing in frequency as a consequence of climate change
Threats	Variation of CECs concentration in secondary effluent	CECs concentration in the secondary effluent may vary according to different factors (namely, countries or region, consumption patterns, level of treatment applied at WWTPs, etc.)
	Attention to aquatic life	Attention to the potential environmental impacts induced by emerging technologies is increasing. Polishing treatments do not have a well-known effect on the reduction of CECs ecotoxicity impacts on freshwater. In general, the formation of transformation products during the polishing treatments (such as ozonation, AOPs, etc.) may increase toxicity
	Other CEC treatment technologies as its main competitors	The SERPIC technology can be compared with others which may be more effective, or consume less energy and be less expensive
	Socio-economic concerns	The most crucial social issues are farmers, retailers, and consumers' acceptance. If their products are not sellable, farmers will not find wastewater reuse viable; consumers will not purchase products where treated wastewater was used unless it has been proven safe

The following strategies were identified for both study areas:

1. **Leverage Strengths**

- **Water Quality Improvement**: Highlight the capability of the SERPIC technology to remove conventional pollutants and a wide spectrum of contaminants of emerging concern (CECs), including antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), ensuring water quality suitable for direct agricultural reuse. This aligns with South Africa and West-Africa's policies promoting water reuse in agriculture.
- **Ease of Control and Automation**: Utilize the technology's automated control systems, including flowrate measurement, pH, and conductivity probes, to facilitate remote operation. This is particularly beneficial in South Africa and Nigeria, where technical expertise may be limited in remote rural areas.
- **On-site Oxidant Production and Zero Waste**: Emphasize the environmental benefits of producing oxidants electrochemically on-site and the absence of waste streams, aligning with global trends towards sustainable and green technologies.

2. **Address Weaknesses**

- **Mitigate performance variability**: Conduct localized pilot studies to assess and optimize SERPIC technology performance under South African and Nigerian conditions, considering variations in feedwater characteristics and operational parameters. Develop clear guidelines for operation and maintenance to manage performance variability.
- **Safety concerns**: Implement stringent safety protocols and training programs to manage risks associated with technology use. Ensure compliance with both countries' occupational health and safety regulations. Implement rigorous training programs for local operators to safely handle the electrochemically production of oxidants and manage operational risks. Provide detailed but understandable safety protocols and monitoring equipment as part of the technology package.

- **Operational challenges**: Develop comprehensive yet easy to follow maintenance schedules and training for local operators to address issues such as nanofiltration membrane fouling and monitoring of equipment. Establish local support centres to provide technical assistance and spare parts.
- **Complexity and energy demand**: Simplify the system design where possible and ensure the integration of solar power, to offset the technology's high energy consumption and make it viable in regions with unreliable electricity. This approach is feasible given South Africa's abundant solar resources as well as in Nigeria. Create a country, or even local, specific user manual to address the complexity of the technology.

3. Capitalize on Opportunities

- **Growing demand for sustainable technologies**: Position SERPIC technology as a green and cost-effective solution for removing CECs, appealing to the country's stakeholders interested in sustainable agricultural practices.
- **Alignment with European and national policies**: Leverage the European Union's directives encouraging water reuse and the European Green Deal initiatives to attract funding and support for implementing SERPIC technology in South and West Africa. Collaborate with authorities to align the technology with national water reuse policies.
- **Public interest in water reuse**: Engage with local farmers and communities to raise awareness about the benefits of treated wastewater reuse, addressing water scarcity and promoting acceptance of SERPIC technology. Capitalize on farmers' interest by conducting educational campaigns to demonstrate the environmental and economic benefits of using treated wastewater for irrigation. Use testimonials or case studies from European farms to build trust and acceptance among African farmers and to highlight environmental and economic benefits through case studies from Europe.
- **Position as a Green Technology:** Promote SERPIC technology as part of South Africa's commitment to sustainable development under global frameworks like the Paris Agreement and sustainable development goals. Seek funding under national or international green technology initiatives.

4. Mitigate Threats

- **Variability in CEC concentrations**: Conduct thorough assessments of local wastewater to understand CEC profiles and adjust technology operations accordingly to ensure consistent treatment efficacy. For example, include robust monitoring and adaptive control systems to handle variations in feed water quality. In parallel, conduct ongoing research to study and mitigate local water quality challenges.
- **Water quality monitoring**: Recent findings from a study funded by the Water Research Commission indicate that South Africa lacks sufficient laboratories capable of conducting routine analyses on emerging contaminants in water (Swartz et al., 2018). To ensure the safety of product water, it is essential to invest in bio-sensors, in addition to the automated water monitoring sensors mentioned above, that can effectively monitor water quality in real-time and ensure that polishing treatments do not inadvertently increase the toxicity of the water. The absence of water quality regulations specifically addressing water reuse is a significant barrier to building public trust in this practice. Currently, neither the South African Water Quality Guidelines nor SANS 241 (the standard for drinking water quality) nor the Nigerian water quality guidelines sufficiently address emerging contaminants or establish clear water quality requirements for the various uses of reclaimed water.
- **Competition from alternative technologies**: Differentiate SERPIC technology by emphasizing its unique features, such as on-site/in-situ oxidant production and zero waste generation. Highlight modularity, flexibility in operation and control, and compatibility with renewable energy sources. Demonstrate high effectiveness and cost-effectiveness through pilot projects and case studies.
- **Socio-economic acceptance**: Develop certification programmes to assure consumers of the safety of crops irrigated with treated wastewater. Engage with retailers and farmers to build trust and acceptance of SERPIC-treated water in agricultural production. Position the technology as a long-term cost-effective solution by demonstrating savings from reduced waste disposal and chemical use. Implement pilot projects with subsidies or co-funding to reduce initial costs for farmers. Partner with

retailers and certification bodies to create a "sustainable farming" label, boosting consumer confidence in products irrigated with treated wastewater.

- **Risk-sharing agreements**: Create agreements to share risks (e.g., economic, operational) between European providers and South African partners.
- **Contingency planning**: Develop robust strategies to address potential challenges like droughts or power outages that could impact system functionality.

5. Address Financial Constraints

- **Innovative financing models**: Introduce cost-sharing schemes, public-private partnerships (PPPs), or subsidies to encourage adoption.
- **Tiered implementation**: Break the technology transfer into affordable phases to spread out costs.
- **Attract International Funding:** Apply for grants and subsidies from organizations like the African Development Bank, Green Climate Fund, or EU initiatives supporting green technology adoption in Africa.

The proposed roadmap to implement the technology involves the following:

Phase 1 (Year 1):
Identify locations: Conduct feasibility studies and identify regions in South Africa/Nigeria with urgent water scarcity and suitable conditions for SERPIC technology.
Stakeholder Engagement: Initiate dialogues with South African/Nigerian government agencies, agricultural bodies, and local communities to build support for SERPIC implementation. Develop partnerships with local authorities, agricultural cooperatives, and funding bodies.
Host workshops: Introduce SERPIC technology and gather feedback.

Phase 2 (Year 2-3):
Engage local technology providers: Upon sufficient interest during the workshop and from the relevant stakeholder's, engage with local technology providers potentially interested in the transfer and implementation of the SERPIC technology.
Pilot projects: Implement localized pilot projects to refine technology performance under South African and Nigerian conditions.
Training: Provide training programs for local operators and technicians to handle the system safely and efficiently to ensure proper operation and maintenance of SERPIC systems.
Policy Integration: Work with policymakers to incorporate SERPIC technology into national water reuse strategies and obtain necessary regulatory approvals.

Phase 3 (Year 4-5):
Scale up successful pilots and expand installations across water-stressed regions.
Establish local manufacturing partnerships to reduce costs and simplify construction.
Ongoing:
Implement continuous monitoring systems to assess performance, environmental impact, and socio-economic acceptance, allowing for iterative improvements. Establish long-term monitoring and evaluation frameworks.
Engage in community outreach to build long-term acceptance and adoption.
Conduct regular training and awareness programs to ensure sustained adoption.

4.4 CONCLUSIONS

Water reuse presents a viable solution for addressing Africa's, such as South Africa and Nigeria's, pressing water challenges. By implementing the proposed roadmap in both countries, there is potential for successful implementation of the SERPIC technology. While there is potential for significant progress through public awareness, overcoming regulatory, infrastructural, and funding barriers is crucial for successful implementation in both countries and beyond. A coordinated approach involving government, private sector participation, and community engagement will be essential for advancing water reclamation efforts.

CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS

5.1 CONCLUSIONS

The contribution of Stellenbosch University in the SERPIC project assisted in the achievement of the objectives.

- Objective 1 was to minimize the spread and transformation of CECs, including ARB and 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.

Objective 1 was achieved since SERPIC successfully developed a CEC removal technology up to prototype level (TRL5), offering a knowledge base for further development until real application in WWTPs (TRL9). When this is reached, CECs, including ARB and ARGs, will be removed, resulting in a minimization of their spread in both urban and natural water cycles.

- Objective 2 was to reduce CECs from WWTP effluent by developing an innovative treatment technology, based on membrane filtration and light-driven electrochemical processes. For the two application routes, different reduction targets were followed, defined as a percentage referred to the WWTP effluent that will feed the SERPIC system:
  - Route A: for irrigation purposes, we aimed at a reduction of the target CECs by at least 90%;
  - Route B: for the discharge into the aquatic environment, we aimed for more than an 80% reduction. We have followed these reduction targets: For Route A, SERPIC achieved reduction rates for the six target CECs between 89.9 % and 99.4 % (see chapter 3.4.3), i.e., a full achievement.

For Route B, SERPIC achieved reduction rates between 96.1% and 99.6% for five of the six target compounds, and 70.3% for Venlafaxine (see booklet in appendix). Thus, the SERPIC consortium achieved 11 of the 12 reduction targets, i.e., 92% of objective 2.

A long-term field test by irrigating carrots and potatoes with route A stream was performed to control the fate of the few remaining CECs and AMR bacteria into soil and plants. SERPIC treatment removed sulfamethoxazole, venlafaxine and diclofenac from WWTP effluent to almost undetectable levels. Therefore, low or undetectable levels of these CECs were detected in soil and crops irrigated with SERPIC-treated water. The recalcitrant organic compound Venlafaxine could be detected in the leaves of potatoes and carrots irrigated with SERPIC-treated water, but in lower concentrations in comparison to the crops irrigated with WWTP effluent.

- Objective 3 was to elaborate methodologies and tools for monitoring, health and environmental risk assessment and for the implementation of new reuse concepts, including new treatment technologies, as a basis for better policy and decision-making, for regulatory issues and new standards.

SERPIC elaborated on a methodology to select relevant target CECs based on four criteria: occurrence, persistence, bioaccumulation and toxicity. This method can be used not only for investigations in treatment technologies, but also for many other purposes, like monitoring and risk assessment.

5.2 RECOMMENDATIONS AS SUMMARIZED IN THE SERPIC TECHNICAL REPORT

5.2.1 Lessons learnt and outlook

Considering the project results and the feedback from the stakeholders during workshops held in Europe and South Africa, three lessons and outlooks can be concluded:

A) The disinfection by ozone gas that is produced electrochemically is regarded as a promising alternative to gas ozonation with ozone produced from liquid oxygen or air. The second and third options need a lot of energy, so that the electrochemical gas production has the potential to be an energy and cost-saving alternative. Additionally, it would be much less technically complex and thus more fail-safe, and would require less maintenance. Also, no liquid oxygen would be needed as a consumable, which would create a huge advantage for regions where it is not easily available. The application of this disinfection method is not limited to the disinfection of wastewater effluent for irrigation. It could similarly be applied, e.g., for disinfection during drinking water production.

B) B) Nanofiltration is often not regarded as a useful technology for CEC mitigation in WWTPs. One reason that is mentioned is that the CECs are not degraded but just separated; up to now, no good methods exist to handle the retentate with the concentrated CECs. Our results show not only a very good NF separation performance, but SERPIC also offers a new method to degrade the CECs in the concentrate in a very efficient manner. This offers new opportunities for nanofiltration as the central CEC separation method. NF also has the advantage that a very clean permeate is created continuously that could not only be used for irrigation but also in other applications where high-quality water is needed, including industry (cooling, process water) and also, with some minor additional treatment, for drinking water.

C) C) An upscaling of the SERPIC technology to full WWTP size would need an increase of the flow rate by five orders of magnitude. This will require huge further developments and resources. However, we see a special advantage also for applications that require the treatment of smaller flow rates, e.g., decentralised wastewater treatments, like in remote places or rural areas where no sewer system is available. A development of SERPIC until TRL9 and thus a real application and impact creation would be much faster on small scales, correspondingly.

5.2.2 Transfer

As the results of SERPIC are on TRL5, a lot of further development is necessary until the technology can be applied in real life.

The reliability of the technology is related to its continuous working and its ability to respect the limits set by local and national regulations. During its future development, an environmental and human health risk assessment, carried out according to the recent EU guidelines of the whole systems (treatments, storage and final destination), will identify the right way and confirm the current preventive safety measures of the whole process.

The technology has proven that the CEC removal rates are sufficient and promising. The most important step for further development is to scale up the technology and show that it is working well, also with higher volume flows. The SERPIC prototype plant had a volume flow of 34 litres per hour. A medium-sized WWTP has a volume flow in the range of 1000 cubic metres per hour. That means that for application in WWTPs, the technology must be scaled up by five orders of magnitude. This can only be implemented in several steps. The first step would be to scale it up to demonstrator level, with a volume flow of app. one cubic metre per hour (1000 litres). This affords another funded project and the participation of engineering equipment manufacturers, like membrane manufacturers, diamond electrode manufacturers, or manufacturers of electrochemical cells. Funding programmes like LIFE+, Horizon Europe, or Water4All might be suitable for this step. Such a project affords, amongst others, quite a lot of hardware budget for the bigger scale. Therefore, ERA-NETs will most likely not be suitable, as they usually do not provide large hardware budgets. If the technology proves also successful in reducing CECs on this volume flow level, more reliable estimations of energy demand and investment costs can be made.

This will give the precondition for the next step: the upscaling to a near-technical level of 100 cubic metres per hour. On this level, not only equipment manufacturers but also WWTPs might be ready to take part and provide the necessary demand and boundary conditions concerning energetic efficiency, affordability, and practical operations. As funding programmes, KICs might be suitable for this step, or the German BMBF programmes, under the precondition that the necessary non-German partners can be integrated.

When the regulatory limits are in force, WWTPs will need to install additional treatment processes to keep these limits for their discharge or to provide water for reuse. Then, as a next step, manufacturing companies for water treatment equipment will regard this as a meaningful market and develop corresponding water treatment products based on the SERPIC technology. Then, finally, WWTPs can invest in these products, install them and put them into operation.

Concerning the involvement of stakeholders, we made the experience that the collaboration is best done by the local partners, based on existing relationships and in their language. A central communication and interaction from the coordinator might not be so effective. Furthermore, individual communication, e.g., through interviews, has proven very effective and might be more effective than larger events with multiple stakeholders.

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APPENDIX A: RECOVERY STUDIES

Table 1 Recovery studies performed on soil irrigated with WWTP effluent

Chemical	Soil WWTP Carrots 1b	Recovery Soil WWTP Carrot 1b 50 ppb	Recovery %	Recovery Soil WWTP Carrot 1b 500 ppb	Recovery %
Sulfamethoxazole	<MQL	54,9	109,8	585,7	117,14
Venlafaxine	0,25	59,3	118,6	581	116,2
Diclofenac	<MDL	65,3	130,6	619,8	123,96
Iopromide	<MDL	<MQL	<MQL	789,3	157,86

Table 2 Recovery studies performed on ultra-pure water

Chemical	Recovery Water Blank	Recovery Water 50 ppb	Recovery %	Recovery Water 500 ppb	Recovery %
Sulfamethoxazole	<MDL	43,1	86,2	473,5	94,7
Venlafaxine	<MQL	49	98	477,4	95,48
Diclofenac	<MDL	41,8	83,6	442,4	88,48
Iopromide	<MDL	59,3	118,6	450,7	90,14

Table 3 Recovery studies performed on potato roots

Chemical	Recovery Potato Roots Blank	Recovery Potato Roots 50 ppb	Recovery %	Recovery Potato Roots 500 ppb	Recovery %
Sulfamethoxazole	<MDL	55,1	110,2	546,1	109,22
Venlafaxine	0,25	61	122	567,1	113,42
Diclofenac	<MDL	120	240	1063,4	212,68
Iopromide	<MDL	<MQL	<MQL	3037,6	607,52

Table 4 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	<MQL	<MQL	444,8	88,96
Venlafaxine	0,14	60,4	120,8	588,2	117,64
Diclofenac	<MDL	63,5	127	754,3	150,86
Iopromide	<MDL	22	44	647,5	129,5

Table 5 Recovery studies performed on potato leaves

Chemical	Recovery Potato Leaves Blank	Recovery Potato Leaves 50 ppb	Recovery %	Recovery Potato Leaves 500 ppb	Recovery %
Sulfamethoxazole	<MDL	49,7	99,4	542,4	108,48
Venlafaxine	0,1	62,6	125,2	557,7	111,54
Diclofenac	<MDL	102	204	1058,3	211,66
Iopromide	<MDL	<MDL	<MDL	<MQL	<MQL

Table 6 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	44,9	89,8	497,8	99,56
Venlafaxine	0,6	54,7	109,4	529,3	105,86
Diclofenac	<MQL	47,9	95,8	526,4	105,28
Iopromide	<MDL	36,8	73,6	740,7	148,14

Table 7 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	44,6	89,2	518,3	103,66
Venlafaxine	0,13	54,2	108,4	512,4	102,48
Diclofenac	<MQL	54,7	109,4	511	102,2
Iopromide	<MDL	<MQL	<MQL	9037,7	1807,54

Table 8 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	42,1	84,2	497,9	99,58
Venlafaxine	0,07	54,1	108,2	532,8	106,56
Diclofenac	<MQL	46,5	93	511,6	102,32
Iopromide	<MDL	<MDL	<MDL	<MQL	<MQL

APPENDIX B: Published booklet of SERPIC project

Published booklet of SERPIC project

Project Name & Acronym	Sustainable Electrochemical Reduction of contaminants of emerging concern and Pathogens in WWTP effluent for Irrigation of Crops – SERPIC
Project coordinator (Name, E-Mail, Organisation, Country)	Dr Jan Gäbler, jan.gaebler@ist.fraunhofer.de, Fraunhofer Institute for Surface Engineering and Thin Films IST, Germany
Project partner organisations, country	Fraunhofer-Gesellschaft e.V., Germany Fraunhofer Institute for Surface Engineering and Thin Films IST Fraunhofer Institute for Solar Energy Systems ISE SolarSpring GmbH, Germany Università degli Studi di Ferrara, Italy (UNIFE) Universidad de Castilla-La Mancha, Spain (UCLM) Universidade do Porto, Portugal (UP) AdP VALOR, Serviços Ambientais, SA, Portugal (AdP) Norwegian Institute for Water Research, Norway (NIVA) Stellenbosch University, South Africa (SU)
Project Duration	1.9.2021 - 31.12.2024
Project website	serpic-project.eu
Main project theme(s) – please highlight relevant ones	Monitoring, Analytics, Substance Behaviours, Assessing Exposure Routes, Methods, Human Health & Environmental Effects, Management Strategy, Regulatory Issues, Technical Solutions

Motivation behind the project – What is the overall problem your project addressed

Increasing water scarcity is one of the biggest challenges worldwide. The provision of safe water in sufficient amounts is essential for both human health (drinking water and sanitation) and – via irrigation of crops – for a sufficient human nutrition. SERPIC connects the urban water cycle with the water cycle in agriculture and the natural water cycle. The reuse of the effluents of wastewater treatment plants (WWTPs) constitutes a significant and constantly available water resource that can be safely used for irrigation. This is the motivation for the SERPIC project to develop technologies to reduce CECs from conventional WWTP effluent.

Project Objectives

- Objective 1: Minimize the spread and transformation of CECs, including ARB and 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;
- Objective 2: Reduce CECs from WWTP effluent by developing an innovative treatment technology, based on membrane filtration and light-driven electrochemical processes. Reduction targets: Route A > 90 %, Route B > 80 %
- Objective 3: Elaborate on methodologies and tools for monitoring, health and environmental risk assessment and for the implementation of new reuse concepts,

including new treatment technologies, as a basis for better policy and decision-making, for regulatory issues and new standards.	
Research methodology and implementation summary With the SERPIC technological multibarrier approach, secondary effluent of a municipal wastewater plant underwent a sequence of reclaiming treatments: A nanofiltration separated the effluent into a high-quality permeate stream (Route A), and a more polluted concentrate stream (Route B). Route A stream was disinfected by ozone gas, electrochemically generated using diamond electrodes, for safe irrigation of crops. The CECs in the Route B stream were degraded by persulfate, electrochemically generated using diamond electrodes, for safe discharge into the aquatic environment. A prototype plant was set up and tested in a long-term field test by irrigating crops with the treated water.	
Summary of main results A methodology was developed to select relevant target CECs for the analysis to prove the performance of the technology. Process components were developed and built. A prototype plant was commissioned and put into operation. It could process 34 L/h of water with an energy consumption of 630 W. The CECs in the Route A water for irrigation were reduced by 90-99 %. The CECs in the Route B water for discharge were reduced by 70-99 %. LCA and LCC were done, as well as a risk analysis of the plant operation. An exploitation of the results was planned and discussed with stakeholders.	
Key exploitable outcome / output / result 1 *Target CEC selection methodology*	*Designed to assess the usefulness of a technology for a specific location and the risk for the environment and crops. It is based on occurrence, persistence, bioaccumulation and toxicity.*
Key exploitable outcome / output / result 2 *SERPIC prototype and performance*	*This output includes a full setup of a water treatment on a prototype level to reduce CECs and AMR bacteria.*
Key exploitable outcome / output / result 3 *Nanofiltration selection and performance*	*Knowledge has been elaborated on the CEC removal performance of a specifically selected nanofiltration membrane*
Key exploitable outcome / output / result 4	*- Computer-aided design (CAD), computational fluid dynamics simulation (CFD) and 3D printing to support the production process and achieve maximum electrogeneration efficiencies.* *- Design features that increase persulfate production efficiency: minimum distance between electrodes, uniform fluid distribution by distribution fins, conical inlets and outlets for the evacuation of*

Persulfate electrolyser design and performance	*gases generated in the process. Easily adapted to a single or double compartment.* *- Definition of optimal operating conditions, achieving production efficiencies higher than similar reactors without the need for any turbulence promoter. Capable of working in various discontinuous and continuous operation modes, regulating the inlet and outlet flow rate for future industrial application.*
Key exploitable outcome / output / result 5 *Ozone gas electrolyser design and performance*	*- Custom design of an electrochemical cell for ozone production using Computer Aided Design and printed with 3D printing technology.* *- Proton Exchange Membrane type cell with a commercial Membrane Electrode Assembly specially designed to evacuate the gases formed during the electrolysis reaction, avoiding decomposition reactions and at the same time maintaining a good flow distribution within the electrochemical cell.* *- Optimization of the system variables to obtain the maximum ozone production conditions, but with the minimum energy consumption required.*
Key exploitable outcome / output / result 6 *Persulfate oxidation photoreactor design and performance*	*- Technology optimized for the advanced treatment of the nanofiltration retentate stream* *- Homogeneous oxidant distribution (radial and axial) via dosing through porous ceramic* *- Simulation tool for optimization of reflective surfaces in tubular UVC-photoreactors* *- Definition of operational conditions to ensure compliance with legal discharge limit values*
Outlook / Perspectives *Disinfection by electrogenerated ozone gas is regarded as a promising alternative to ~~gas~~ ozonation with ozone produced from liquid oxygen.* *The results offer new opportunities for nanofiltration as a central CEC separation method. Nanofiltration also enables a very clean permeate that could be used for irrigation and industry.* *Water treatment with electrochemically generated persulfate offers a low-energy alternative with much higher CEC degradation rates compared to activated carbon adsorption and ozonation.* *A special advantage of SERPIC is offered for applications requiring the treatment of smaller flow rates, e.g., decentralised wastewater treatments, like remote places or areas without a sewer system.*	

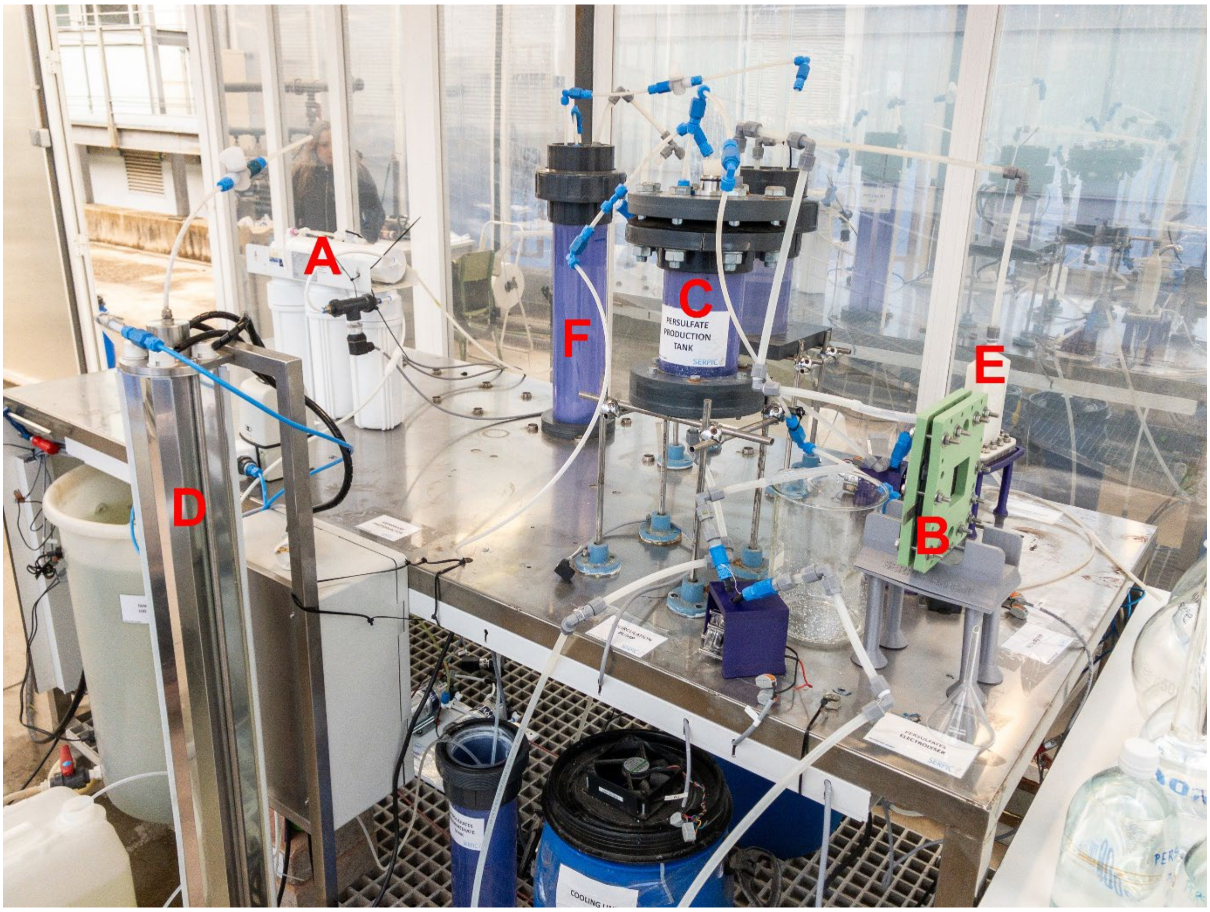

Figure 1. SERPIC prototype plant: A nanofiltration, B persulfate electrolyser, C persulfate intermediate tank, D UV photoreactor, E ozone gas electrolyser, F disinfection unit with ozone contactor.

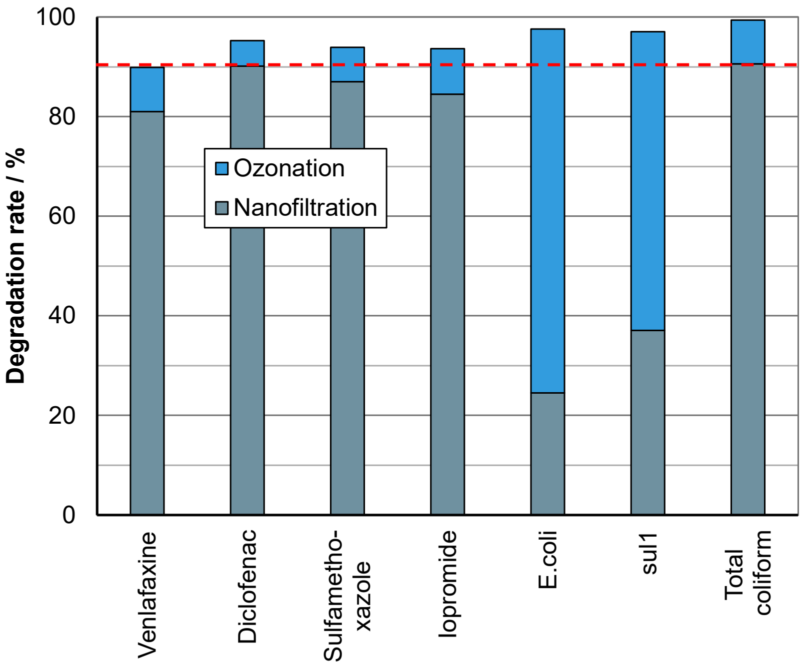

Figure 2. Degradation rates of the target CECs in Route A (disinfected nanofiltration permeate, for the use in irrigation); red: SERPIC goal.

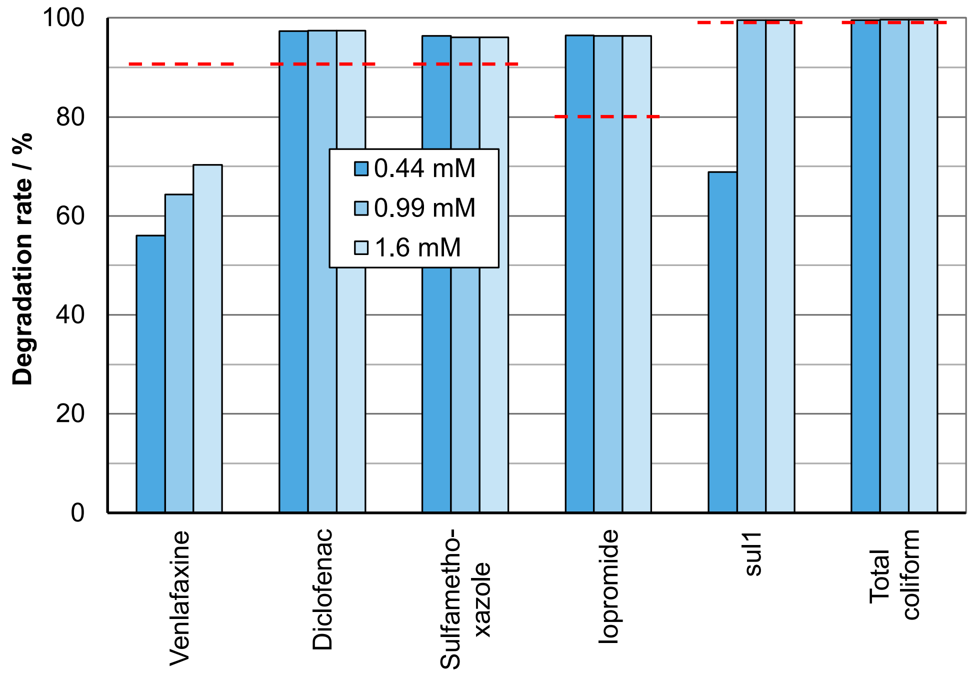

Figure 3 Degradation rates of the target CECs in Route B (nanofiltration concentrate treated with persulfate, for discharge into environment) for three different persulfate concentrations; red: SEPIC goals.