UPTAKE, TRANSLOCATION AND RISKS OF CONTAMINANTS OF EMERGING CONCERN IN TOMATO (*SOLANUM LYCOPERSICUM* L.) CULTIVATION USING TREATED WASTEWATER AND SLUDGE

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Doctoral Dissertation
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PRIVZEM IN PRENOS ONESNAŽEVAL, KI VZBUJAJO ZASKRBLJENOST, V PARADIŽNIK (*SOLANUM LYCOPERSICUM* L.) TER OCENA TVEGANJ PRI UPORABI PREČIŠČENE ODPADNE VODE IN BLATA V KMETIJSTVU

Doktorska disertacija

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"All we have to decide is what to do with the time that is given to us."
J.R.R. Tolkien

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«'Cause there were pages turned with the bridges burned,
Everything you lose is a step you take.»
You're On Your Own, Kid, Taylor Swift

Γιάννη, Δέσποινα, Φωτεινή, Ρότζερ, ευχαριστώ για την υπομονή,
Ντίνα, Γιώργο ευχαριστώ για τη στήριξη,
Βάγια, Ορφέα, Γεωργία, Ηρώ, Δέσποινα, Πάνο ευχαριστώ για την έμπνευση,
Όλους σας για την αγάπη που μου δίνετε καθημερινά.

Abstract

The reuse of treated wastewater (TWW) and sewage sludge represents an important practice that supports sustainable agriculture, conserves freshwater resources, and supports environmental sustainability through circular economy principles. However, both matrices can introduce contaminants of emerging concern (CECs) into agroecosystems, potentially affecting soil health, crop production, and food safety, while their environmental fate, plant uptake, and associated risks remain poorly understood.

This thesis investigated the uptake, translocation, and bioaccumulation of selected model CECs, such as pharmaceuticals, personal care product ingredients, industrial chemicals, hormones and pesticides, in tomato plants (*Solanum lycopersicum* L. cv. Rally) across five experiments representing key wastewater- and sludge-reuse scenarios: (i) a 2020 field experiment with soil-grown tomatoes irrigated with potable water (PW), TWW, or TWW spiked at 0.1 mg L^{-1} CECs; (ii) a 2022 field experiment with soil-grown tomatoes irrigated with PW, TWW, or PW/TWW spiked at 1 mg L^{-1}; (iii) a 2020 hydroponic experiment using nutrient solution (NS, control) or NS spiked at 0.1 mg L^{-1}; (iv) a 2022 hydroponic experiment using NS, TWW, NS spiked at 0.1 or 1 mg L^{-1}, or TWW spiked at 1 mg L^{-1}; and (v) a peat-substrate experiment using stabilised anaerobically digested (dried) sludge applied at two agronomic rates (to meet N and P requirements), sludge spiked with CECs at the same rates, CEC-spiked peat, or peat amended with non-dried sludge. To support these experiments, different sample preparation methods were optimised and validated for tomatoes, plant tissues, soil, sludge, TWW and PW followed by liquid chromatography coupled to mass spectrometry (LC–MS/MS) analysis. For TWW, a green dispersive micro-solid phase extraction method based on a newly synthesised acrylic copolymer was evaluated and compared with conventional extraction methods.

In the 2020 field experiment, uptake of CECs into edible tissues was minimal: no compounds were detected in fruits under PW or TWW irrigation, and only bisphenol S (BPS) was quantified in fruits irrigated with spiked TWW. In 2020, BPS, bisphenol F (BPF), and naproxen were quantified in tomatoes grown hydroponically in spiked NS. Across various experiments, CEC uptake and translocation were significantly influenced by the compound's physicochemical properties, matrix composition, and cultivation conditions. Under realistic field irrigation conditions (TWW), bioaccumulation in edible tissues was limited, with carbamazepine being the only CEC quantified. In spiked media, acetamiprid, azithromycin, BPS, carbamazepine, clarithromycin, and dimethomorph were above the limit of quantification in tomato fruits, while up to 17 CECs were quantified in roots > leaves > stems.

In the 2022 hydroponic experiment, reduced plant growth was observed, including leaf chlorosis and a poor root system, which led to minimal fruit production (not ripe). However, uptake by roots was extensive, with concentrations reaching up to 118 µg/g for specific compounds. In plant parts, 21 out of 27 CECs were found, with a distribution pattern of roots > leaves > stems > fruits. In fruits, between 6 and 12 CECs were quantified depending on the treatment (TWW or spiked media).

In pot experiments with sludge amendment, tomato fruits accumulated a range of CECs, depending on the treatment and whether spiking was applied. In tomato fruits, ibuprofen and triclosan were quantified at the low amendment rate, while caffeine, carbamazepine, ciprofloxacin and ibuprofen were quantified at the high amendment rate. For carbamazepine, the only compound detected in each tissue in spiked treatments, translocation from roots to leaves was highest, followed by stems and fruit.

Across all experiments, dietary exposure assessments indicated that consuming tomatoes grown with TWW irrigation or sludge amendment generally posed limited risks to human health. For the majority of compounds found in fruits, the calculated estimated intake remained below the respective health-based guidance values, for two consumption rates and all evaluated age groups. Carbamazepine, the only CEC detected in soil-grown tomato fruits under realistic TWW irrigation, had an estimated daily intake of nearly half the respective acceptable daily intake (Hazard Quotient up to 0.017), even for vulnerable population groups, such as toddlers. Across all the experiments, risk was identified only for bisphenols (BPS, BPF, and bisphenol A (BPA)), due to the comparison with the tolerable daily intake proposed for BPA, as a conservative approach.

Ecological risk was evident in both soil and peat substrate experiments. Under TWW irrigation, selected CECs, azithromycin and triclocarban, remained in soil up to 30 months after TWW irrigation, and exceeded risk thresholds. In sludge-amended peat, when dried sludge was applied at two levels, risks were identified for five compounds. Drying sludge before amendment resulted in a lower risk compared to adding non-dried sludge, with seven compounds exceeding the risk threshold. The results highlight that the repeated use of TWW and sludge can lead to the accumulation of CECs in soils, facilitating their uptake by plants.

Overall, the thesis contributes to the understanding of the uptake and translocation of CECs in tomato plants. Findings confirm that wastewater irrigation and sludge amendment can lead to CEC uptake in tomato plants; however, accumulation in fruits is generally low and matrix-dependent. This thesis highlights the importance of degradation and sorption processes in TWW, soil, and sludge, which seem to limit the fraction that remains bioavailable for plant uptake. The results support the need for updated regulatory frameworks, improved monitoring of CECs and further research on long-term reuse scenarios to ensure the safe agricultural reuse of wastewater and sludge.

Povzetek

Ponovna uporaba prečiščene odpadne vode (POV) in komunalnega blata predstavlja pomembno prakso, ki podpira trajnostno kmetijstvo, ohranja vodne vire ter spodbuja okoljsko trajnost v okviru krožnega gospodarstva. Kljub temu lahko obe matriki (POV in blato) v agroekosisteme vnašata onesnaževala, ki vzbujajo zaskrbljenost (Contaminants of Emerging Concern, CECs), kar lahko vpliva na zdravje tal, kvaliteto poljščin in varnost hrane. Njihova usoda v okolju, privzem v rastline in s tem povezana tveganja, pa so še vedno pomanjkljivo raziskani.

V okviru doktorske naloge smo preučevali privzem, prenos in bioakumulacijo izbranih modelnih onesnaževal, ki vzbujajo zaskrbljenost, kot so zdravilne učinkovine, učinkovine iz izdelkov za osebno nego, industrijske kemikalije, hormoni in pesticidi, v rastline paradižnika (*Solanum lycopersicum* L. cv. Rally) v petih eksperimentih, ki predstavljajo ključne scenarije ponovne uporabe odpadne vode in komunalnega blata: (i) poljski poskus, izveden leta 2020, z gojenjem paradižnika v tleh, namakanih s pitno vodo (PV), prečiščeno odpadno vodo ali POV, obogateno z 0,1 mg L^{-1} CECs; (ii) poljski poskus iz leta 2022 z gojenjem paradižnika v tleh, namakanih s PV, POV ali PV/POV, obogateno z 1 mg L^{-1} CECs; (iii) hidroponski poskus iz leta 2020 z gojenjem paradižnika v hranilni raztopini (HR, kontrola) ali HR, obogateni z 0,1 mg L^{-1} CECs; (iv) hidroponski poskus iz leta 2022 s HR, POV, HR obogateno z 0,1 ali 1 mg L^{-1} CECs ter POV obogateno z 1 mg L^{-1} CECs; ter (v) poskus s šotnim substratom, v katerega smo dodali anaerobno stabilizirano (posušeno) blato v dveh agronomskih odmerkih (glede na zahteve po N in P), blato obogateno s CECs v istih odmerkih, s CECs obogateno šoto ter ločeno šoto, pomešano z neobdelanim (nesušenim) blatom. Za spremljanje prenosa CEC smo preučili in validirali različne postopke priprave vzorcev paradižnika, rastlinskih tkiv, tal, blata in POV ter analize z uporabo tekočinske kromatografije, sklopljene z masno spektrometrijo (LC–MS/MS). Za POV smo preizkusili tudi disperzijsko mikroekstrakcijo na trdnem nosilcu, ki temelji na novo sintetiziranem akrilnem kopolimeru. Uspešnost te zelene ekstrakcijske metode smo primerjali z običajno ekstrakcijo na trdnem nosilcu.

V poljskem poskusu iz leta 2020 je bil privzem CECs v paradižnikove plodove minimalen: v plodovih, namakanih s PV ali POV, nismo določili nobene izmed preučevanih spojin, medtem ko je bil v plodovih, namakanih z obogateno POV, kvantificiran le bisfenol S (BPS). V hidroponskem poskusu iz leta 2020 so bili v paradižnikih, gojenih v obogateni hranilni raztopini, nad mejo kvantifikacije (> meja kvantifikacije (LOQ)) BPS, bisfenol F (BPF) in naproksen. Na privzem in vnos CEC so pomembno vplivale fizikalno-kemijske lastnosti spojin, značilnosti matrike ter pogoji gojenja. Pri realističnih poljskih pogojih namakanja s POV je bila bioakumulacija v plodovih omejena, pri čemer je bil karbamazepin edini CEC, ki je bil določen nad mejo kvantifikacije. V obogatenih medijih so bili v paradižnikovih plodovih >LOQ acetamiprid, azitromicin, BPS, karbamazepin, klaritromicin in dimetomorf, medtem ko je bilo v koreninah > listih > steblih kvantificiranih do 17 CECs.

V hidroponskem poskusu iz leta 2022 smo opazili zmanjšano rast rastlin, klorozo listov in slabo razvit koreninski sistem, kar je negativno vplivalo na razvoj plodov (nezreli

paradižniki). Kljub temu je bil privzem CECs v korenine znaten, pri čemer so koncentracije posameznih spojin dosegle do 118 µg/g. V rastlinskih delih smo kvantificirali 21 od 27 CECs, z značilnim vzorcem porazdelitve: korenine > listi > stebla > plodovi. V plodovih je bilo kvantificiranih od 6 do 12 CECs, odvisno od obravnave (POV ali obogateni mediji).

V lončnih poskusih z dodatkom komunalnega odpadnega blata so plodovi paradižnika privzeli različne CECs, odvisno od vrste obravnave in tega, ali je bil medij dodatno obogaten. Pri nižjem odmerku blata sta bila v plodovih kvantificirana ibuprofen in triklosan, pri višjem odmerku pa kofein, karbamazepin, ciprofloksacin in ibuprofen. Za karbamazepin, edino spojino, ki je bila v obogatenih obravnavah zaznana v vseh rastlinskih tkivih, je bil prenos iz korenin v liste najvišji, sledila so stebla in nato plodovi.

V vseh poskusih so ocene prehranske izpostavljenosti pokazale, da uživanje paradižnikov, namakanih s POV ali gojenih z dodatkom blata, na splošno predstavlja omejeno tveganje za zdravje ljudi. Pri večini spojin, kvantificiranih v plodovih, je bil ocenjeni vnos pod ustreznimi na zdravju temelječimi usmeritvenimi vrednostmi, pri obeh ocenjenih količinah zaužitja in za vse starostne skupine. Za karbamazepin, edini CEC, ki smo ga kvantificirali v plodovih paradižnika, namakanih s POV, je bil ocenjeni dnevni vnos približno polovica ustreznega sprejemljivega dnevnega vnosa (količnik nevarnosti do 0.017), tudi pri ranljivih skupinah prebivalstva, kot so otroci, stari 1–3 leta. V vseh poskusih je bilo tveganje ugotovljeno le za bisfenole (BPS, BPF in bisfenol A (BPA)), najverjetneje zaradi uporabe dopustnega dnevnega vnosa, predlaganega za BPA (konservativni pristop).

Ekološko tveganje je bilo očitno tako v poskusih s tlemi kot s šotnim substratom. Pri namakanju s POV sta bila azitromicin in triklokarban v tleh prisotna še 30 mesecev po koncu namakanja in sta presegla mejne vrednosti tveganja. V šoti, obogateni z blatom, so bila tveganja ugotovljena za pet spojin pri dodatku sušenega blata v obeh odmerkih. Sušenje blata pred vnosom v substrat je vodilo do nižjega tveganja v primerjavi z dodatkom neposušenega blata, pri čemer je sedem spojin preseglo mejno vrednost tveganja. Rezultati poudarjajo, da lahko ponovna uporaba POV in komunalnega blata vodi do kopičenja CECs v tleh, kar omogoča njihov privzem v rastline.

Na splošno doktorska naloga prispeva k boljšemu razumevanju privzema in vnosa CECs v rastline paradižnika. Ugotovitve potrjujejo, da lahko namakanje z odpadno vodo in dodatek komunalnega blata povzročita vnos CECs v rastline paradižnika; vendar je njihovo kopičenje v plodove na splošno nizko in izrazito odvisno od uporabljene matrike. Naloga poudarja tudi pomen procesov razgradnje in sorpcije v prečiščeni odpadni vodi, tleh in blatu, ki omejujejo delež CECs, ki ostanejo (bio)dostopni za privzem v rastline. Rezultati podpirajo potrebo po posodobitvi regulatornih okvirov, izboljšanem spremljanju CECs ter nadaljnjih raziskavah ponovne uporabe odpadne vode in komunalnega blata, zlasti pri dolgoročnih scenarijih, z namenom zagotavljanja varne kmetijske rabe teh obnovljivih virov.

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Abbreviations

µ-SPE	... Micro-Solid Phase Extraction
ADI	... Acceptable Daily Intake
APCI	... Atmospheric Pressure Chemical Ionisation
ASE	... Accelerated Solvent Extraction
BCF	... Bioconcentration Factor
BMD	... Benchmark Dose
BMDL	... Benchmark Dose Lower Confidence Limit
BOD	... Biochemical Oxygen Demand
BPA	... Bisphenol A
BPAF	... Bisphenol AF
BPF	... Bisphenol F
BPS	... Bisphenol S
CECs	... Contaminants of Emerging Concern
CNTs	... Carbon Nanotubes
D-µ-SPE	... Dispersive Micro-Solid Phase Extraction
DG-SANTE	... Directorate-General for Health and Food Safety
DNEL	... Derived No-Effect Level
d-SPE	... Dispersive SPE
E1	... Estrone
E2	... 17-Beta-Estradiol
EDCs	... Endocrine Disrupting Chemicals
EDI	... Estimated Daily Intake
EE2	... 17-Alpha-Ethinylestradiol
EF	... Extraction Efficiency
EFSA	... European Food Safety Authority
EPM	... Equilibrium Partition Method
ERA	... Ecological Risk Assessment
ESI	... Electrospray Ionization
EU	... European Union
GAC	... Green Analytical Chemistry
GC	... Gas Chromatography
GCB	... Graphitised Carbon Black
HF-LPME	... Hollow Fibre Liquid-phase Microextraction
HLB	... Hydrophilic–Lipophilic Balanced
HPLC	... High-Performance Liquid Chromatography
HQ	... Hazard Quotient
HRMS	... High Resolution Mass Spectrometry
HS-SPME	... Headspace Solid-Phase Microextraction
LC	... Liquid Chromatography
LOAEL	... Lowest Observed Adverse Effect Level

LOD ... Limit of Detection
$\log K_{ow}$ ... Logarithm of Octanol/Water Partition Coefficient
LOQ ... Limit of Quantification
MAE ... Microwave-Assisted Extraction
MCX ... Mixed-mode Cation Exchange
ME ... Matrix Effect
MEC ... Measured Environmental Concentration
MIPs ... Molecularly Imprinted Polymers
MOFs ... Metal Organic Frameworks
MRM ... Multiple Reaction Monitoring
MS ... Mass Spectrometry
MS/MS ... Tandem Mass Spectrometry
MSPD ... Matrix Solid-Phase Dispersion
NOAEL ... No Observed Adverse Effect Level
NOEC ... No Observed Effect Concentration
PEC ... Predicted Environmental Concentration
PHWE ... Pressurised Hot Water Extraction
pK_a ... Acid Dissociation Constant
PLE ... Pressurised Liquid Extraction
PNEC ... Predicted No-Effect Concentration
PPCPs ... Pharmaceuticals and Personal Care Products
PSA ... Primary-Secondary Amine
PW ... Potable Water
Q-Orbitrap ... Quadrupole-Orbitrap
QSAR ... Quantitative Structure-Activity Relationship
qTOF ... Quadrupole Time-of-Flight
QuEChERS ... Quick, Easy, Cheap, Effective, Rugged and Safe
RCF ... Root Concentration Factor
RP ... Reversed Phase
RQ ... Risk Quotient
RSD ... Relative Standard Deviation
S/N ... Signal-to-Noise Ratio
SAX ... Strong Anion Exchange
SLE ... Solid Liquid Extraction
SPE ... Solid-Phase Extraction
SRM ... Selected Reaction Monitoring
TDI ... Tolerable Daily Intake
TF ... Translocation Factor
TPs ... Transformation Products
TQ ... Triple Quadrupole
TSS ... Total Suspended Solids
TTC ... Threshold of Toxicological Concern
TWW ... Treated Wastewater
UAE ... Ultrasound Assisted Extraction
UPLC ... Ultra-Performance Liquid Chromatography
WAX ... Weak Anion Exchange
WCX ... Weak Cation Exchange
WFD ... Water Framework Directive
WHO ... World Health Organisation
WWTP ... Wastewater Treatment Plant

Chapter 1

Introduction

1.1 Background

Freshwater scarcity is one of the most pressing global challenges of the 21st century, directly threatening food production, ecosystem stability, and socio-economic development [1]. The growing population, projected to exceed 9.7 billion by 2050, along with urbanisation and industrialisation, is significantly increasing global freshwater demand [2, 3]. Additionally, climate change has an additional effect on the hydrological cycle by altering precipitation patterns, raising evapotranspiration rates, and increasing the frequency of droughts [4]. These factors have already resulted in over 40% of the global population, accounting for 4 billion people, currently living under water-stressed conditions for at least one month of the year [5].

Agriculture is a major sector contributing to this water crisis, annually extracting approximately 70% of available freshwater resources [6, 7]. The situation is particularly critical for arid and semi-arid regions, where irrigation remains crucial for maintaining agricultural productivity. The Mediterranean basin, for instance, faces increasing water stress due to limited rainfall, overexploitation of groundwater, and seasonal droughts [8, 9]. As a result, agricultural productivity may be jeopardised, with potential implications for global food security [2]. Alpine countries, such as Slovenia, despite having sufficient water resources nationwide, still experience water scarcity. Water shortages typically affect the North East and South West regions, due to the uneven distribution of precipitation. A shift in water shortages towards central Slovenia has been noted over time. It is estimated that 15% of Slovenia's surface area is at risk due to soil water deficit [10].

The concept of a circular economy is a framework that can also be applied to water management by adopting sustainable water use strategies, such as reducing, preserving and optimising its use [11]. Within this framework, the reuse of treated wastewater (TWW) is a practice contributing to achieving the circular economy model, providing an alternative resource that supports both environmental protection and sustainable agricultural production [12]. In the European Union (EU), more than 40 billion m^3 of wastewater is treated annually, from which only 1 billion m^3 of TWW is reused annually, accounting for 2.5% of the total volume produced [13]. However, current levels could be increased by 6 times, which could significantly reduce environmental pressures and enhance resilience to drought and resource scarcity [14]. Increased wastewater reuse in the EU could result in a 10% reduction of direct abstraction from natural resources, including water bodies and groundwater [13].

1.1.1 Treated wastewater reuse in agriculture

Israel is considered a global leader in wastewater reuse due to state-of-the-art technologies, with more than 80% of TWW being reused for agriculture, covering half of the irrigation water demand [15]. In the EU, water reuse practices are more prevalent in southern countries, such as Cyprus, Malta, Italy, Greece and Spain, where water scarcity and irrigation demands are particularly severe [8]. Reuse practices vary among different member states and are influenced by a country's climate zone [16]. Based on data from 2020, Cyprus reused 90% of its TWW, primarily for irrigation in agriculture [16]. Spain is also one of the leading countries in water reuse among EU countries, where over 10% of TWW is reused, out of which over 70% is intended for agricultural irrigation, with the scope of doubling the current reused volumes by 2027. Within the country, reclaimed water production is concentrated in the Mediterranean region to mitigate water scarcity issues [17]. Currently, Slovenia does not support the reuse of TWW.

1.1.1.1 Benefits of TWW reuse

The benefits of TWW reuse in agriculture are multiple. From an environmental perspective, reusing treated effluents is a substitute for other water resources and can reduce the extraction of freshwater from surface and groundwater. Thereby, stress to natural water bodies is reduced, and flows to vital ecosystems are increased [18, 19]. TWW can also supply additional nutrients, including macronutrients (N, P, and K) and micronutrients (Ca, Mg, B, Fe, Mn, or Zn), which are essential for plant growth [20]. From an economic perspective, the application of TWW has the potential to reduce the use of chemical fertilisers by up to 50%, thereby reducing expenses and avoiding the direct return of additional nutrients to the water cycle [21]. TWW can provide a cost-effective irrigation scheme, increasing farmers' profitability through a readily available source of irrigation and a boost in crop yield, with savings of around €280/ha reported for tomato farming utilising TWW [22, 23].

1.1.1.2 Risks and challenges related to TWW

Despite its advantages, TWW reuse practices introduce several challenges that may limit their widespread adoption, which will depend on the quality of the effluent, its physicochemical properties, and its microbial composition. The environmental impacts include the potential to pollute surface water and groundwater, depending on the type of TWW and the treatments applied [24].

The reuse of TWW can also have a negative impact on soil, as it can increase soil salinity and reduce water-holding capacity, thereby reducing agricultural productivity [23]. Alterations in soil microbial communities can also occur, either directly through the introduction of exogenous microorganisms or indirectly through changes in soil physicochemical properties, which impact soil fertility by altering the soil's capacity to supply nutrients and sustain plant growth [25]. As mentioned, the nutrient content can be beneficial for plant growth, but an excess supply may result in phytotoxic effects [26].

Public health impacts are related to the presence of organic compounds [27], microplastics [28], pathogens [29], and potentially toxic elements [30] in TWW. These pollutants may pose a risk to farmers and farmworkers during reuse practices, as well as for consumers through ingestion of contaminated food.

Regarding the economic challenges, TWW reuse requires additional investments to meet specific quality requirements for agricultural reuse, as well as to cover transportation and storage costs. Based on an economic analysis by the European Joint Research Centre, the costs of water reuse practices, including the cost to make TWW safe, are currently

approximately 0.08 Euro/m^3, ranging from 0.50-1 Euro/m^3 if all costs of infrastructure, treatment and pumping are taken into account [31].

1.1.1.3 Legislation

To ensure the safe use of reclaimed water for agricultural irrigation and to guarantee a high level of protection for environmental, human, and animal health, the EU introduced Regulation 2020/741, which defines the minimum requirements for water reuse [32]. This regulation specifies four water quality classes based on the type of crop and irrigation method (Table 1.1). The classification reflects the potential risk of human exposure, requiring each crop category to be irrigated with the corresponding minimum water quality classes, ranging from the most stringent (Class A) to the least (Class D), based on exposure risk. Alternatively, the use of additional barriers may allow the application of a lower water quality class for certain crops, to ensure the safety of water reuse systems.

Table 1.1: Classes of reclaimed water quality and permitted agricultural use and irrigation method (adopted from Regulation (EU) 2020/741 [32]).

Reclaimed water quality class	Crop category	Irrigation method
A	All food crops consumed raw, where the edible part is in direct contact with reclaimed water and root crops consumed raw	All irrigation methods
B	Food crops consumed raw, where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops, including crops used to feed milk- or meat-producing animals	All irrigation methods
C	Food crops consumed raw, where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops, including crops used to feed milk- or meat-producing animals	Drip irrigation (*) or other irrigation method that avoids direct contact with the edible part of the crop
D	Industrial, energy and seeded crops	All irrigation methods

*Drip irrigation: an irrigation system capable of delivering water drops or tiny streams to the plants and involves dripping water onto the soil or directly under its surface at very low rates (2–20 L/h) from a system of small-diameter plastic pipes fitted with outlets called emitters or drippers.

Quality requirements include limits for microbiological indicators (e.g., *E. coli*, and in specific cases also for *Legionella* spp. and intestinal nematodes), biochemical oxygen demand (BOD), total suspended solids (TSS) and turbidity (Table 1.2).

Table 1.2: Reclaimed water quality requirements for quality class A for agricultural irrigation (adopted by Regulation (EU) 2020/741 [32]).

Quality requirements	
E. coli (number/100 mL)	≤ 10
BOD_5 (mg/L)	≤ 10
TSS (mg/L)	≤ 10
Turbidity (NTU)	≤ 5
Legionella spp.	< 1,000 CFU/L where there is a risk of aerosolisation
Intestinal nematodes (helminth eggs)	≤ 1 egg/L for irrigation of pastures or forage

The regulation also defines the frequency of monitoring for different cases of TWW reuse. In addition, before applying for a permit, a comprehensive risk assessment is required as part of wastewater reuse risk management for each wastewater treatment plant (WWTP) when there is clear evidence that the risk originates from the use of reclaimed water. Key elements of risk management should include identifying and managing risks protectively, ensuring the safety of reclaimed water with an emphasis on heavy metals, pesticides, disinfection by-products, pharmaceuticals, antimicrobial resistance, and other emerging contaminants, including micropollutants and microplastics. To date, Slovenia has not implemented this regulation. However, in light of increasing pressure on freshwater resources, it will reconsider this decision in 2027.

Due to increasing evidence of the occurrence of organic micropollutants in TWW [33], discharged to the environment, the Urban Wastewater Treatment Directive (2024/3019) was revised and entered into force in January 2025. The renewed directive aims to improve water quality through additional water treatment requirements and, for the first time, addresses 12 indicator micropollutants [34]. For this purpose, six out of the 12 indicator micropollutants, selected individually for each WWTP, must be removed by greater than 80%; otherwise, additional quaternary treatment must be introduced. The selected micropollutants, referred as substances that can pollute water even at low concentrations, derive from two categories of compounds: (1) substances that can be very easily treated (amisulpride, carbamazepine, citalopram, clarithromycin, diclofenac, hydrochlorothiazide, metoprolol, venlafaxine) and (2) substances that can be easily disposed of (benzotriazole, candesartan, irbesartan, mixture of 4-methylbenzotriazole and 5-methyl-benzotriazole). The scope of the directive is to improve the quality of wastewater effluents and subsequently to protect the receiving waters. The Directive also states that "*Member states shall ensure that urban wastewater that is reused or planned to be reused is treated in accordance with the requirements for quaternary treatment*" [34].

1.1.2 Sludge and biosolids reuse in agriculture

Beyond TWW reuse, the valorisation of sewage sludge represents another critical aspect to promote the circular economy and its use in agriculture. Sewage sludge, also referred to as biosolids, is the main by-product of wastewater treatment, after TWW. It contains high concentrations of organic matter and essential plant nutrients such as nitrogen and phosphorus, also making it a potentially valuable soil amendment [35, 36]. Although the terms "sewage sludge" and "biosolids" are used interchangeably in literature, "sludge" refers to a semi-solid material produced by WWTPs, while the term "biosolids" refers to sludge that has received one or more additional treatments, including, for example, aerobic

or anaerobic digestion, thermal drying, alkaline stabilisation, composting, acid oxidation/disinfection [37].

Generally, when treated sludge (biosolids) is applied to soil, it has been shown to improve its properties (bulk density, porosity, water holding capacity) [35]. This is because sludge is rich in organic matter, macronutrients (N, P, K), and other mineral elements, and can be used to condition the soil, thereby improving its physical, chemical, and biological properties, making it healthier and more fertile for plant growth [35]. In addition, it can also serve as a source of plant nutrients and as a fertiliser. This practice can contribute to the reduction of synthetic organic fertiliser use and the application of non-renewable sources of energy for sludge treatment and disposal, such as incineration, thereby adding to the concept of the circular economy [38-40].

1.1.2.1 Legislation

In the EU, between 6 and 9 million tonnes of dry sewage sludge are generated annually. Out of an estimated 7.5 million tonnes of waste or disposal based on data from 2016, 35% is used in agriculture, while the remaining sludge management routes involve incineration (31%), compost and other applications (12%), landfilling (12%) and other uses (10%) [41]. At the European level, the agricultural use of treated sewage sludge is regulated under Council Directive 86/278/EEC, which sets limits for heavy metals (cadmium, copper, nickel, lead, zinc, mercury, chromium), to protect the health of soil, plants, animals, and humans. However, this Directive was adopted in 1986 and is expected to be revised in the near future, most likely following the example of the Urban Wastewater Treatment Directive, which also regulates organic micropollutants [42].

Generally, after the Directive sets out a goal that all EU countries must achieve, each member state can choose how to implement it in its own legal system. National legislation across EU Member States often sets stringent limitations, leading to differences in the extent to which sludge is reused in agriculture [43]. Limits for pathogens in sludge are set by national legislation, including the presence of *Salmonella, E. coli*, helminth eggs, enterococci, and enterobacteria [44]. However, many countries do not support the use of sludge on fields growing vegetables and fruits, due to the risks posed by heavy metals and pathogens, except for fruit trees and when commercial food crops are grown for animal consumption [45]. For example, Slovenia produces approximately 30,000 tonnes of pre-treated excess sludge [46], of which around 400 tonnes are composted and 600 tonnes are landfilled. Slovenia does not support the application of sludge in agriculture. The remaining sludge is either incinerated (36%) or disposed of through other routes, including export, use in construction materials, or processing for biogas production.

1.1.2.2 Risks related to sludge amendment

Although the Directive aims to prevent harmful effects on soil, vegetation, animals, and humans by controlling heavy metals, it has not been substantially updated in decades, and, among other things, does not include limits for organic micropollutants [41]. A large number of micropollutants are present in sewage sludge and biosolids, including pharmaceuticals, personal care product ingredients, endocrine-disrupting compounds, persistent organic pollutants and microplastics [47-49]. These compounds may leach into the soil and be uptaken by crops, potentially entering the food chain [50]. Long-term applications of sludge can also affect soil chemistry and microbial communities [51]. Surface runoff and tile drainage from sludge-amended soil may convey micropollutants to surface water, posing an acute and chronic risk to aquatic life and groundwater, which serves as a primary source of drinking water, thereby introducing a potential risk to human health [52].

Strategies such as pyrolysis, composting, and biochar stabilisation are being explored to reduce contaminant bioavailability and improve the safety of sludge-derived fertilisers, although operation costs and energy consumption may limit their use [53-55]. Thus, while sludge reuse aligns with the circular economy, its implementation must balance between resource recovery and environmental and health protection.

1.2 Contaminants of Emerging Concern

Contaminants of emerging concern (CECs) are synthetic or naturally occurring substances, either newly synthesised or existing chemicals, that are suspected to be present in environmental compartments for an extended period without adequate monitoring. These contaminants may pose a threat due to their persistence, bioaccumulation, and toxicity, as they can enter the environment through various pathways, such as industrial discharge, agricultural runoff, and improper waste disposal, leading to contamination of air, water, soil, and food [56, 57].

As a group, CECs comprise a broad range of pollutants, including pharmaceuticals and personal care products (PPCPs), hormones, pesticides, artificial sweeteners, industrial additives, and flame retardants, among others. Most of these compounds are discharged as a consequence of human activities, considered a result of the modernised lifestyle and rapid industrialisation. WWTP discharges are considered one of the main pathways through which CECs can get into the environment [58, 59]. Conventional WWTPs are not designed to eliminate CECs, which are frequently detected in both influent and effluent streams, as well as in sewage sludge [60]. Their presence and persistence in the environment, even at trace levels (ng/L to µg/L), are considered a threat to human, animal and ecosystem health, due to their potential toxicity [61].

1.2.1 Legislation

The EU has amended various directives, such as the Water Framework Directive (2000/60) (WFD), together with the Environmental Quality Standards Directive (2008/105/EC) and the Groundwater Directive (2006/118/EC), to ensure surface water bodies achieve good ecological and chemical status [62, 63]. Currently, the WFD includes a list of 45 priority substances that member states are required to monitor in surface waters. The list needs to be reviewed and updated, if necessary, every 6 years. In 2013, the WFD adopted a Watch List mechanism and amended the Water Framework Directive (2000/60/EC) and the Environmental Quality Standards Directive (2008/105/EC).

A Watch List is defined as “a monitoring tool to help identify substances that may pose a significant risk to or via the aquatic environment, but for which existing monitoring data in surface waters are insufficient”. The inclusion of substances is based on the requirements of the Environmental Quality Standards Directive (2008/105). The Watch List is updated every few years, and member states must monitor the listed substances at selected sampling sites, at least once per year for up to four years. Based on the collected data, the EU decides to keep the substance on the Watch List to gather more data. Inclusion can also be based on substances of emerging concern identified based on research projects and literature, as well as recommendations from member states and stakeholders. So far, the Watch List has been updated five times (2015, 2018, 2020, 2022), with the latest update realised in 2025 (Table 1.3) [64-68].

Based on the collected data, the Commission then proposes revisions to the List of Priority Substances. Currently the list includes 25 substances which pose well-documented risks to nature and human health, some of which have been previously included in the

Watch List system including 17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2), acetamiprid, azithromycin, bifenthrin, bisphenol A (BPA), carbamazepine, clarithromycin, clothianidin, deltamethrin, diclofenac, erythromycin, esfenvalerate, estrone (E1), glyphosate, ibuprofen, imidacloprid, nicosulfuron, permethrin, silver, thiacloprid, thiamethoxam, triclosan, per- and poly-fluorinated alkyl substances (PFAS) as sum of 24 and total of active substances in pesticides, including their relevant metabolites, degradation and reaction products. If the proposal is agreed, member states will be required to take measures to reduce the emissions of all these pollutants to meet the quality standards [69].

Table 1.3: Watch List of substances for European Union-wide monitoring.

Watch List	Name of substances	Refs
1st (2015)	17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2), estrone (E1), diclofenac, 2,6-di-*tert*-butyl-4-methylphenol, 2-ethylhexyl 4-methoxycinnamate, erythromycin, clarithromycin, azithromycin, methiocarb, imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetamiprid, oxadiazon, triallate	[64]
2nd (2018)	17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2), estrone (E1), erythromycin, clarithromycin, azithromycin, methiocarb, imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetamiprid, metaflumizone, amoxicillin, ciprofloxacin	[65]
3rd (2020)	metaflumizone, amoxicillin, ciprofloxacin, sulfamethoxazole, trimethoprim, venlafaxine, *O*-desmethylvenlafaxine, clotrimazole, fluconazole, imazalil, ipconazole, metconazole, miconazole, penconazole, prochloraz, tebuconazole, tetraconazole, dimoxystrobin, famoxadone	[66]
4th (2022)	sulfamethoxazole, trimethoprim, venlafaxine, *O*-desmethylvenlafaxine, clotrimazole, fluconazole, imazalil, ipconazole, metconazole, miconazole, penconazole, prochloraz, tebuconazole, tetraconazole, dimoxystrobin, azoxystrobin, famoxadone, diflufenican, fipronil, clindamycin, ofloxacin, metformin, guanylurea, butyl methoxydibenzoyl-methane, octocrylene, benzophenone-3	[67]
5th (2025)	fipronil, clindamycin, ofloxacin, metformin, guanylurea, butyl methoxydibenzoyl-methane, octocrylene, benzophenone-3, octisalate (2-ethylhexyl salicylate), *N*-1,3-Dimethylbutyl-*N*′-phenyl-*p*-phenylenediamine (6PPD), 6PPD-quinon, avermectin B1a, avermectin B1b, bromuconazole, climbazole, cyazofamid, difenoconazole, epoxiconazole, itraconazole, ketoconazole, mefentrifluconazole, propiconazole, triticonazole, etoxazole, fluoxetine, propranolol, oxytetracycline, tetracycline	[68]

1.2.2 Occurrence of CECs in treated wastewater and sludge

Different compounds have been quantified in TWW and sludge in WWTPs worldwide, including PPCPs, pesticides, industrial chemicals, flame retardants and corrosion inhibitors (Table 1.4). Ranges of common CECs in effluent were from a limit of quantification (LOQ, ng/L) to 2.5 µg/L, while for sludge, CEC concentrations range from LOQ (ng/g) to 10 mg/kg [70, 71] based on data from WWTP across different continents, including mainly

Europe (Austria, Belgium, Bosnia-Herzegovina, Czech Republic, Croatia, Cyprus, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovenia, Spain, Sweden, Switzerland, United Kingdom), Asia (China, India, Iran, Japan, Jordan, Kuwait, Malaysia, Singapore, South Korea, Thailand, Turkey), Africa (South Africa, Tanzania, Tunisia), North America (Canada, Mexico, USA), South America (Argentina, Brazil) and Australia.

Table 1.4: Typical ranges of CECs detected in wastewater and sludge reported from different studies worldwide within the last 2 decades.

Compound	**Concentration in wastewater (ng/L)**	**Concentration in sludge (ng/g dry weight)**	**Reference**
17α-ethynylestradiol	0.5-549	<LOQ-17	[72, 73]
β-estradiol	0.53-17.9	9-49	[74, 75]
Amoxicillin	<LOQ-190	<LOQ	[73]
Azithromycin	45.2-597	81-1220	[73, 76]
Bisphenol A	16-1,840	<LOQ-4,700	[73, 77]
Caffeine	30-13,900	<LOQ-805	[73]
Carbamazepine	11-6,940	<LOQ-50	[73, 78]
Ciprofloxacin	43.2-1,100	1,780-160,000	[76, 79]
Clarithromycin	4.5-313	5.6-580	[73, 76]
Diclofenac	42.9-2,090	<LOQ-133	[73, 78]
Erythromycin	17.9-620	6-79	[73, 79]
Estrone	<LOQ-95	70-280	[73]
Ibuprofen	28-10,297	<LOQ-3,988	[73, 78]
Methylparaben	6.1-1,440	15.9–204	[80, 81]
Naproxen	9-5,638	<LOQ -1,022	[73, 78]
Propylparaben	0.5-390	0.5-7.7	[80, 81]
Tonalide	2-7,555	245.7-1,980	[82, 83]
Triclosan	9-6,880	2,000-19,700	[73, 84]

Monitoring studies conducted in Slovenia have confirmed the presence of CECs in wastewater and surface waters, particularly in relation to major treatment plants such as Ljubljana and Domžale-Kamnik, where over 20 CECs have been determined simultaneously [85, 86]. A comparison between influent and effluent samples revealed insufficient removal rates of pharmaceuticals, including carbamazepine, ciprofloxacin, and diclofenac, leading to the occurrence of CEC residues in effluents and surface waters. Compounds that have been detected in effluent of Slovenian WWTPs include caffeine (133-49,600 ng/L), ibuprofen (35.9-6,130 ng/L), naproxen (235-2,190 ng/L), diclofenac (113-4,760 ng/L) and carbamazepine (86.2-5,320 ng/L) [86, 87].

Other compounds quantified in wastewaters around Slovenia included parabens (methylparaben, up to 1,910 ng/L), hormones (E1 up to 1,980 ng/L, E2 up to 713 ng/L) [88] and bisphenols, such as BPA up to 2,620 ng/L, bisphenol S (BPS) up to 435 ng/L, bisphenol AF (BPAF) up to 3.4 ng/L and bisphenol F (BPF) up to 117 ng/L. Bisphenols have also been quantified in anaerobically stabilised sludge in Slovenia, with BPA up to 946 ng/g, BPF up to 83 ng/g and BPS up to 45 ng/g [48], although data for other CECs, such as pharmaceuticals, are limited. Pharmaceuticals have also been quantified in surface waters, including river waters and lakes [88, 89].

These findings can serve as evidence of the persistence of CECs through conventional wastewater treatment and their presence in TWW, which, during reuse for agriculture, can pose a threat. Thus, CECs uptake and their potential accumulation in the food chain, following irrigation with TWW or amendment with sludge, should be examined, as it is one of the major concerns regarding how these practices can affect public health.

1.3 Uptake of CECs Using Treated Wastewater and Sludge

1.3.1 Uptake of CECs using treated wastewater

A literature review on the uptake of CECs in plants following TWW irrigation found a significant increase in the number of publications over the last decade, as retrieved from the Web of Science database (Figure 1.1). A high variability was identified between studies, including uptake and accumulation of CECs in different crops (leafy vegetables, fruit vegetables, root crops), growing conditions (greenhouse, field, and hydroponic systems), and matrices (irrigation with TWW, spiked treated TWW, spiked water, or river water receiving high loads of TWW).

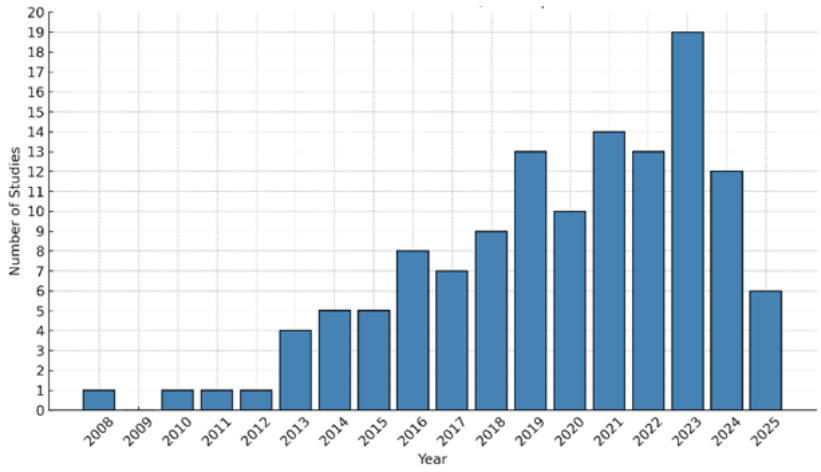


Figure 1.1: Histogram showing the number of publications studying wastewater reuse/CEC uptake based on the Web of Science database screening from 2008 to 2025 (April).

1.3.1.1 Crop types

The most frequently studied category of crop is leafy vegetables, followed by fruit and root vegetables (Figure 1.2). The extensive inclusion of leafy vegetables is due to their wide cultivation, short growth cycles, high transpiration rates and large surface areas that favour contaminant uptake, making them ideal model crops for experimental studies [90]. Individual crops studied across the literature are shown in Figure 1.3.

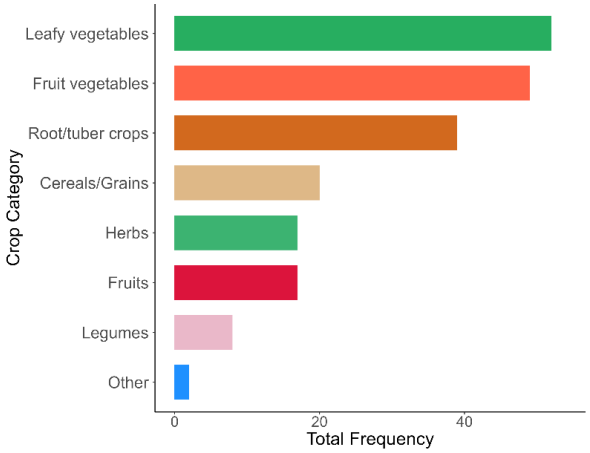


Figure 1.2: Crop categories studied across different publications from 2008 to 2025 (April).

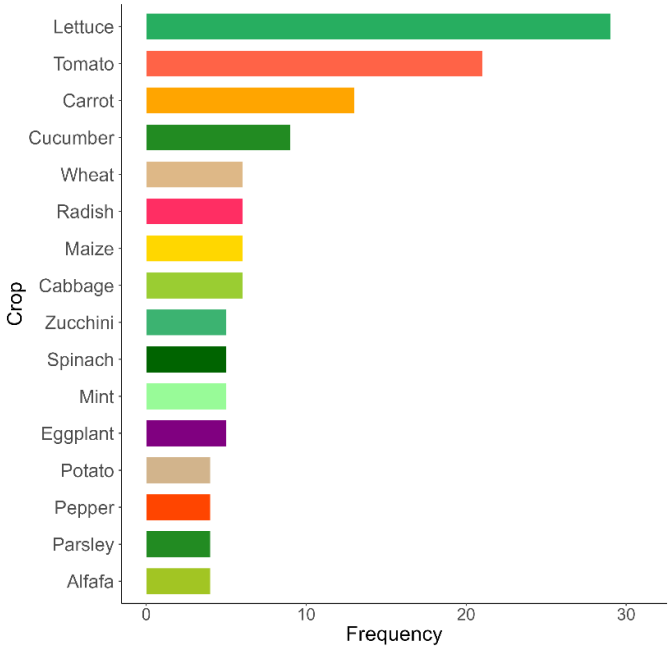


Figure 1.3: Individual crops studied across different publications from 2008 to 2025 (April).

1.3.1.2 Compounds

To date, over 700 individual compounds have been studied, varying in physicochemical properties (e.g., water solubility, acid dissociation constant (pK_a) and logarithm of octanol/water partition coefficient ($\log K_{ow}$)). Among them, the most studied compound was carbamazepine, followed by sulfamethoxazole, caffeine, trimethoprim, diclofenac, naproxen, ibuprofen, gemfibrozil, atenolol and metoprolol, based on their inclusion frequency (Figure 1.4). Although it is known that different CECs are present in TWW, studies usually focus on a limited number of CECs, with approximately 70% of studies investigating the simultaneous uptake of < 20 CECs.

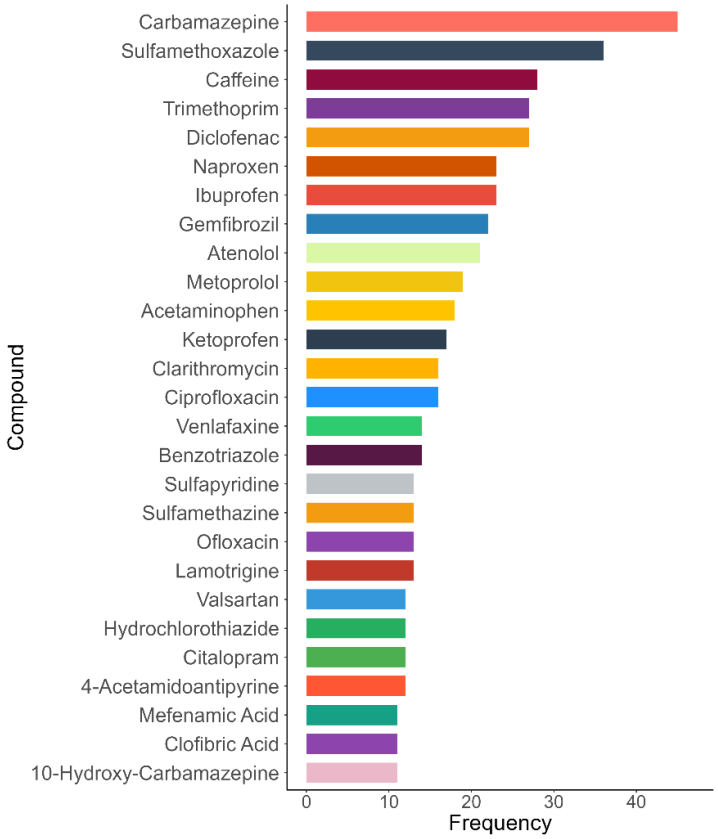


Figure 1.4: Individual studied compounds across different publications publications from 2008 to 2025 (April).

1.3.1.3 Uptake and translocation of CECs in tomato plants

Tomato is one of the most widely produced and consumed vegetable crops worldwide and within the EU [91]. Tomatoes are a good source of vitamins, minerals, and antioxidants, and are consumed both raw and in a wide range of processed products such as sauces, soups, and juices [92]. However, among the available studies evaluating uptake in vegetables, only a limited number of them included tomato either in field conditions (Table 1.4) or greenhouse experiments [93-99]. Among those, only five have evaluated the uptake of CECs in tomatoes under real TWW application scenarios, in Israel, Saudi Arabia and Spain [100-104]. Moreover, uptake experiments typically focus on a single irrigation matrix, such as plants irrigated with TWW. Comparative studies involving different irrigation media, such as potable water (PW) (control), TWW, and spiked media, are lacking. The inclusion of spiked media can guarantee the presence of CECs in sufficient concentrations, unlike TWW. There is still a lack of field studies on different vegetable crops that clearly distinguish between the effects of irrigation type and crop species (Sections 1.3.1.3.1 and 1.3.1.3.2).

The simultaneous determination of CECs in different matrices during the agricultural process (soil, plant parts, water) allows for the estimation of the bioconcentration factor (BCF), where concentrations in plant organs (root, stem, leaf, and fruit) and the top layer of soil are taken into account [105]. The BCF is used to express the ability of a plant to accumulate CECs either as the ratio of concentration in the crop tissue to the concentration in the soil or water (Eq. 1.1 and 1.2). Concentration in water is used when plants are directly exposed to water, or when drip irrigation is applied to soil, where the potential efficiency of water absorbed by plants is assumed to be over 90% [95].

$$BCF \ = \frac{Concentration\ in\ crop\ tissue\ (\mu g/kg)}{Concentration\ in\ soil\ (\mu g/kg)} \tag{1.1}$$

$$BCF\ (L/kg) \ = \frac{Concentration\ in\ crop\ tissue\ (\mu g/kg)}{Concentration\ in\ water\ (\mu g/L)} \tag{1.2}$$

The root concentration factor (RCF) is used to evaluate a plant's ability to accumulate CECs in its roots, employing the same principles as BCF (Eq. 1.3 and 1.4).

$$RCF \ = \frac{Concentration\ in\ roots\ (\mu g/kg)}{Concentration\ in\ soil\ (\mu g/kg)} \tag{1.3}$$

$$RCF\ (L/kg) \ = \frac{Concentration\ in\ roots\ (\mu g/kg)}{Concentration\ in\ water\ (\mu g/L)} \tag{1.4}$$

Also important is the translocation factor (TF), which is used to estimate the translocation of CECs from roots to other plant tissues, including stems, leaves and fruits, depending on the crop type. The TF is calculated as the ratio of the concentration in leaves/stems/fruits to the concentration in roots (Eq. 1.5).

$$TF \ = \frac{Concentration\ in\ leaves, stems, fruits\ (\mu g/kg)}{Concentration\ in\ roots\ (\mu g/kg)} \tag{1.5}$$

1.3.1.3.1 Field studies

Although experiments performed in the field, studying the uptake of CEC in tomato fruits, are limited, they demonstrate that uptake strongly depends on the selected conditions (Table 1.5). The available data indicate that tomato fruits generally accumulate lower concentrations of CECs compared to leafy and root vegetables. Riemenschneider et al. studied the uptake of 28 CECs and their metabolites in different field-grown vegetables (including tomatoes), where the results revealed the highest total concentrations in leafy vegetables, such as parsley (533 ng/g dry weight), lettuce (402 ng/g dry weight) and rucola (247 ng/g dry weight) with reduced uptake in root and fruit-bearing vegetables, respectively [106]. Although different plant parts were analysed including roots, shoots, leaves and fruits, data were not reported for leaves in tomatoes and peppers, preventing an accurate evaluation of the distribution of CECs in the plants. In these vegetables only carbamazepine was quantified in fruits (5 and 8.3 ng/g dry weight, respectively). The authors suggested that the influence of contaminants' physicochemical properties ($\log K_{ow}$, pK_a) and plant specificity should be further studied.

Uptake and translocation in tomatoes have also been addressed by Gatta et al., who studied 22 CECs in tomato and wheat irrigated with freshwater and TWW, over two growing seasons. Eight CECs were found in irrigated soil; however, accumulation in tomatoes was limited, with only three compounds quantified: carbamazepine (in roots, stems, and leaves), fluconazole (in stems and leaves), and flecainide (in roots and leaves), with higher accumulation after the second growing cycle [107].

Christou et al. conducted a three-year study on tomato plants irrigated with TWW, in which diclofenac, sulfamethoxazole, and trimethoprim were measured in the tomatoes. The authors demonstrated that uptake also depends on the duration of irrigation, as diclofenac was detected in tomato fruits after the second growing season of consecutive TWW

application, but not when TWW irrigation was applied for a single growing period. Thus, it is considered that the prolonged treatment with TWW may increase the concentration of CECs in fruits [108].

Table 1.5: Field studies on CECs uptake in tomatoes.

Studied compounds	**Experimental conditions**	**Studied crops**	**Evaluation of translocation**	**Compounds in tomato fruits irrigated with TWW**	**References**
6 pharmaceuticals	TWW	Pepper, tomato, melon, lettuce, watermelon, spinach, carrot	×	-	[109]
3 pharmaceuticals	TWW, consecutive application for 3 years	Tomato	×	Diclofenac, sulfamethoxazole, trimethoprim	[108]
28 CECs (pharmaceuticals, personal care products, industrial chemicals, pesticides)	River water (TWW as a significant source)	Tomato, eggplant, zucchini, pepper, cabbage, lettuce, parsley, rucola, potato, carrot	Roots, leaves, stems	Carbamazepine	[106]
Carbamazepine and 2 metabolites	TWW, TWW with biosolids amendment	Tomato, wheat, lettuce	Leaves	Carbamazepine, dihydroxy-carbamazepine	[110]
56 CECs (pharmaceuticals, personal care products, industrial chemicals)	TWW	Tomato, carrot, lettuce	×	23 CECs	[111]
22 CECs (pharmaceuticals, personal care products)	PW, TWW	Tomato, wheat	Roots, leaves, stems	-	[107]
32 CECs (pharmaceuticals, personal care products, industrial chemicals, PFAS)	PW, TWW	Radish, lettuce, tomato	×	16 CECs	[112]

Among the published studies, TWW was usually the only medium used, without inclusion of potable water (PW) to compare the levels of CECs between the two. Furthermore, only a subset of studies analysed plant compartments; thus, the information on the translocation of CECs within tomato plants remains limited. Additionally, soil contamination was shown to be a side effect of TWW reuse in agriculture. This fact was highlighted by Fatta-Kassinos et al., who found that soils irrigated with wastewater have higher contaminant levels than non-treated soils, which points to the potential accumulation of CEC in soil from TWW reuse [27, 113].

1.3.1.3.2 Soil-based studies including spiked media

The uptake in plants irrigated with spiked media (spiked potable water or spiked wastewater) in field- or greenhouse-grown tomatoes is also addressed in available studies (Table 1.6). Such studies are helpful for further understanding the distribution of CECs across plant parts, as TWW may contain low concentrations of CECs, which can result in uptake at concentrations below the limit of detection (LOD) and LOQ. Because the CEC content in TWW varies considerably, spiking the irrigation media with known concentrations of target compounds allows researchers to achieve measurable uptake and to relate translocation and bioaccumulation patterns to the physicochemical properties of the CECs.

Table 1.6: Studies on CECs uptake in tomatoes with spiked media.

Studied compounds	**Experimental conditions**	**Studied crops**	**Evaluation of translocation**	**Compounds in tomato fruits irrigated with TWW**	**References**
30 CECs (pharmaceuticals, pesticides)	Spiked TWW (1 µg/L)	Tomato	Roots, leaves, stem	19 CECs	[95]
19 CECs (pharmaceuticals, personal care products)	TWW, spiked TWW (0.25 µg/L)	Carrot, celery, lettuce, spinach, cabbage, cucumber, pepper, tomato	×	Carbamazepine	[114]
10 CECs (pharmaceuticals)	PW, spiked TWW (200 & 600 µg/L)	Tomato	Roots, leaves, stem	Carbamazepine, metoprolol, clarithromycin, ketoprofen, fluconazole, naproxen	[115]
Triclosan	PW, spiked PW (0.015, 0.15, and 1.5 µg/L)	Onion, tomato	Roots, shoots	Triclosan	[116]
5 CECs (antibiotics)	Spiked TWW (2 and 20 µg/L)	Lettuce, carrot, tomato	Roots, leaves, stem	Tetracycline, norfloxacin, chloramphenicol	[117]
Diclofenac, sulfamethoxazole, trimethoprim	Nutrient solution, spiked nutrient solution (10 µg/L)	Tomato	×	Sulfamethoxazole, trimethoprim	[118]
Abacavir, nevirapine, efavirenz	Spiked PW (2000 and 5000 µg/L)	Beetroot, spinach, tomato	Roots, leaves, stem	Abacavir, nevirapine, efavirenz	[119]
11 CECs (pharmaceuticals, PFAS)	PW, spiked PW (5 µg/L)	Radish, lettuce, tomato	Roots, leaves, stem	10 CECs	[120]

Wu et al. studied the uptake of 19 PPCPs in eight vegetables, including tomato irrigated with spiked water (0.25 µg/L), and reported a general accumulation order of root > leaf > stem > fruit for the studied crops [114]. The study revealed that carbamazepine,

a recalcitrant compound in wastewater treatment processes, was detected in all edible plant parts. It was also the only compound quantified in tomato fruits irrigated with spiked TWW. Denora et al. evaluated the uptake of 10 CECs in tomatoes irrigated with wastewater spiked with CECs (0.6 mg/L) and identified clarithromycin, carbamazepine, ketoprofen, metoprolol, fluconazole, and climbazole in tomato fruits and plant parts [115]. The study suggested that even at high concentrations, specific compounds (naproxen and diclofenac) may still not be present in plants or soil due to degradation, either in the soil or the irrigation media.

Irrigation with spiked TWW (1 µg/L) was used to test the uptake and translocation of 30 CECs in tomato plants by Bueno et al., who identified 19 compounds; 7 pharmaceuticals (caffeine, hydrochlorothiazide, 4-formylamino-antipyrine, epoxide-carbamazepine, carbamazepine, furosemide, diclofenac) and 12 pesticides (pymetrozine, thiamethoxam, imidacloprid, acetamiprid, thiacloprid, carbendazim, diuron, thiabendazole, azoxystrobin, fluxapyroxad, myclobutanil, diazinon) in the fruits and reported higher CEC content (in µg) in the fruits compared to leaves and roots [95]. Pan et al. also reported a distribution of tetracycline, norfloxacin and chloramphenicol as fruit > leaf/shoot > root. The trend was opposite for sulfamethazine and erythromycin, with root > leaf/shoot > fruit, suggesting that uptake is concentration-dependent, as indicated by the comparison of the two different spiking levels [117].

In general, using spiked media tends to provide less insight into realistic irrigation scenarios, and the range of compounds evaluated remains limited. As in field applications, the selected irrigation media studied simultaneously are limited, since studies either included only a single spiked condition (e.g., TWW spiked) or limited comparable treatments (PW and TWW without further spiking). Similarly, the irrigation media included in most studies are restricted, often focusing on a single spiked condition (e.g., TWW with added CECs) or only a few comparable treatments (e.g., PW and TWW), which limits the ability to assess differences under practical field conditions. Although spiked media may lead to overestimation of exposure, they can be used to study CECs uptake mechanisms.

1.3.1.3.3 Uptake during real TWW applications in soil-grown tomatoes

As mentioned, only a few studies included sampling from regions where TWW is systematically used for irrigation, such as Spain and Israel. Mordechay et al. evaluated the presence of CECs in 445 commercial fields irrigated with reclaimed wastewater (2-35 years), where leafy greens, carrots, potatoes, bananas, tomatoes, avocados, and citrus fruits are grown. The study aimed to quantify sixty-five CECs and reported the presence of venlafaxine, gabapentin, caffeine, carbamazepine, and its two metabolites (epoxide-carbamazepine and dihydroxy-carbamazepine) in tomato fruits, with concentrations up to 10 ng/g. The data indicate that soil can act as a "sink" for CECs, following continuous TWW application [102].

A field study in the peri-urban area of Barcelona (Spain) evaluated the uptake of 33 CECs in lettuce, tomato, cauliflower, and broad beans, following the irrigation with well water, compared with river water receiving discharges from 80 WWTPs [121]. Five compounds were detected in tomato fruits, including dimethomorph, bisphenol F (BPF), carbamazepine, methylparaben and tris(2-chloroethyl) phosphate (TCEP). However, the presence of specific CECs in tomatoes grown in peri-urban areas was not attributed solely to TWW irrigation, but rather to the direct application of fungicides (dimethomorph), atmospheric deposition of TCEP on the vegetable surface and the leaching of BPF from plastic pipelines [100]. Another study from a different region of Spain, Almeria, where TWW is applied for crop irrigation in greenhouses, investigated 293 substances. Out of

those, 44 CECs were quantified in irrigation water, while carbamazepine, lidocaine and caffeine were found in cherry tomatoes [103].

These studies, in which crops were sampled from fields or greenhouses systematically irrigated with TWW, supported the finding that although pharmaceuticals and other CECs can be found in edible parts, the quantities are below the risk threshold. A further observation, when comparing the results among different applications, is that CEC uptake depends on various factors, including CEC concentration in irrigation water, soil properties, and plant type.

1.3.1.3.4 Uptake of CECs in hydroponically grown tomatoes in spiked substrate

Hydroponics is a method of growing plants without soil, using mineral nutrient-rich solutions dissolved in water [122]. Experimentally, hydroponic systems offer better repeatability for evaluating the uptake and stress response of plants. Hydroponic conditions enable precise control over the amounts of nutrients and parent compounds, and plants are directly exposed to contaminants, unlike in soil experiments. So far, hydroponic experiments can be separated based on the cultivation solution (plants in spiked solution, in wastewater or spiked wastewater) or plant type, such as edible crops or plants that may be used in constructed wetland systems to remove a variety of contaminants [123, 124]. Several studies on hydroponically grown plants are available, evaluating the uptake of industrial chemicals, PPCPs, and endocrine disrupting chemicals (EDCs). However, fruit-bearing plants are less studied compared to leafy vegetables (Table 1.7), and to our knowledge, no studies to date have investigated CEC uptake in tomatoes grown hydroponically with TWW.

Although the hydroponics setup offers controlled cultivation conditions, the duration of experiments usually remains short (3-6 weeks), as this monitoring period is sufficient to measure the uptake of CECs in plant parts; however, there remains a lack of data on fruit accumulation for plants such as tomatoes. Lu et al. evaluated the uptake and translocation of BPA and nonylphenol in tomatoes and lettuce grown in a CEC-spiked nutrient solution. In the tomato plants, the distribution within the plant showed higher accumulation in the roots, followed by the fruits, leaves, and stems for both compounds. The exposure through roots was also compared with foliar application. Foliar application resulted in higher accumulation of compounds in tomato leaves compared to roots, suggesting that during TWW application, contact of the irrigation media with aerial plant parts should be minimised [125].

Dodgen et al. studied the uptake of 16 PPCPs/EDCs in carrots, lettuce, and tomatoes, and reported that based on TFs, non-ionic compounds accumulated in root tissues without further translocation, while in tomato leaf tissues, cationic and neutral compounds were more prevalent (carbamazepine, meprobamate) [126]. However, it was emphasised that further studies, including those on edible portions of crops, are necessary to understand the accumulation in plants.

Experiments with hydroponically grown plants typically include only spiked nutrient media, which often overestimate the plant's exposure to CECs due to the addition of high concentrations of added CECs and the absence of a complex matrix, such as TWW. This approach, though, can provide valuable insights into translocation and how it is affected by compound physicochemical properties (such as ionisation state). Most studies neglect to test TWW and instead focus solely on the nutrient solution, so the acquired results may not accurately reflect the potential matrix interaction affecting CEC uptake.

Table 1.7: Studies on CECs uptake, including hydroponically grown tomatoes.

Studied compounds	**Experimental conditions**	**Studied crops**	**Plant parts tested**	**Compounds in tomato fruits grown in spiked media**	**References**
Carbamazepine and 13 carbamazepine metabolites	Nutrient solution + spiking at 500 µg/L	Tomato	Roots, leaves, stems, fruits	Carbamazepine and 11 carbamazepine metabolites	[127]
16 CECs (pharmaceuticals, personal care products, endocrine-disrupting chemicals)	Nutrient solution + spiking at 1 µg/L	Carrot, lettuce, tomato	Roots, leaves	-	[126]
Nonylphenol, BPA	Nutrient solution + spiking at 50 µg/L	Lettuce and tomato	Roots, leaves, stems, fruits	Nonylphenol, BPA	[125]
7 CECs (pharmaceuticals, personal care products, hormones, industrial chemicals, insect repellents)	Nutrient solution + spiking at 4 mg/L	Tomato, corn and wheat	Roots, leaves, stems	-	[128]
11 CECs (pharmaceuticals, PFAS)	Nutrient solution + spiking at 5 µg/L	Lettuce and tomato	Roots, stems	-	[129]

1.3.1.3.5 Studies evaluating metabolites and transformation products

So far, few studies have investigated the uptake of parent compounds, as well as metabolites and transformation products. Martínez-Piernas et al. have applied a novel analytical workflow based on target analysis (60 compounds) and suspect screening for over 1300 compounds in tomato crops irrigated with reclaimed water. The study detected eight contaminants in fruits (five of which were detected for the first time) and in plants (namely 4-acetylaminoantipyrine, mepivacaine, paraxanthine, tramadol, and venlafaxine) [94]. The same authors have also conducted a further study on suspect screening on 262 transformation products (TPs) from 20 parent CECs, in reclaimed water, soil, and tomato samples from four production greenhouses. Eighteen TPs were tentatively identified in reclaimed water, soil and tomato leaves in field samples, while no transformation products

were detected in tomato fruits. The results stressed the wide variety of CECs and respective transformation products that may be present in wastewater [130].

Uptake of carbamazepine and the presence of its metabolites were evaluated by Riemenschneider et al. in hydroponically grown tomato plants using a spiked nutrient solution. Carbamazepine, a common compound included across different experimental designs, was transformed into its respective metabolites by 33%, with 11 phase-I and 10 phase-II metabolites identified, with the highest ratio observed in fruits and leaves [127]. Metabolites of three non-steroidal anti-inflammatory drugs (ketoprofen, naproxen and mefenamic acid) were tested in a fast-screening approach by Mlynek in eleven edible plants, including tomatoes, grown hydroponically in spiked media. The study tentatively identified five naproxen, eight ketoprofen and 11 mefenamic acid metabolites in tomatoes [131]. These studies indicate a shift toward broader monitoring efforts to enhance understanding of previously uncharacterised CECs and their metabolites. Accordingly, the potential of non-target approaches to elucidate CEC metabolic pathways, transformations, and to postulate the structures of metabolite/transformation products has yet to be fully applied in TWW reuse studies.

1.3.2 Uptake of CECs during sludge amendment

The uptake of CECs in edible crops following sludge amendment has been evaluated in various publications, focusing on the application of sludge, spiked sludge, or spiked soil (Fig. 1.5). The establishment of regulations for water reuse could be the driver for the increase in studies on TWW reuse. In contrast, the regulatory framework for sludge has remained unchanged since its adoption in 1986, resulting in relatively stable research activity on sludge-amended crops, which also depends strongly on national legislation (1.1.2.1).

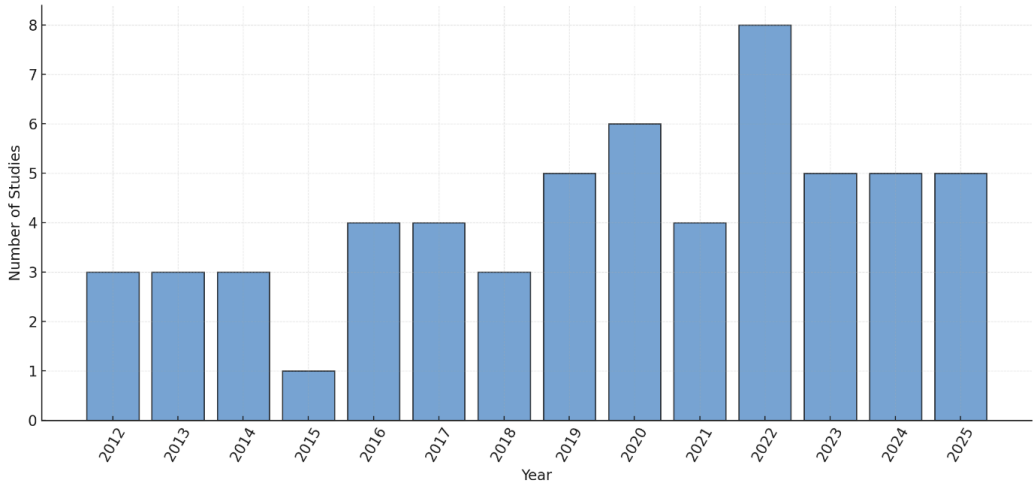


Figure 1.5: Histogram showing the number of publications studying sludge amendment/CEC uptake based on the Web of Science database screening from 2012 to 2025 (April).

Similarly, as in TWW reuse studies, the most studied category was leafy vegetables. Lettuce was the most studied crop, followed by wheat, radish, and tomato. Regarding the evaluated CECs, most studies have addressed only a limited number of compounds (≤ 8 studied simultaneously). Triclosan and triclocarban were identified as the most studied compounds in sludge amendment experiments, since both are consistently detected in sewage sludge [47, 132].

1.3.2.1 Uptake and translocation of CECs in tomato plants

Only a few studies so far (Table 1.8) have examined the translocation of CECs in tomatoes grown in soils amended with sludge/ biosolids (definitions provided in Section 1.1.2) [133, 134]. PFAS have also been studied in tomato plants amended with biosolids, with higher translocation of short-chain ones to aerial tissues [135-137]. Ramos et al. studied 11 musks and six UV-filters in tomato fruits amended with commercially available biosolids and spiked biosolids. They found that the selected compounds bioaccumulated in tomato fruits (1-147 ng/g dry weight) and that a potential health risk is minimal. However, mass balance calculations showed a loss of compounds, which was attributed to potential metabolism, degradation, volatilisation, and accumulation in other tomato tissues, although the rest of the plant parts were not analysed [134].

Table 1.8: Studies on CECs uptake, including tomatoes grown in sludge-amended soil.

Studied compounds	Experimental conditions	Amendment rate	Studied crops	Evaluation of translocation	Compounds in tomato fruits	References
Carbamazepine, 10,11-epoxide-carbamazepine, dihydroxy-carbamazepine	Composted biosolids	60 m^3/ha	Tomato, lettuce, wheat	Leaves	Carbamazepine, dihydroxy-carbamazepine	[110]
Triclosan and triclocarban	Dewatered anaerobically digested biosolids	8 Mg/ha	Carrot, cucumber, tomato, green bell pepper	×	Triclocarban	[132]
19 CECs (musks and UV filters)	Composted biosolids (spiked up to 500 ng/g)	25 tons/ha	Tomato	×	15 CECs	[134]
Carbamazepine, diphenhydramine, triclocarban	Spiked biosolids (100 ng/g)	1.04 kg/m^2	Pepper, tomato, collard, lettuce, radish	Root, shoot	Diphenhydramine, triclocarban	[133]
30 PFAS	Biosolids	9 g per kg of soil	Tomato	Leaves	-	[135]
49 CECs (PFAS, PBDEs, dechloranes)	Anaerobically digested thermally dried sludge	150 kg N/ha	Spinach, tomato	Roots, leaves, stems	21 PFAS and PBDEs	[136]
12 PFAS	Biosolids	12.5-50 Mg/ha	Lettuce, tomato, corn	×	7 PFAS	[137]
141 CECs (PPCPs, hormones, transformation products)	Dewatered municipal biosolids	8000 dry kg/ha	Barley, carrot, potato, sweet corn, tomato	×	6 CECs	[138]

One study with an extensive target list of CECs was conducted by Sabourin et al., who investigated the growth of four different edible crops (tomato, potato, carrot, and sweet corn) in soil fertilised with dewatered municipal biosolids, characterised as "normal farming conditions". Crops were analysed for 141 CECs (PPCPs or PPCP transformation products, six parabens, and 17 hormones or hormone transformation products). Out of the evaluated compounds, only six (atenolol, *N,N*-diethyl-meta-toluamide, minocycline, sulfamerazine, testosterone, trimethoprim) were detected in tomatoes harvested one year after biosolids application, suggesting a low risk of CEC uptake under the proposed conditions, which differs from the outcomes of most other studies [138].

Studies also showed that uptake and translocation do not depend only on a CEC's physicochemical properties (e.g., $\log K_{ow}$, sorption affinity) but also on soil characteristics, e.g., pH, $CaCO_3$ content, organic matter and cation exchange capacity [50, 139], as well as sludge properties including pH, total organic carbon, TSS and potentially toxic elements content [140, 141]. Wu et al. evaluated the uptake of carbamazepine, diphenhydramine, and triclocarban by crops grown in soil amended with aerobically treated biosolids. Triclocarban was present in root>shoot>fruit in tomato plants, while carbamazepine had the highest concentrations in the shoots, with relatively low concentrations in fruits [133].

1.3.2.2 Studies with simultaneous application of sludge amendment and TWW irrigation

A limited number of studies have evaluated CEC uptake under experimental conditions where TWW and sludge are applied simultaneously or individually to compare them. Ben-Mordechay et al. showed that carbamazepine was taken up by various vegetables, including tomatoes, under all three scenarios: TWW irrigation alone, biosolids amendment alone, and the combined application of biosolids with TWW [110]. However, the amount quantified in tomatoes irrigated with TWW was higher than that in tomatoes grown with sludge amendment. When both sources were applied together, sludge acted as an additional organic phase in the soil, hypothesised to increase sorption sites, thus reducing the bioavailability of carbamazepine compared to application of TWW alone.

Apart from carbamazepine, which remains bioavailable mainly in TWW, other pharmaceuticals may adsorb onto biosolids or sludge, reducing their bioavailability to plants. However, because existing studies vary in the CECs analysed and in the rates of sludge or wastewater application, further research is needed to clarify how the exposure route (via TWW or sludge) influences the bioavailability of CECs and the associated risks.

Overall, the literature demonstrates that plants, including tomatoes, can take up a wide variety of CECs from both TWW and sludge. The extent of uptake depends on crop type, compound properties, irrigation or amendment conditions, and plant metabolic processes, which can transform CECs and influence their bioavailability and accumulation in edible tissues. Despite the growing number of studies, most focus on a limited set of CECs and often neglect sampling of specific plant parts (e.g., edible tissue), which limits the understanding of CEC translocation. Furthermore, the inconsistency in the selection of irrigation or amendment matrices across studies hampers direct comparisons and a comprehensive evaluation of exposure pathways.

1.4 Methodological Approaches for the Determination of CECs in Uptake Studies

1.4.1 Sample preparation

1.4.1.1 Treated wastewater

1.4.1.1.1 Sample pretreatment

Sampling of wastewater is a crucial step in studies involving CECs. The most common sampling technique is grab sampling [103], which involves taking a sample at a specific time and point, usually at the beginning of the experiments, followed by periodic sampling at specified intervals, most often weekly [107]. Another approach is to sample directly from the drip outlets when drip irrigation is applied [95]. Sampling at multiple timepoints serves to evaluate the effect of storage on water quality, a common practice during reuse, as TWW must be transported and stored before reuse [142, 143].

Pre-treatment of wastewater samples is also very much compound-dependent and typically involves filtration or centrifugation, to remove suspended substances (including suspended soils, microorganisms and colloids) [144, 145]. Similarly, the choice of filter depends on the type of analyte and should be optimised to minimise losses. Suitable filters include glass microfiber filters, nylon filters, cellulose acetate filters, and nitrocellulose filters, with two consecutive filtration steps often employed [146]. Adjustment of pH may also be necessary to prevent analyte degradation by microorganisms present in the samples [147, 148].

Depending on the studied CECs, e.g., pharmaceuticals, the addition of preservatives may also be necessary to prevent microbial degradation, such as formaldehyde and sodium azide [149, 150], and/or antioxidants (ascorbic acid, sodium thiosulfate) [151, 152]. The use of EDTA, a chelating agent, added to samples at a final concentration of 0.1-5% (g/L) is also reported. Its addition can improve extraction efficiency (EF) by preventing the formation of complexes between pharmaceuticals and metals in water [153-155].

Internal standards are chemical compounds added to samples or sample extracts in a known quantity to verify the correct execution of the analytical protocol. An isotopically labelled internal standard is a compound, usually with the same chemical structure and elemental composition as the target analyte. Still, one or more of the atoms of the molecule of the target analyte are substituted by isotopes. The addition of internal standards before extraction (procedural) is used to correct for errors arising from various steps in the method, including sample preparation and analysis, and to compensate for matrix effects that can influence the measurement of extracted compounds (further explained in Section 1.4.3). An internal standard can also be added to the final extracts to assess variability in injection volume and instrument response.[156, 157].

1.4.1.1.2 Sample extraction

A common step in sample preparation is the pre-concentration of analytes through sample extraction. Among the available techniques, liquid–liquid extraction (LLE) separates analytes based on their partitioning between two immiscible liquid phases, and solid-phase extraction (SPE) isolates analytes on a solid sorbent from the liquid matrix. Compared to LLE, SPE offers higher analyte recovery and pre-concentration capability, while using smaller amounts of organic solvents, making it the most commonly employed technique for determining organic contaminants in wastewater analysis [158]. Reversed phase (RP) is the most common choice for SPE sorbents, with Oasis Hydrophilic–Lipophilic Balanced (HLB)

being the most used sorbent (60 mg and 200 mg) due to good extraction efficiency, stability in wide pH ranges and retention of acidic, basic, and neutral analytes [145, 159]. At the same time, Oasis mixed-mode cation exchange (MCX), weak anion exchange (WAX), and weak cation exchange (WCX) are also reported in the literature to cover the range of different physicochemical properties of selected analytes [160-162]. For instance, Qian et al. performed a non-target analysis on influent and effluent wastewater from 16 different WWTPs and utilised three types of cartridges: Oasis HLB, WAX, and WCX, to achieve the highest overall recoveries for unknown emerging pollutants [163].

Alternative methods are currently prioritised to develop more environmentally friendly protocols, according to Green Analytical Chemistry (GAC) rules [164, 165]. Micro-SPE (µ-SPE) and dispersive micro-SPE (D-µ-SPE) can be employed, allowing for the use of alternative materials to enhance the sensitivity and efficiency of extraction techniques while adhering to green sample preparation principles [165]. Materials that can be used as green SPE sorbents include metal-organic frameworks (MOFs) [166, 167], molecularly imprinted polymers (MIPs) [168], carbon nanotubes (CNTs) [169], graphene-based materials [170], and other polymeric sorbents [171]. The option to modify the surface of materials through chemical or physical functionalization can facilitate the extraction of various analytes. So far, these advanced SPE sorbents have been successfully applied to environmental matrices, but usually focus on environmental water samples (e.g., drinking water, lake water) rather than wastewater, with limited target compounds [172, 173].

1.4.1.2 Sludge

Sewage sludge is a more challenging matrix than wastewater due to its heterogeneous and chemically complex composition. Sludge contains components derived naturally (lipids, humic substances) or from processing (surfactants, ferric chloride, polymeric colloids, or lime) that are potential interferences for all stages of the analytical process, but mainly during clean-up procedures [140, 174]. Pretreatment of sludge may vary, depending on the type of sludge (primary sewage sludge, activated sludge, digested sewage sludge, or treated sludge) [36]. For example, primary or secondary sludge may require centrifugation or filtration before extraction [175]. The most common storage technique is application of freeze-drying, while air or oven drying, and direct storage at 4 °C or -20 °C have also been reported. Freeze-drying is preferred because it is time-efficient, ensures minimal loss of analytes, and requires small sample amounts. However, the effect of different pre-treatment approaches on sludge has not been extensively studied [176]. Additional steps included homogenization with a mortar and pestle and sieving through a mesh [177].

The most commonly applied extraction techniques are ultrasound-assisted extraction (UAE), pressurised liquid extraction (PLE) and Soxhlet with organic solvents or mixtures with an aqueous solvent [178]. The UAE is considered the most common technique, as it is preferable to traditional extraction methods like Soxhlet extraction, since it requires less extraction time and solvent volume [179, 180]. PLE, also known as accelerated solvent extraction (ASE), is an automated extraction method that utilises solvents at high pressure (up to 200 bar) and temperature (up to 200 °C). The increased extraction temperature can promote analyte solubility and mass transfer rate, while it can be optimised based on the extraction solvent, pressure, temperature, number and time of cycles [181]. An additional clean-up step may be necessary to reduce potential interferences due to matrix complexity, rather than simply diluting the extracts [182]. The most common clean-up procedure is SPE, since it offers a great variety of sorbents, while for CECs extraction, reversed-phase SPE with Oasis HLB was the most preferred [183, 184].

There is also a trend toward applying environmentally friendly (green chemistry) techniques for the extraction of solid sludge samples, such as matrix solid-phase dispersion

(MSPD) [185], Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) [186] and microwave-assisted extraction (MAE) [187, 188]. MSPD does not require expensive instrumentation and requires moderate consumption of organic solvents, although extraction conditions (sorbent and solvent) need to be optimised to achieve acceptable recoveries [182]. QuEChERS combines salting-out LLE and dispersive SPE (d-SPE) as a clean-up, offering good recoveries and high selectivity through a low time-consuming procedure [178], while MAE can be automated with a shorter extraction time compared to other techniques, such as UAE [187].

Alternative methods that have been used for CECs extraction include pressurised hot water extraction (PHWE) for pharmaceuticals [189], headspace solid-phase microextraction (HS-SPME) for volatile organic pollutants [190] and hollow fibre liquid-phase microextraction (HF-LPME) for non-steroidal anti-inflammatory drugs [191]. Their application can limit the use of organic solvents, as it eliminates the need for further clean-up steps.

Overall, although sewage sludge is a complex matrix, different methods can be adopted, depending on the selected analytes, to achieve high recoveries, minimise matrix effects, and improve detection.

1.4.1.3 Fruits and plant parts

Across the reviewed uptake studies, sampling protocols were designed to minimise contamination and allow the accurate quantification of CECs in plant tissues. In most experiments, crops were harvested once they reached marketable size and physiological maturity. For instance, lettuce, carrots, and tomatoes were typically collected 30-120 days after sowing/planting, depending on the crop type, with roots, shoots, and fruits separated [117, 129, 192, 193]. Cleaning plants with water rinsing typically included sequential washes with distilled or deionised water, followed by blotting and drying [194, 195]. For soil-grown plants, thorough rinsing of the roots is necessary to remove any remaining soil particles. In the case of hydroponically grown plants, the roots have to be washed multiple times to ensure the removal of spiked nutrient solution from the root surface [47, 196, 197].

After fruits and plant parts are harvested, freeze-drying is the most common pretreatment technique used prior to grinding and storage [110, 198, 199] to remove water, preserve analytes, and produce a homogeneous matrix for extraction [200]. Freeze-drying is preferred since it ensures high efficiency in water removal while retaining the CECs content and stops metabolic reactions [200]. Some studies also used oven drying at 40 °C [201, 202], drying with liquid nitrogen [100, 203] or natural drying [47, 204]. However, these approaches may lead to analyte losses [205].

After drying, samples were stored at room temperature (freeze-dried), or frozen, commonly at -20 °C or -80 °C [47, 206-208], until analysis. Direct storage of fresh plants at -20 °C before analysis can also be selected to process fresh tissues [94, 134, 209]. To ensure representative sampling, composite samples generated from multiple plants are commonly prepared [100, 210].

The extraction of CECs from fruits and vegetables has been studied extensively in the literature over the last decade to evaluate food quality [211-213]. Additionally, sample extraction and clean-up steps are required to determine the presence of CECs in plants, at low concentrations of analyte residues (mainly in the ng/g dry weight range). Compared to other samples, the sample preparation of plant materials may be challenging due to the presence of pigments, fats, and waxy materials, which can lead to a higher matrix effect [214].

Among the available studies, solid-liquid extraction (SLE) approaches were reported more often. In most cases, plant tissues were extracted using sonication, which employs

ultrasonic vibration to ensure high proximity between the sample and solvent (methanol, acetonitrile, acetone, ethyl acetate, etc. and mixtures with water) [214]. Sonication is usually preferred over PLE since using high temperatures and pressure may result in the degradation of thermally unstable CECs [215]. Other methods reported in the literature include those employed for other solid samples (e.g., sewage sludge), such as MSPD, MAE, and PHWE [216]. SPE is used as a purification step to achieve further clean-up and preconcentration of analytes. HLB cartridges are typically used to purify plant extracts [194, 217], while the use of MCX cartridges has also been reported for the extraction of paracetamol [195] and beta blockers [139].

QuEChERS, which is an official method for pesticide extraction from food matrices, can also be used for the determination of CECs in plant materials [103, 218]. The use of primary-secondary amine (PSA), C-18, and graphitised carbon black (GCB) sorbents during the d-SPE step can effectively remove matrix interferences. However, purification sorbents and their respective quantities should be chosen based on optimisation, as they may have a strong affinity for specific CECs, resulting in low recoveries [219]. The QuEChERS procedure can further be improved by introducing automated workflows, such as automatic micro-solid-phase extraction, to improve robustness and repeatability [220, 221].

1.4.1.4 Soil

Soil can be extracted with similar methods already mentioned for plant parts or sludge. Suggestions regarding storage of soil include amber glass containers to avoid interaction of CECs with plastic and photodegradation [222]. Compared to aqueous matrices, storage time is generally considered less critical for soils; nevertheless, samples should be kept at low temperature and protected from light to limit microbial activity and transformation of CECs. Accordingly, soil samples are typically stored refrigerated (4 °C) for short periods or frozen (-20 °C or lower) until analysis [223]. Prior to extraction, soil samples should be homogenised and sieved. In many studies, air-drying or freeze-drying is performed to reduce the moisture content and improve sample consistency. However, excessive heating should be avoided, since it may lead to degradation or volatilisation of thermally labile compounds [224].

Sample extraction protocols reported in the literature mainly include UAE, PLE, MAE and different variations of SLE, such as sonication, QuEChERS and MSPD. The majority of techniques can successfully extract the desired CECs; however, a high proportion of matrix components may also be extracted, including humic and fulvic substances, pigments, carbohydrates, proteins and lipids, which can increase matrix interferences and reduce sensitivity [225]. As in the other matrices, purification of extracts with SPE using HLB sorbent is the most common technique employed [108, 226], since it is advantageous for both polar and nonpolar CECs, while a combination of Strong Anion Exchange (SAX) and HLB [192] has also been reported for the extraction of antibiotics, and Florisil for the extraction of musks [227]. However, these multi-step procedures can be time-consuming, requiring the use of large volumes of organic solvents, which highlights the need to apply green chemistry principles to develop faster, simpler, and more environmentally friendly extraction and cleaning alternatives [228].

1.4.2 Instrumental methods

The primary technique for determining organic contaminants in various matrices involves chromatographic separation coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS). Liquid chromatography (LC) is the preferred method for

separating CECs, covering a wide range of polar and less volatile compounds [94, 95, 100, 210], compared to gas chromatography (GC), which is mainly suited for volatile compounds or CECs that can be derivatised [229, 230]. High-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) have been employed to analyse organic compounds, primarily utilising reverse-phase chromatographic columns. While both techniques rely on the same principles, UPLC uses columns packed with much smaller particles (<2 µm) and operates at substantially higher pressures than HPLC, allowing faster separation and increased sensitivity.

Both LC and GC are typically coupled to MS or MS/MS to determine emerging contaminants in environmental samples during target analysis, with triple quadrupole (TQ) being the most common type of MS [165]. Electrospray ionisation (ESI) is the most frequently used ionisation technique, as it allows mild ionisation of target analytes [175, 231]. Atmospheric pressure chemical ionisation (APCI) is recommended for nonpolar compounds [232]. To improve compound ionisation, the inclusion of chemical additives in mobile phases is suggested, such as formic acid, acetic acid, ammonium formate and ammonium fluoride in concentrations ranging from 0.01% to 0.1% [233].

Mass spectrometric detection can be performed in selected reaction monitoring (SRM) or multiple reaction monitoring (MRM), where the latter offers improved detection and quantification in complex environmental matrices [93, 234]. In SRM, only one transition (a product ion) is monitored for each analyte, whereas MRM monitors two or more ion transitions, providing both quantification and confirmation. Thus, MRM can improve selectivity and quantification accuracy in complex matrices such as wastewater.

High-resolution mass spectrometry (HRMS) is a powerful analytical technique that allows characterisation of complex environmental mixtures by acquiring full-scan data with high mass accuracy and exceptional resolving power [235]. These capabilities enable the distinction of ions with very similar m/z values, a critical feature for reliable identification in complex matrices such as TWW, sludge, and soil. Instruments such as quadrupole time-of-flight (QTOF) and Orbitrap (Q-Orbitrap) provide the resolution and exact-mass capability needed to detect low-molecular-weight contaminants. These features make HRMS particularly valuable for non-target and suspect screening, enabling the detection and identification of known targets, suspected compounds, and previously unknown transformation products or metabolites [235]. By determining the presence and fate of these compounds in the environment, TWW, sludge, and soil, HRMS facilitates a deeper understanding of their environmental degradation and potential uptake by crops.

1.4.3 Method validation

To ensure the quality of an analytical method and the integrity of the results obtained, method validation is required. Validation can be based on different guidelines, from Eurachem, Directorate-General for Health and Food Safety (DG-SANTE) and International Council for Harmonisation [236-238] depending on the scope and type of the analysis, but it usually includes similar parameters, such as linear range, recovery, sensitivity, selectivity, precision, LOD and LOQ.

Calibration curves are created to establish a correlation between the instrumental response and the concentration of the analyte in the sample, allowing for subsequent analyte quantification. Different approaches can be used, depending on the sample. External calibration is the simplest method among the available options, as it only requires the addition of an analyte standard in neat solvent. Matrix effects, primarily matrix-induced ion suppression or enhancement, can affect analyte signal and lead to inaccurate determination of concentration [239].

In matrix-matched calibration, analytes are added to extracts of a blank matrix. By using a matrix-matched calibration, matrix effect can be compensated on chromatographic and MS instruments, while accuracy can be improved as the calibration standards closely mimic the sample matrix. Standard addition is an alternative approach to matrix-matched calibration, including the analysis of a non-spiked sample and one or more test portions spiked with known amounts of the selected analyte, with spiking prior to extraction. According to SANTE guidelines, standard addition involves a sample divided into at least three test portions. One portion is analysed directly and increasing amounts of the analyte are added to the other analytical test portions, immediately prior to extraction (amount one to five times higher than the estimated amount present in the sample). Another alternative is the use of a procedural standard calibration, which is prepared by spiking a series of blank test portions with different amounts of analyte before extraction. This approach compensates for matrix effects and low extraction recoveries associated with specific analytes [157].

Trueness can be measured as the mean recovery/extraction efficiency of analytes from spiked samples. Extraction efficiency (EF) can be calculated by comparing the responses of samples spiked before and after extraction based on equation 1.6:

$$EF\ (\%) = \frac{Response(prior)}{Response(post)} \times 100 \tag{1.6}$$

Based on the guidance document of the DG-SANTE, acceptable mean recoveries are those within the range 70-120%, with a relative standard deviation (RSD) below 20%. However, in specific cases, mean recovery rates outside this range are acceptable if they are consistent (RSD $\leq$ 20 %) and the reason for this is well established (e.g., due to analyte distribution in a partitioning step), but the mean absolute recovery should not be lower than 30% or above 140%. Matrix effect (ME) can also be determined as the % of enhancement or suppression of the analyte signal, by comparing the response of samples spiked post-extraction and solvent samples, based on equation 1.7:

$$ME\ (\%) = \left(\frac{Response(post)}{Response(solvent)} - 1\right) \times 100 \tag{1.7}$$

LOD and LOQ are both relevant parameters included in all method validations. LOD is defined as the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified as an exact value. LOQ is defined as the lowest concentration tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable RSD is obtained. Different approaches can be applied depending on the selected guidelines. A common approach includes the signal-to-noise ratio (S/N), where the LOD is typically set to S/N = 3 and LOQ to S/N = 10. Other guidelines consider the LOQ as the lowest calibration point. The DG-SANTE guidance applies a stricter criterion, setting the LOQ to the lowest concentration that meets all validation criteria (e.g., trueness and precision). Another approach is to calculate the LOD based on analysis of a blank sample. If blank samples are not available, a "naturally contaminated" (native) pseudo-blank sample may be used instead. In this case, ten replicates of the sample should be analysed. Then LOD and LOQ are estimated based on the variability of signal values, expressed as standard deviation, obtained from the analysis of ten blanks. LOD is then calculated based on equation 1.8, where $S_{y,b}$ is the standard deviation of the blank (pseudo-blank) signals, and b is the slope of the calibration curve:

$$LOD = 3.9 \times \frac{Sy, b}{b} \tag{1.8}$$

1.5 Risk Assessment

Currently, the EU has approximately 40 laws governing chemicals, which aim to protect citizens and the environment [240]. According to an estimate by the World Health Organisation (WHO), 24% of the global burden of disease is related to environmental factors, including exposure to toxic chemicals [241]. Thus, various tools have been developed to assess the health and environmental risks associated with the use of chemicals [242]. These assessments are important in TWW reuse and sludge amendment practices, since it is known that CECs can accumulate in edible plant parts and enter the food chain [33].

1.5.1 Human health risk assessment

According to the WHO, "Risk assessment consists of scientific analyses, the results of which are quantitative or qualitative expressions of the likelihood of harm associated with exposure to a chemical substance" [242]. The main steps that need to be followed include:

i) hazard identification, which identifies the type and nature of adverse health effects,

ii) hazard characterisation, including qualitative or quantitative description of inherent properties of an agent having the potential to cause adverse health effects,

iii) exposure assessment, for a selected population to a particular agent,

iv) risk characterisation, to advise for decision making.

Risk assessment is closely related to the science of toxicology and its descriptors, which examine the adverse effects of chemicals on humans, animals, and the environment [243]. The risk of long-term exposure to substances can be determined based on toxicity studies, which can be divided based on the type of toxicity and adverse response of the organism, including: i) acute toxicity, which develops quickly, usually with severe symptoms; ii) subacute toxicity, where effects are the same as in acute response, but with weaker symptoms over a more extended period and iii) chronic toxicity, which develops slowly, during systematic long-term absorption of small amounts of a substance [244].

The human health hazard classification encompasses a broad range of potential health effects, including acute toxicity, carcinogenicity, and reproductive toxicity [245]. Quantitative hazard characterisation often consists of a dose-response assessment, as a point of departure from animal toxicology data for use in human health risk assessment [246], including:

- No observed adverse effect level (NOAEL) is the greatest concentration or amount of a substance at which no detectable adverse effects occur in an exposed population.
- The lowest observed adverse effect level (LOAEL) is the lowest level of a substance that has been observed to cause harm in an exposed population.
- Derived no effect level (DNEL) is the level of exposure to a substance above which humans should not be exposed.

- The benchmark dose lower confidence limit (BMDL), which is the lower confidence limit of the benchmark dose (BMD), refers to the dose associated with a predefined degree of adverse response.

Those toxicological descriptors can be used to develop and determine guidance values commonly used in chemical evaluations, with the application of uncertainty factors to account for interspecies and intraspecies (interindividual) variability. Health-based reference values include the acceptable daily intake (ADI) and tolerable daily intake (TDI), which are often not established for compounds such as pharmaceuticals and personal care products [247]. ADI and TDI values can provide an estimate of the amount of chemical that can be ingested orally (mainly through food and drinking water) or absorbed by inhalation or dermal contact without appreciable health risk.

For an exposure assessment of CECs, concentration data from the analysis of foods, such as edible plants irrigated with TWW, can be used by both deterministic and probabilistic approaches [248, 249]. The deterministic approach uses single values, or point estimates, as inputs, resulting in an output of a single point value for exposure, which provides estimates of exposure within a defined population. The probabilistic approach utilises distributions of data, from which multiple points are selected as inputs to the exposure equation, throughout multiple simulations. Consequently, the output is a distribution of potential exposure values [249]. Compared to the deterministic approach, it offers an increased ability to characterise uncertainty and variability; however, it requires more resources, such as sufficient input of concentration data. Monte Carlo simulation is the most common tool for estimating exposure using a probabilistic approach [250].

The deterministic approach is extensively applied in studies about the uptake of CEC due to TWW reuse [108, 210], where hazard quotient (HQ) is calculated as the ratio of estimated daily intake (EDI) to health-based reference values. EDI (mg/kg per body weight per day) is calculated based on equation 1.9:

$$EDI = \frac{CEC\ (mg/kg\ fresh\ weight) \times Food\ consumption\ (kg/day)}{Body\ weight\ (kg)} \tag{1.9}$$

The European Food Safety Authority (EFSA) provides default values, including body weight (bw) of different population groups, to be used. The default average body weights of the European adult (>18 years), adolescent (14-18 years), and toddler (1-3 years) populations are 70, 53 and 12 kg, respectively [251]. Consumption levels can be derived from the EFSA Comprehensive European Food Consumption Database, where "chronic food consumption" values are available for different European countries, calculated based on individual average consumption over the total survey period [252]. While several combinations of data can be applied, a high consumer scenario is usually calculated with higher percentiles (90^{th} and 95^{th}) of food consumption data. The level of concern in the risk assessment can be set to HQ ≥ 1 or 0.1, depending on the level of conservatism/protection that needs to be included in the assessment. The lower HQ level of concern (0.1) is set to recognise that humans may be exposed to CECs via other pathways, e.g., ingestion of other matrices, dermal, and inhalation [253].

An alternative approach for risk assessment, reported in literature associated with CECs through the consumption of vegetables irrigated with TWW, is the threshold of toxicological concern (TTC) approach using the Toxtree software [94, 254], based on the Cramer classification tree [255]. The TCC is a conservative estimate that is based on 5% of the level at which there are no observed adverse effects, with an additional 10^{-6} uncertainty factor and includes defining compounds' structural class based on their genotoxic potential. Class I compounds contain simple structures and are easily

metabolised, thus presenting minimal toxicity concerns. Class II compounds contain substances that may be more harmful than those in Class I, but they do not contain structural features that are suggestive of toxicity. Class III compounds contain reactive functional groups and present greater toxic concern [256]. When the consumption of a specific compound exceeds the TTC value, this indicates a potential risk of exposure and requires specific toxicity analysis [257].

The aforementioned approaches for human health risk assessment typically focus on individual compounds and a single exposure route (oral, when applied in the context of TWW reuse), where risks may be over- or underestimated [100]. However, the effects of cumulative exposures must also be considered, as well as their potential interactions (either synergistic or antagonistic) [258]. Although predicting the risk for mixtures can support a more realistic risk assessment, the current state-of-the-art is limited due to a lack of data and harmonisation [259].

1.5.2 Ecological risk assessment

Ecological risk assessment (ERA) is conducted to evaluate the potential environmental risks associated with specific pollutants, to protect aquatic and terrestrial ecosystems, including surface water, groundwater, and soil. ERA is based on physicochemical, ecotoxicological, and environmental fate properties of a substance and includes both a risk assessment and a hazard assessment. Risk assessment reflects the possibility of an effect occurring. It involves an evaluation of both the exposure of organisms in the environment to a substance and their susceptibility to its ecotoxicity. The hazard assessment involves identifying the intrinsic properties of a substance that could render it harmful to the environment, regardless of the level of exposure [260].

The risk quotient (RQ), ratio between measured (MEC) or predicted environmental concentrations (PEC) with Predicted No-Effect Concentration (PNEC), is the most common approach to identify high- or low-risk ecotoxicological situations associated with the presence of CECs in the terrestrial environment, especially in soil, following the application of TWW or sludge [140]. Following the calculation of RQ values, the proposed classification by Hernando et al. is used to identify risk as low (RQs < 0.1), medium (RQs between 0.1 and 1), and high (RQs > 1) [261]. PNEC values are calculated using the lowest acute and chronic toxicity data (no observed effect concentration (NOEC)) in model organisms (fish, *Daphnia magna* and algae) and dividing them by an assessment factor, between 1000 and 10, respectively, to consider the worst-case scenario [262].

However, PNEC values in soil are usually limited, especially for organic contaminants, due to the lack of acute toxicity data for terrestrial organisms such as earthworms, plants and soil microorganisms [36]. Instead, PNEC values for soil can be derived from PNEC values for water using the equilibrium partition method (EPM), as suggested by the EU [263]. PNEC values for freshwater organisms (µg/L) can be obtained from the literature or available databases, such as the Norman Ecotoxicology Database, including experimental data or quantitative structure–activity relationship (QSAR) predictions suitable for preliminary screening and prioritisation [264]. PNEC for soil can be calculated based on equation 1.10, considering $PNEC_{water}$ (mg/L), bulk density of wet soil (RHO_{soil}, kg/m^3), soil-water partition coefficient ($K_{soil\text{-}water}$), and $PNEC_{soil}$ (mg/kg):

$$PNECsoil = \frac{Ksoil - water}{RHOsoil} \times PNECwater \times 1000 \tag{1.10}$$

A more simplified version of calculation incorporates default values for soil parameters, such as bulk density of wet soil (1700 kg/m^3), density of solid phase (2500 kg/m^3), organic

carbon-water partition coefficient, volume fraction of air, water, solid and organic carbon in soil, using equation 1.11:

$$PNECsoil = (0.1176 + 0.01764 \times Koc) \times PNECwater \quad (1.11)$$

This method is regarded as a "screening" approach and is suitable for substances with $\log K_{ow}$ values between 3 and 6. For compounds with a $\log K_{ow} > 5$, or for compounds with a corresponding adsorption or binding behaviour, the ratio between MEC and PNEC should be increased by a factor of 10 [265]. Overall, EPM may either overestimate or underestimate actual risk to soil organisms, but it is accepted as a conservative proxy [266].

1.6 Knowledge Gaps and Research Needs

Despite the promotion of TWW and sludge reuse as valuable strategies for water conservation and waste valorisation, knowledge gaps remain regarding the safe implementation of those practices. Although the wastewater reuse regulation includes a risk management plan, more data are needed regarding the fate of organic pollutants in the soil-plant-consumer continuum, particularly based on the application of TWW in realistic conditions [267]. Additionally, the sewage sludge directive has not been renewed since 1986 [42], but it is expected to include micropollutants, following the WFD and Urban Wastewater Treatment Directive [34, 62].

Following the literature review, most existing studies focus on specific types of crops, primarily leafy vegetables (Figures 1.2 and 1.3), while neglecting fruit-bearing vegetables. Insights on the uptake and translocation patterns are lacking, since not all plant parts are systematically analysed across different studies (Tables 1.4-1.7). Current studies usually focus on a limited number of compounds. Therefore, extended monitoring, adopting multiresidue analytical methods in wastewater and sludge reuse in agriculture, is important to estimate the range of CECs that can be present in fruits and other plant parts [104, 214].

An additional gap identified is that limited treatments are applied across experimental designs, usually limited only to TWW reuse. Although the main aim of uptake studies is to evaluate the TWW effect in conditions that resemble real application scenarios, the inclusion of comparable spiked treatments with selected CECs is rare. The application of both irrigation media in the same experimental design is necessary to elucidate uptake mechanisms and quantify human health and ecological risks under both low-dose (realistic) and high-exposure scenarios [268]. Although spiking with higher concentrations may not reflect environmental concentration levels, the practice remains useful since the use of TWW may result in limited detection of CECs in irrigation media and also across plant parts, limiting the ability to conclude the fate of pollutants, which may remain in environmental compartments (soil, water) or be present across different plant parts. Spiked treatments can also help to understand uptake and translocation mechanisms in plants.

The long-term environmental fate and accumulation of organic contaminants in soil-plant systems are still insufficiently understood. Furthermore, comparisons across cultivation systems (soil and hydroponics) focusing on the same analytes are scarce, despite being essential to understanding how the growing medium, influenced by the presence or absence of soil, modulates the uptake of different compounds. Additionally, TWW and sludge reuse are rarely compared across the same experimental designs, which addresses the question of which CECs may be available to plants depending on the source of exposure [110].

Another aspect neglected within the available studies is the influence of TWW reuse systems, including transportation and storage of TWW before application [269]. Storage of

TWW is important, since the conditions may result in CEC degradation or transformation in the media, and therefore influence the fate and potential uptake in TWW reuse systems [142].

Overall, future research should incorporate both realistic field conditions and spiked treatments, providing complementary insights into the fate of CECs in wastewater and sludge reuse systems. Spiked experiments can allow the evaluation of compound behaviour, while field studies capture actual environmental dynamics and variability. Together, these studies are necessary to accurately assess human and environmental risks under both typical and extreme scenarios [270].

Chapter 2

Aims and Hypotheses

The review of the current literature highlights the importance of reusing TWW and sewage sludge in agriculture as a key strategy to reduce water demand and promote more sustainable water management practices. However, such practices can lead to the introduction of CECs into agricultural systems and the food chain, posing an environmental and food safety risk. Despite significant progress in analytical techniques, regulatory efforts, and significant interest within the scientific committee, knowledge gaps remain.

The aims of this thesis were to investigate the fate, uptake, and potential risks of model CECs in tomato cultivation systems irrigated with treated wastewater and amended with sludge. Tomato (*Solanum lycopersicum* L. cv. Rally) was chosen as a model plant because it is one of the most popular and widely consumed vegetables in the EU and is widely eaten fresh (raw) as well as in various processed forms. In 2020, 14 CECs were studied, including pharmaceuticals (ketoprofen, ibuprofen, naproxen, diclofenac), stimulants (caffeine), hormones (estrone, 17-beta-estradiol, 17-alpha-ethynylestradiol, estriol) and industrial chemicals; bisphenol F isomers (2,2-BPF, 2,4-BPF, 4,4-BPF), BPA and BPS. Compounds were selected based on occurrence data in Slovenian WWTPS and availability of standards before the experiment.

For 2022, the selected 27 target CECs were finalised based on previous uptake studies, combined with their occurrence in wastewater effluents, and their prior inclusion in the EU Watch Lists (Section 1.2.1). Out of 14 CECs included in 2020, 10 were included in the 2022 experiment based on the potential uptake, excluding one hormone (estriol), one pharmaceutical (ketoprofen) and two BPF isomers (since only 4,4-BPF was uptaken). The primary aim was to incorporate compounds from diverse compound classes with varying physicochemical properties (Appendix A). Accordingly, the selected CECs comprised the following: industrial chemicals (BPA, BPF, BPS, bisphenol AF), parabens (methylparaben, propylparaben), antimicrobials and biocides (triclosan, triclocarban), hormones (estrone, 17-beta-estradiol, 17-alpha-ethynylestradiol, progesterone, testosterone), pharmaceuticals (carbamazepine, diclofenac, naproxen, ibuprofen, ciprofloxacin, amoxicillin, azithromycin, erythromycin, clarithromycin), stimulants (caffeine), UV filters (benzophenone), pesticides (acetamiprid, dimethomorph) and musks (tonalide).

To achieve these aims, the study pursued six interrelated objectives:

1. To conduct three distinct tomato cultivation experiments representing different reuse scenarios: (1) lysimeter field experiment irrigated with treated wastewater, (2) hydroponic system supplied with treated wastewater, and (3) greenhouse pots amended with sludge.

2. To develop and apply analytical methods for quantifying selected model CECs (27) across different matrices, including wastewater, potable water, sludge, soil, tomato fruits, and plant parts.

3. To evaluate the use of newly synthesised sorbents for extracting CECs from wastewater and compare their performance to conventional solid-phase extraction materials.

4. To study the uptake, translocation, and bioaccumulation of selected CECs in tomato plants under the different cultivation systems.

5. To estimate potential dietary exposure to CECs through consumption of tomatoes grown with alternative methods (wastewater irrigated, amended with sludge).

6. To evaluate the environmental risks of CEC accumulation in soil following wastewater irrigation and sludge amendment.

These objectives directly informed the following four hypotheses:

H1: Irrigation of tomato plants with treated municipal wastewater and/or cultivation in sludge-amended substrate results in the uptake and translocation of CECs into plant parts, including tomato fruit, at concentrations measurably higher than in plants irrigated with potable water or grown in non-amended substrate.

H2: Uptake and translocation of CECs differ between cultivation systems due to variations in exposure pathways, plant-matrix interactions, and compound bioavailability.

H3: Dietary exposure to individual CECs from tomatoes grown with wastewater irrigation or sludge amendment remains below established risk thresholds for the general population.

H4: Agricultural reuse of treated wastewater and sludge leads to accumulation of CECs in soils, potentially increasing environmental risks.

Chapter 3

Publications

The work is presented as five scientific papers. Four papers have been published in peer-reviewed journals, and the fifth will be submitted for peer review in December 2025 in Environmental Research.

The objectives are divided into three thematic sections:

3.1 Development and application of analytical methods for the determination of contaminants of emerging concern (CECs)

3.1.1 Synthesis and evaluation of a new acrylic copolymer for dispersive solid-phase microextraction of organic contaminants from urban wastewater

3.2 Uptake of CECs in tomatoes irrigated with treated wastewater: differences between hydroponically and field-grown tomatoes

3.2.1 Contaminant uptake in wastewater irrigated tomatoes

3.2.2 Uptake of Emerging Contaminants in Tomato Plants: A Field Study on Treated Wastewater Reuse

3.2.3 Hydroponically grown tomatoes in treated wastewater: Uptake of CECs and impact on plant growth

3.3 Uptake of CECs in tomatoes grown in sludge-amended peat and implications for soil accumulation and food safety

3.3.1 Contaminants of Emerging Concern in Tomatoes Grown in Sludge-Amended Peat: Uptake, Translocation and Risk Assessment

3.1 Development and Application of Analytical Methods for the Determination of Contaminants of Emerging Concern (CECs)

3.1.1 Synthesis and evaluation of a new acrylic copolymer for dispersive solid-phase microextraction of organic contaminants from urban wastewater

The paper "Synthesis and evaluation of a new acrylic copolymer for dispersive solid-phase microextraction of organic contaminants from urban wastewater" by E. Andreasidou, L. Martello, D. Heath, D.N. Bikiaris, D.A. Lambropoulou and E. Heath was published in Microchemical Journal in March 2025 (doi.org/10.1016/j.microc.2025.113038). My role in this joint effort included performing experimental work for extraction optimisation, method development, instrumental analysis, method validation, data evaluation, and manuscript preparation. L. Martello performed the synthesis and characterisation of the copolymer.

The presence of contaminants of emerging concern (CECs) in treated wastewater effluents may pose a risk to aquatic ecosystems and human health, emphasising the need for developing innovative and greener methods for their analysis. This study reports the synthesis, characterisation, and application of a novel acrylic copolymer, Poly(TMPA-co-TEGDMA-co-AA-co-NIPAM-co-HEMA) (PTTANH), functionalised with acrylic acid (AA), *N*-isopropyl acrylamide (NIPAM), and 2-hydroxyethyl methacrylate (2-HEMA). Characterised by FTIR, SEM, BET, and DSC, confirming a high cross-linking degree and thermal stability up to 300 °C. An LC-MS/MS method was developed for the analysis of 27 model CECs. Meanwhile, the copolymer was applied in dispersive micro-solid phase extraction (D-μ-SPE), which was optimised and validated for the analysis of 24 CECs in treated urban wastewater. The obtained LODs and LOQs were between 2 and 92 ng/L and 6 and 279 ng/L, respectively. The average recovery was 85 %, intra-day precision was 7 % RSD, and inter-day precision was 11 % RSD. The method utilised a reduced sample volume (12 mL), sorbent (40 mg), and solvent (4 mL), making it both time- and resource-efficient, and achieved a score of 6.0/10 on the Sample Preparation Metric of Sustainability (SPMS). When applied to real wastewater effluents, several CECs were detected above LODs, and six were quantified above LOQs across different wastewater treatment plants. Overall, the PTTANH-based protocol offers a green, cost-effective, and robust alternative to conventional SPE approaches.

This publication addresses one aim of the thesis, i.e., to evaluate the use of newly synthesised sorbents for the extraction of CECs from wastewater compared to conventional methods. The paper also achieves the secondary, but equally important, aim of developing and validating an analytical LC-MS/MS method for model CECs, including pharmaceuticals, personal care product ingredients, hormones, industrial chemicals, stimulants, and pesticides.

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Synthesis and evaluation of a new acrylic copolymer for dispersive solid-phase microextraction of organic contaminants from urban wastewater☆

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ABSTRACT

Contaminants of emerging concern (CECs) in wastewater effluents pose significant risks to aquatic ecosystems and human health, underscoring the urgent need for effective monitoring. This study introduces a novel acrylic copolymer, Poly(TMPA-co-TEGDMA-co-AA-co-NIPAM-co-HEMA), referred to as PTTANH, functionalized with acrylic acid (AA), N-isopropyl acrylamide (NIPAM), and 2-hydroxyethyl methacrylate (2-HEMA). The copolymer was designed for dispersive solid-phase microextraction (D-μ-SPE) to extract CECs from aqueous media. The copolymer was characterized using FTIR, SEM, BET, and DSC, with key parameters optimized to develop an efficient and high degree of cross-linking and thermal stability (0–300 °C). The developed PTTANH was subsequently employed in a multi-analyte D-μ-SPE extraction method that was optimized and validated for 24 CECs from urban wastewater effluent. Samples were analyzed using LC-MS/MS. The LOD and LOQ were between 2–92 ng L^{-1} and 6–279 ng L^{-1}, respectively. The average recovery was 85 %, intra-day precision was 7 % RSD, and inter-day precision was 11 % RSD. The linear range was 100–33300 ng L^{-1}. The optimized protocol requires a small sample volume (12 mL), a minimal amount of sorbent (40 mg), organic solvent (4 mL), and analysis time (25 min), achieving a score of 6.0/10 on the Sample Preparation Metric of Sustainability (SPMS).

1. Introduction

Contaminants of emerging concern (CECs) are unregulated substances that are not commonly monitored but have the potential to persist, bioaccumulate and pose a risk to aquatic organisms and human health through contamination of drinking water and entry into the food chain [1–3]. The presence of CECs in waters is a global issue and includes a wide range of organic compounds with varying physicochemical parameters, including pharmaceuticals [4,5], pesticides [6], personal care products [7] and industrial chemicals [8], among others [9]. A significant source of CECs is treated municipal and industrial wastewater effluent since wastewater treatment plants are not typically designed to remove CECs [10]. Moreover, their presence in treated wastewater complicates its safe reuse for irrigation, an essential agricultural practice in regions facing water scarcity [11,12]. Consequently, developing innovative, rapid, robust, and greener methods for analyzing CECs in wastewater is crucial for their effective monitoring.

Many studies have investigated the presence of CECs in wastewater. In all cases, adequate sample preparation is essential for enhancing the selectivity and sensitivity of the detection methods. The extraction and preconcentration of organic pollutants from wastewater are most commonly achieved using solid-phase extraction (SPE) [13]. However,

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alternative methods, such as dispersive solid-phase microextraction (D-μ-SPE), where sorbent is introduced directly in the sample, and Solid Phase Microextraction (SPME), which utilizes a fiber coated with a stationary phase, are promising techniques offering improved efficiency and simplicity [14–17].

The benefits of D-μ-SPE compared to SPE are its simplified procedure, lower sample volumes (0.1–25 ml), reduced solvent use, and higher sensitivity for detecting low concentrations of contaminants. The method involves uniformly dispersing a sorbent in an aqueous sample, thereby enhancing extraction kinetics by increasing the surface interaction between the sorbent and the analytes. The sorbent is then separated by centrifugation and eluted with a solvent to recover the analytes of interest [14–18]. It also offers versatility in adapting to various analytes, improved recovery rates, and cost-effectiveness from using smaller amounts of sorbent and solvents. However, given the small amount of material used, the continued development of sorbent materials is essential for enhancing extraction efficiency and selectivity.

Recent advancements in D-μ-SPE have led to the development of different novel sorbents, each with distinct advantages and limitations. For example, metal–organic frameworks require complex synthesis and exhibit reduced stability in aqueous samples [19,20], carbon nanotubes have low dispersion efficiency in aqueous solution due to their non-polar nature [21], molecularly imprinted polymers (MIPS), show limited efficiency for water-soluble compounds [22,23], and the sorption properties of chitosan-derived nanomaterials can be affected by sample pH [28,29]. With the exception of chitosan, they are also relatively costly to produce. Polymeric sorbents, in contrast, offer good stability, simple synthesis and multifunctional structure, which permits the extraction of analytes with various physicochemical properties, making it promising for analyzing contaminants of emerging concern (CECs) in complex matrices like wastewater [21,24].

This study introduces a novel acrylic copolymer, Poly(TMPA-co-TEGDMA-co-AA-co-NIPAM-co-HEMA), referred to as PTTANH, functionalized with acrylic acid (AA), N-isopropyl acrylamide (NIPAM), and 2-hydroxyethyl methacrylate (2-HEMA), cross-linked with trimethylolpropane triacrylate (TMPA) and triethylene glycol dimethylacrylate (TEGDMA) as efficient, cost-effective greener D-μ-SPE sorbent. The new sorbent was characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), Brunauer-Emmett-Teller analysis (BET) and differential scanning calorimetry (DSC). As a proof of concept, the sorbent was tested as part of an optimized and validated D-μ-SPE method for the extraction of 24 CECs with a broad range of physicochemical properties present in treated urban wastewater.

2. Materials and methods

2.1. Chemicals, standards, and materials

Triethylene glycol dimethacrylate (TEGDMA), acrylic acid (AA), trimethylolpropane triacrylate (TMPA) and azobisisobutyronitrile (AIBN) were supplied by Sigma-Aldrich (St. Louis, MO, USA). 2-hydroxyethyl methacrylate (2-HEMA) and N-Isopropyl acrylamide (NIPAM) were supplied by Tokyo Chemical Industry (Haven, Belgium). Acetonitrile (ACN) (HPLC grade) and methanol (MeOH) (HPLC grade) were supplied by Merck (Darmstadt, Germany). Acetic acid and trichloromethane (TCM) (HPLC grade) were acquired from Fisher Scientific (Waltham, MA, USA), and hydrochloric acid 37 % was supplied by Carlo Herba (Emmendingen, Germany). For analysis, all LC-MS grade solvents were obtained from J. T. Baker (Deventer, Netherlands). Formic acid (FA; 99 %) and ammonium fluoride (NH_4F, 99.8 %) were purchased from Sigma Aldrich (St. Louis, USA).

Analytical standards (> 95 % purity) of acetamiprid, azithromycin, benzophenone, 4,4′-bisphenol F (4,4-BPF), bisphenol AF (BPAF), bisphenol S (BPS), caffeine, carbamazepine, ciprofloxacin, clarithromycin, diclofenac sodium salt, dimethomorph, erythromycin, estrone (E1), 17α-ethynyl estradiol (EE2), ibuprofen, methylparaben, naproxen, progesterone, propylparaben, testosterone, tonalide, triclocarban and triclosan were purchased from Sigma-Aldrich (Steinheim, Germany), whereas, 4,4′-(2,2-Propanediyl)diphenol (BPA), was purchased from Merck (Darmstadt, Germany). Amoxicillin trihydrate was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and beta-estradiol (beta-E2) was purchased from Thermo Fischer Scientific (Waltham, MA, USA).

Isotopically labelled surrogate standards: BPA-d_{16}, $^{13}C_3$-caffeine, carbamazepine-d_{10}, clothianidin-d_3, $^{13}C_6$-diclofenac and ibuprofen-d_3 were purchased from Sigma Aldrich (Steinheim, Germany), oxybenzone-d_5 was purchased from C/D/N Isotopes Inc. (Quebec, Canada), $^{13}C_{12}$-triclosan from Cambridge Isotope Laboratories (Tewksbury, MA, USA), $^{13}C_2$-beta-estradiol from Campro Scientific (Berlin, Germany) and $^{13}C_{12}$-BPS and $^{13}C_{12}$-BPF were obtained from CanSyn Chem. Corp. (Toronto, Canada).

Stock solutions (1 mg mL^{-1}) were prepared by dissolving each compound in MeOH. Working standards (10, 100 and 1000 ng mL^{-1}) were prepared by making appropriate serial dilutions of the stock solutions with MeOH. Similarly, an internal standard solution (1.0 μg mL^{-1}) in MeOH was prepared from mixing solutions of each isotopically labelled compound. Once prepared, all standards were stored in amber glass vials at 4 °C.

2.2. Sorbent synthesis

PTTANH was synthesized (Fig. 1) by combining approaches described in [25,26]. Radical activation, commonly used in developing sorbent materials [27] and a mixture of solvents ACN:TCM (19:1 v/v), was used as the porogen agent, as reported in [28]. Since most compounds traceable in wastewater are hydrophilic, it was decided to functionalize the copolymer with hydrophilic groups embedded mostly on the aliphatic backbone to enhance the interaction between polar analytes and the sorbent while avoiding sorbent swelling resulting from water adsorption.

The synthesis was as follows: 48 mmol of TMPA were dissolved in 250 mL of ACN:TCM (19:1 v/v) in a round-bottom flask and purged for 1 h at 25 °C. Subsequently, 25 mg of AIBN dissolved in 1 mL of ACN:TCM (19:1 v/v) was added to the reaction mixture, and the temperature was raised to 60 °C. After 30 min of stirring, 144 mmol of TEGDMA were added. The mixture was maintained at 60 °C with stirring for three hours. AIBN (25 mg) was then added to the reaction mixture, along with 16 mmol of NIPAM, AA, and 2-HEMA in 5 mL of ACN:TCM (19:1 v/v). The reaction vessel was kept under a nitrogen (N_2) atmosphere for 30 min, then sealed and stirred for 24 h at 70 °C. The white product was collected, broken into small pieces, and subjected to Soxhlet extraction with ACN:MeOH (70:30 v/v) for 48 h. The material was air-dried, weighed, and homogenized using a mortar and pestle. Synthesis yield was assessed by comparing the mass of the polymer after purification with the mass of the monomers used for the reaction, resulting in a 60.16 % yield.

2.3. Sorbent characterization

The sorbent in the form of a KBr pellet was characterized using FT-IR (FT-IR-2000, Perkin Elmer, Dresden, Germany) by acquiring, for each sample, 32 scans from 450 to 4000 cm^{-1} at a spectral resolution of 4 cm^{-1}. The data were processed using R software [29]. Morphological analysis was performed using a JEOL JSM-840 Scanning Electron Microscope (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 5 kV. Before analysis, a carbon coating was applied to the sample. Micrographs were captured at ×5,000, ×10,000, and x25,000 magnification. Porosity was assessed using nitrogen adsorption–desorption at 77 K employing an Autosorb-1MP Quantachrome porosimeter (Anton Paar QuantaTec Inc., Boynton Beach, FL, USA). The surface area was calculated using the multi-point Brunauer-Emmett-Teller (BET) method, utilizing data from the adsorption isotherm. The total pore volume was

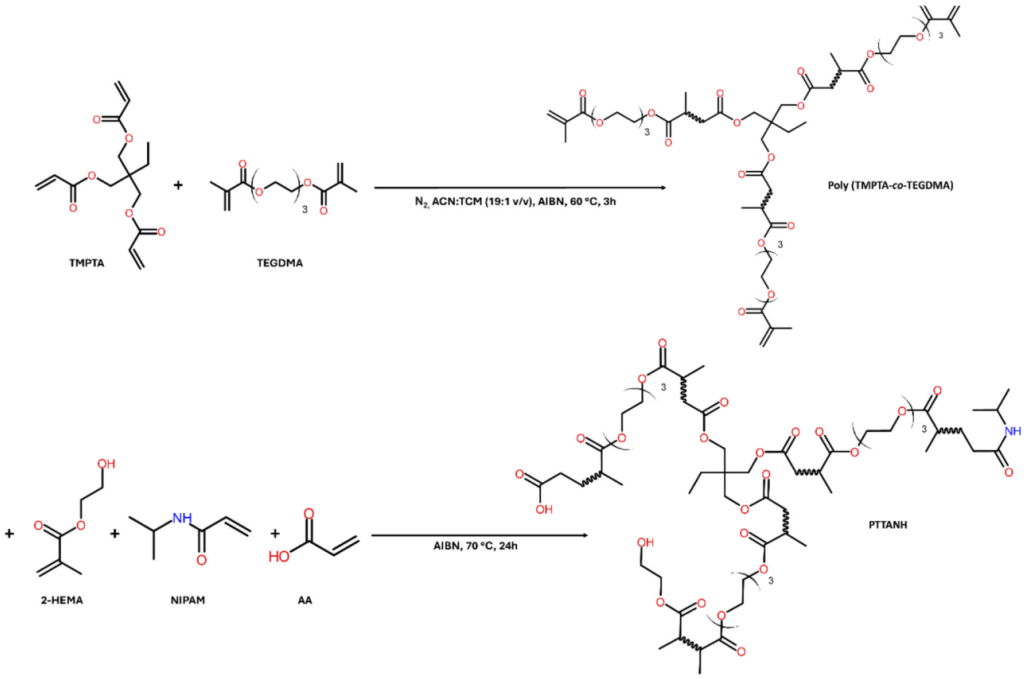


Fig. 1. Reaction scheme for the synthesis of PTTANH.

determined based on adsorbed nitrogen at $P/P_0 = 0.99$. All samples were first outgassed at 150 °C for 12 h. For differential scanning calorimetry, a Shimadzu DSC 60 (Shimadzu Corporation, Kyoto, KYT, Japan) was calibrated using pure indium and zinc standards. The sample (5 mg) was heated at 20 °C min^{-1} up to 300 °C under a nitrogen atmosphere.

2.4. LC–MS/MS analysis

As a proof of concept, 27 CECs were selected as target analytes (Table S1). The CECs were selected based on their occurrence and fate in water, potential adverse effects on human health, and their inclusion in regulatory frameworks such as the Water Framework Directive (2000/60/EC) [30], Groundwater Directive (2006/118/EC) [31], Directive on Environmental Quality Standards (2008/105/EC) [31], Drinking Water Watchlist (2020/2184/EU) [32], Wastewater Reuse Regulation (2020/741) [33] and Watch lists of substances for Union-wide monitoring in the field of water policy (CID 2020/2184 and 2022/1307) [34,35]. Analysis using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was performed using a Nexera X2 UHPLC (Shimadzu, Kyoto, Japan) coupled to a linear ion trap quadrupole 4500 QqLIT-MS/MS (Sciex, Framingham, MA, USA). Separation was achieved using a Poroshell 120 EC-C18 column (100 × 3 mm; 2.7 μm particle size, Agilent Technologies, USA) at room temperature. The sample injection volume was 2 μL. For positive mode, the mobile phases were acetonitrile (A) and 0.1 % formic acid in ultrapure water (B), while for negative mode, the mobile phases were 0.1 % NH_4F in methanol (A) and 0.1 % NH_4F in ultrapure water (B), both at a constant flow rate of 0.3 mL min^{-1}. The optimized gradient programs are provided in the Supplementary (Table S2).

The 4500 QTRAP analyzer was equipped with electrospray ionization (ESI) operating in both positive and negative modes, with the following settings: curtain gas (CUR): 40 (positive) and 35 (negative) arbitrary units; collision-activated dissociation (CAD): −2 for both; ionization voltage (IS): 5500 V and −4500 V; ion source temperature (TEM): 450 °C and 500 °C; ion source gas 1 (GS1): 40 psi and 35 psi; and ion source gas 2 (GS2): 40 psi and 20 psi. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Transitions were optimized for the compound-dependent parameters, including declustering potential (DP), collision energy (CE) and collision exit potential (CXP), presented in Supplementary (Table S3). Data analysis was performed using Analyst v1.6.3.

2.5. Multivariate optimization of extraction conditions

The adsorption performance of PTTANH was evaluated using a multivariate approach [36], with the average recovery of the analytes from spiked wastewater as the response variable based on seven independent factors: 1) *Sorbent amount*, 2) *Sample pH*, 3) *Adsorption time*, 4) *Desorption time*, 5) *Sample volume*, 6) *Desorption solvent*, and 7) *Desorption volume*. The *Sorbent amount*, *Adsorption time*, *Desorption time*, *Sample volume*, and *Desorption volume* were selected based on insights from prior D-μ-SPE protocols [37,38]. Preliminary experiments were conducted to determine the two levels required for optimizing *pH* and *Desorption solvent*. These were performed using 6 mL of deionized water spiked with CEC (33330 ng L^{-1}), 30 mg of PTTANH, 2 mL of desorption solvent, and adsorption and desorption times of 10 and 60 min, respectively, at a pH 4, 6.5 and 10. The sample pH was adjusted using 0.1 mol/L NaOH or HCl. Six combinations of *Desorption solvent* were tested: ACN, MeOH, ACN:MeOH (1:1), ACN 0.1 % v/v FA, MeOH 0.1 % v/v FA, and ACN:MeOH (1:1) with 0.1 % v/v FA.

The extraction protocol was optimized using a two-level Plackett-Burman non-geometric experimental design (PBD) since it allows for the simultaneous evaluation of a large number of factors (k = 7) with relatively few experiments (n = 12) and was generated through R software [29]. The first run included 12 experiments, where independent factors were assessed. All experiments were conducted using a municipal wastewater treatment plant effluent (WWTP-Ljubljana, Slovenia) and spiked with 27 CECs (range: 16,600 and 66,600 ng L^{-1}, depending

on sample and desorption volume).

The sorbent was tested at two levels for each factor (Table 1). Each experiment involved adding a predetermined amount of PTTANH (20 or 40 mg) to a series of falcon tubes containing wastewater (6 or 12 mL), with the pH adjusted accordingly (levels selected after the preliminary experiments). The falcon tubes were oscillated at 300 rpm on a shaking table at room temperature for 20 min, followed by centrifugation (9000 rcf, 10 min) to separate the sorbent material from the supernatant, which was discarded. For analyte desorption, solvent (ACN or ACN:MeOH 0.1 FA, 2 or 4 mL) was added, followed by further mixing (5 min) and centrifugation (9000 rcf, 10 min). The eluate was evaporated under a gentle stream of nitrogen and reconstituted to 0.4 mL with ACN:MeOH (1:1) with 0.1 % FA. The sample was then filtered through a regenerated cellulose filter vial (0.2 μm) before being analyzed by LC–MS/MS.

The quantitative parameters for the twelve experiments were defined by the experimental design. Each experiment was performed in duplicate and randomized to reduce the influence of uncontrolled variables. Linear regression and the effects of factors were evaluated based on the average analyte recovery. Moreover, since two-factor interactions in this design are allied with the main effects (resolution III design), the full PBD matrix (11 columns) was used when generating the half-normal plot and normality of the residuals was tested using the Shapiro-Wilk and the Kolmogorov-Smirnov tests. The optimal extraction conditions were 40 mg of PTTANH dispersed in 12 mL of wastewater at pH 4, with a 20-minute adsorption time and a 5-minute desorption time using 4 mL of ACN:MeOH (1:1) with 0.1 % FA for elution.

2.6. Method performance evaluation

The performance of the PTTANH-D-μ-SPE-LC-MS/MS method was evaluated in terms of recovery, reproducibility, repeatability, linearity, LODs, LOQs and matrix effect following SANTE guidelines [39]. Effluent from a wastewater treatment plant (Ljubljana, Slovenia) was used as the matrix. To improve sensitivity, the sample (4 mL) was evaporated to dryness under N_2 and then resuspended in 0.4 mL of ACN:MeOH (1:1) with 0.1 % FA.

For each target analyte, recovery was determined as the ratio of the signal after extraction of CECs from wastewater samples spiked at two concentrations (500 and 10,000 ng L^{-1}) to that of wastewater samples post-spiked at the final expected concentration. Repeatability and reproducibility were determined as the RSD of intra-day and inter-day recovery experiments, intra-day precision was determined based on the same-day analysis of three spiked samples (500 ng L^{-1}), and inter-day values were obtained from the analysis of three spiked samples (500 ng L^{-1}) on three consecutive days. Linearity was assessed from the procedural calibration curve obtained by spiking analytes in wastewater from 100 to 33000 ng L^{-1}. LODs and LOQs were based on the standard error of the intercept, and the slope of the calibration curves generated from wastewater spiked at 30, 100, and 500 ng L^{-1}. The matrix effect was determined by comparing the slope of the procedural calibration curves obtained in wastewater and the slope of the calibration curves obtained in solvent. For quality control, a mixture of internal standards (1 μg mL^{-1}) was spiked at 5 μg L^{-1}.

Table 1
Factors and levels for Plackett-Burman Experimental Design.

Factors	Levels	
1. Sorbent Amount (mg)	20	40
2. Sample pH	4	6.5
3. Adsorption time (min)	20	60
4. Desorption time (min)	5	20
5. Sample volume (mL)	6	12
6. Desorption Solvent	ACN	ACN:MeOH 0.1 FA
7. Desorption Volume (mL)	2	4

2.7. Evaluation of sustainability of the method

Two green metric tools were employed to evaluate the developed protocol: the sample preparation metric of sustainability (SPMS) [40] and the analytical greenness metric for sample preparation (AGREEprep) [41]. These tools have recently gained recognition for evaluating the greenness of various sample pretreatment procedures [42–44]. By applying these metrics, its alignment with green chemistry principles can be evaluated.

The SPMS metric evaluates sample preparation techniques based on nine parameters that impact the method's greenness, each assigned a different weighting score and organized into four categories: (i) sample information (sample amount), (ii) extractant information (amount, toxicity, and reusability of solvents), (iii) sample preparation procedure information (number of steps, extraction time, need for additional steps post-extraction, and sample throughput), and (iv) energy consumption and waste generation. The SPMS assigns a score on a scale of 1 to 10, with 10 representing the best performance.

Although similar, AGREEprep is a metric based on twelve principles of Green Sample Preparation (GSP) principles. It considers the limiting factors within the method and the respective analytical instrumentation used after sample preparation. Among others, the criteria include the use of safer solvents, "green" configuration for analysis and reagents, and minimized waste, sample, chemical and material amounts. Each criterion is evaluated with an impact score on a scale from 0 (not fulfilling) to 1 (fulfilling).

2.8. Sample analysis

The method was tested on treated wastewater effluent samples sampled in February 2024 from four wastewater treatment plants based on mechanical–biological treatment but with varying wastewater sources (municipal and industrial) and population equivalents (P.E.) in order to determine a wide range of CECs. Further details about wastewater treatment plants are presented in Supplementary (Table S4). Effluent samples were collected in polypropylene bottles and stored in the dark at 4 °C for up to 12 h prior to extraction.

3. Results and discussion

3.1. Characterization

FT-IR characterization of PTTANH revealed insights into the chemical structure of the sorbent (Fig. 2). The broad band spanning 2900–3400 cm^{-1} indicates the stretching vibrations of hydroxyl groups (AA, 2-HEMA), overlapping at 3443 cm^{-1} with the N–H stretching characteristic of NIPAM. The peaks at 2954 cm^{-1} and 2853 cm^{-1} were attributed to asymmetric stretching and symmetric stretching vibration of the C-H bond, respectively, the sharp peak at 1731 cm^{-1} to the presence of ester groups (TMPTA, TEGDMA), and the –COO– bond was confirmed by multiple absorption peaks between 130 cm^{-1} and 1100 cm^{-1}. The bending vibration of methyl groups (TEGDMA, 2-HEMA, NIPAM) was attested by the peak at 1469 cm^{-1}. Also, a peak at 1632 cm^{-1}, typical of the C=O stretching vibration of amides, is evidence of the successful functionalization with NIPAM. The aforementioned characteristic peaks indicate the effective functionalization with AA, NIPAM and 2-HEMA, while the absence of a sharp peak at 1650 cm^{-1} (C=C) confirms the successful acrylic polymerization of TMPTA and TEGDMA. The FT-IR spectra of the monomeric precursors (AA, 2-HEMA, and NIPAM), as well as a table gathering the spectral changes in characteristic bands confirming the successful copolymerization, are included in Supplementary Material (Fig. S1 and Table S5, respectively).

The DSC thermogram (Fig. S2) revealed notable characteristics, i.e., the absence of a distinct glass transition (as evidenced by the lack of baseline shifting), the non-appearance of exothermic peaks indicative of crystallization, and the absence of endothermic peaks associated with

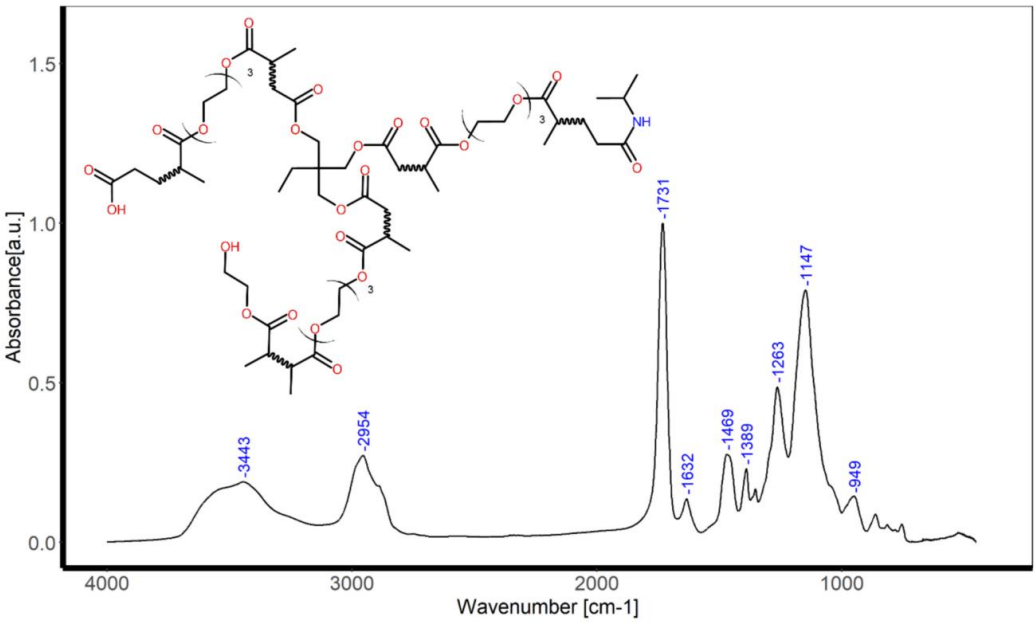


Fig. 2. FT-IR spectrum and suggested chemical structure of PTTANH.

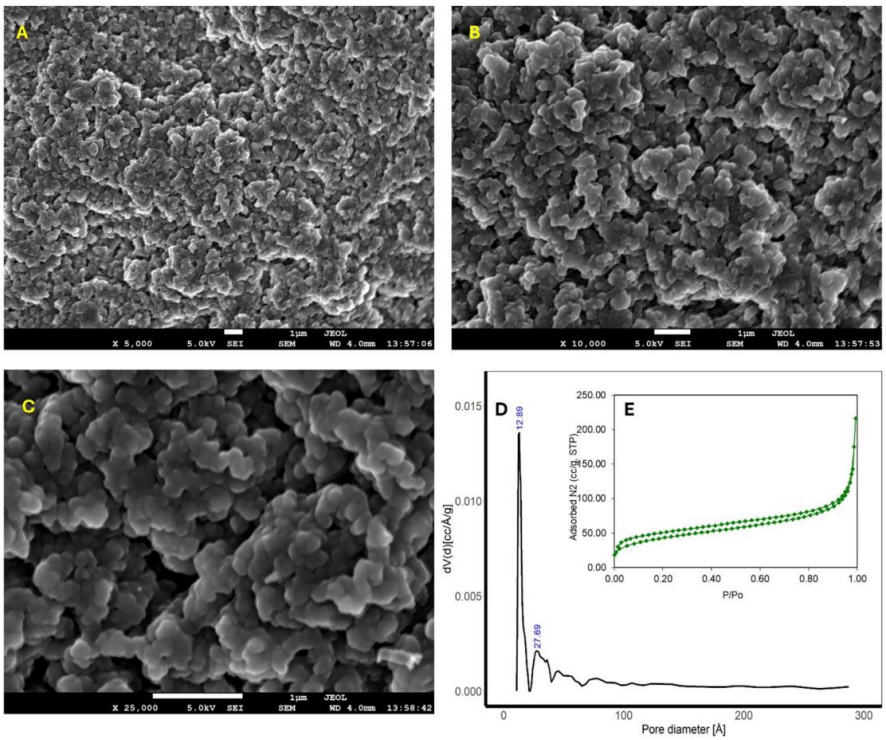


Fig. 3. SEM micrographs at ×5000 (A), ×10,000 (B), and ×25,000 (C) for PTTANH. Micropore distribution as calculated using the NLDFT (D). Nitrogen adsorption–desorption isotherm (E).

melting. These observations collectively suggested a high degree of cross-linking, resulting in high thermal stability (0–300 °C).

The SEM micrographs, captured at magnifications of ×5000, ×10,000, and ×25,000 (Fig. 3A–C), revealed a granular appearance, irregular surface morphology, and the presence of small internal pores dispersed within a three-dimensional rigid polymeric network, confirming the existence of a complex, interconnected network of multilevel pores composed of merged nanoparticles. PTTANH displayed a hierarchically structured porosity, as evidenced by its distinctive isotherm profile (Fig. 3D), which combines features of both type I and type IV isotherms [45], as previously reported for other hierarchically porous hyper-cross-linked polymers [46]. Specifically, a large volume of nitrogen adsorbed at low pressure indicated microporosity, while a hysteresis loop in the medium-pressure range was suggestive of mesopores. Additionally, continued nitrogen uptake was observed in the high-pressure range (0.8–0.99 P/P_0), suggesting the presence of macropores, confirmed by SEM micrographs. From the isotherm, a BET surface area of 111 $m^2\ g^{-1}$ and a micropore area of 43 $m^2\ g^{-1}$ were calculated [47]. Extensive BET parameters are reported in Table S6. The micropore-size distribution curve (Fig. 3D), obtained using NLDFT [48], showed two peaks, at 12.89 Å and 27.69 Å, similar to that reported for another carboxyl-functionalized hyper-cross-linked microporous polymer [49].

3.2. Interaction between PTTANH and target analytes

Various intermolecular forces may mediate the interaction between target analytes and PTTANH. With consideration given to the three primary functional groups incorporated into the polymeric structure (AA, NIPAM, and 2-HEMA), it is reasonable to infer that the predominant interactions involve the polar functionalities present in both the target analytes (typically carbonyl, carboxyl, amide, and halogen groups) and the functionalization of the polymer (amide, carboxyl, and hydroxyl groups), leading to hydrogen bonds and dipole–dipole interactions. Hydrophobic interactions may also involve the aromatic rings within the target analytes, the aliphatic segments of the polymer backbone, and the isopropyl group in NIPAM. The suggested main interactions of PTTANH-CECs are shown in Fig. 4; however, other interactions, such as dispersion forces, likely contribute to the overall interaction profile.

The material's hierarchical porosity may enhance interactions between the analytes and the sorbent [50,51]. This pore structure may function as a sieve, i.e., blocking larger impurities or particles (e.g., microplastics and algae) [52], thus allowing the smaller pores to remain accessible for effective interaction with micropollutants. However, further research is needed to test such a hypothesis.

3.3. Multivariate optimization of PTTANH-D-μ-SPE conditions

During the preliminary experiments, lower recoveries were observed for several analytes at pH 10 compared to pH 4 and 6.5. As a result, pH levels 4 and 6.5 were selected for inclusion in the PBD (Fig. S3). For the *desorption solvent*, acetonitrile (ACN) and a 1:1 mixture of ACN and methanol (MeOH) with 0.1 % formic acid (FA) were chosen as the two solvent options in the design, out of the six combinations tested during preliminary experiments (Fig. S4). The effects of various factors, including *Sample pH* (4 and 6.5), *Sample volume* (6 and 12 mL), *Sorbent amount* (20 and 40 mg), *Adsorption time* (20 and 60 min), *Desorption time* (5 and 20 min), *desorption solvent* (ACN and ACN:MeOH (1:1) with 0.1 % FA), and *Desorption solvent volume* (2 and 4 mL) were assessed. Analyte recoveries served as the response variable for the design. The details of the generated PBD design are provided in Table S7. The factors, *Sample volume* and *Adsorption time* were not significant and were excluded from the model by backward elimination.

The linear model was calculated considering the remaining factors. *Sorbent amount*, *Sample pH* and *Desorption solvent* were the most influential factors affecting the recovery of the analytes (the half-normal plot is reported in Fig. S5). The calculated regression equation is given by Eq. (1).

$$y = 56.8 + 4.7^{*}\alpha - 4.5^{*}\beta - 4.2^{*}\gamma + 3.4^{*}\delta - 2.9^{*}\varepsilon \quad (1)$$

Here, y represents the average recovery of the 27 target analytes from spiked wastewater and α, β, γ, δ, and ε represents *Sorbent amount*, *Sample pH*, *Desorption solvent*, *Desorption volume, and Desorption time,* respectively. The model resulted in an R^2 value of 0.96 and a p-value of 0.0003611, while the influence of the main effects is reported in Table S8. The residuals were normally distributed according to the Shapiro-Wilk and Kolmogorov-Smirnov tests (Table S9). Contour maps obtained through the design are reported in Fig. S6, displaying the relationship between sample pH and sorbent amount with ACN (A) and ACN:MeOH (1:1), 0.1 % FA (B) as desorption solvent.

3.3.1. Effect of sorbent amount

Examination of the PBD results showed a significant effect on the amount of PTTANH employed in the D-μ-SPE procedure (Fig. 5).

The use of 20 mg of PTTANH resulted in statistically lower recoveries for the majority of analytes from spiked wastewater, possibly due to

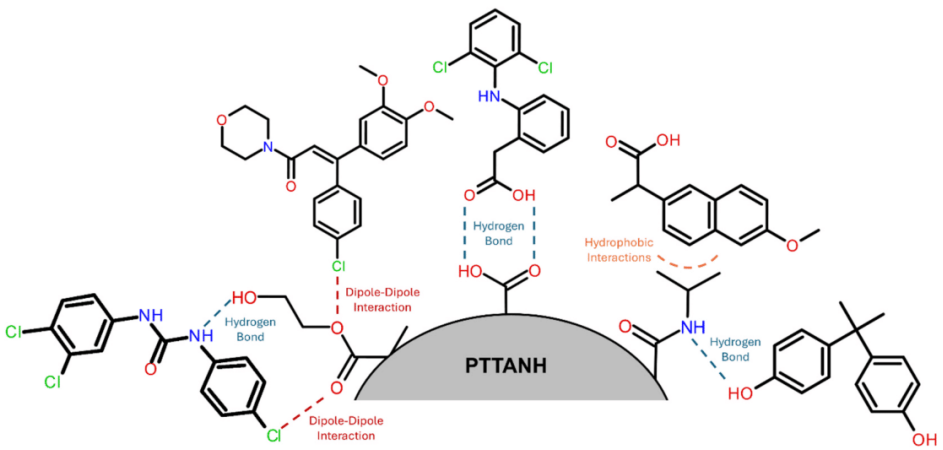


Fig. 4. Suggested interaction between the main functional groups in PTTANH and five analytes, specifically from right to left, BPA, naproxen, diclofenac, dimethomorph, and triclocarban.

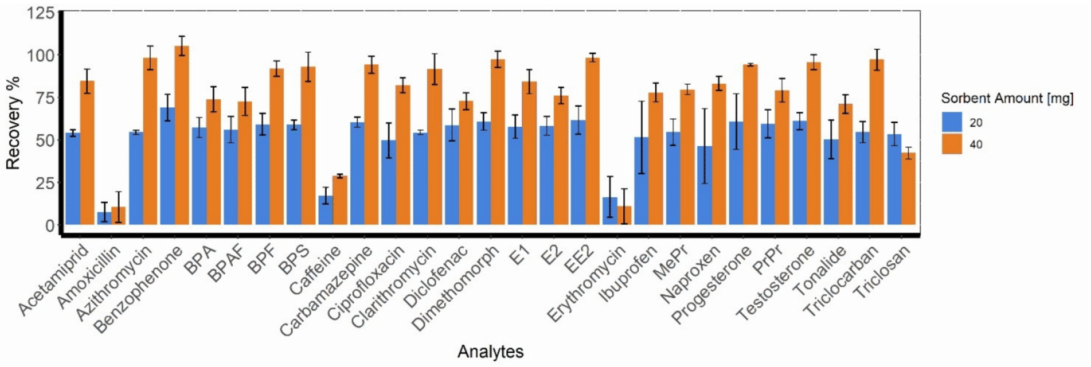


Fig. 5. Effect of PTTANH amount [mg] on recovery of the analytes. n = 3.

overloading the sorbent. However, the use of 40 mg remains competitive in terms of material consumption, i.e., still aligning with the principles of miniaturization [16].

3.3.2. *Effect of sample pH*

Sample pH can influence both the existing state of the target analytes in solution and the surface of the sorbent [53,54]. Experiments performed at pH 4 resulted in higher recoveries compared to the ones performed at pH 6.5 (Fig. S6). The improved adsorption of PTTANH at pH 4 could be attributed to a combination of factors. The acidic environment may also enhance hydrogen bonding by protonating the acrylic acid groups present, allowing them to interact more effectively with hydroxyl and carboxyl groups in the analytes. Additionally, many analytes like bisphenols, parabens, and certain pharmaceuticals are in their non-ionized form, increasing their hydrophobicity and favoring interactions with the hydrophobic regions of the sorbent.

3.3.3. *Effect of desorption solvent*

The ACN:MeOH (1:1) mixture acidified with 0.1 % FA desorbed the analytes from PTTANH better compared to pure ACN (Fig. S6). This behavior can be attributed to several factors, i.e., the selected mixture creates a balanced solvent polarity, which is often more effective at desorbing both polar and non-polar analytes from the sorbent [55,56]. Methanol, being more polar and protic, can enhance the solubility of polar analytes and participate in hydrogen bonding, helping to break the interactions between the analytes and the sorbent. Also, the addition of 0.1 % formic acid lowers the pH of the desorption solvent, thereby disrupting hydrogen bonding between the sorbent and analytes and facilitating their release [57–59].

Since recoveries obtained in PBD ranged from 70 %-120 % for most CECs, meeting SANTE guidelines [60], no further optimization designs were employed. The optimum conditions for PTTANH-D-μ-SPE for CECs recovery from wastewater were the following: 40 mg of PTTANH dispersed in 12 mL of sample at pH 4, adsorption time of 20 min,

Table 2
Validation results on wastewater for the developed PTTANH-D-μ-SPE-LC-MS/MS method.

Analyte	LOD [ng L^{-1}]	LOQ [ng L^{-1}]	Linearity [ng L^{-1}]	R^2	Intra-day [RSD%]	Inter-day [RSD%]	RE% (Low)[a]	RE% (High)[b]	ME%
Acetamiprid	59	178	100–33,300	1.000	4.7	5	77	84	−5
Amoxicillin	579	1755	5000–33,300	0.998	1.3	15	16	11	−85
Azithromycin	5	16	30–33,300	1.000	8.6	19	78	98	−25
BPA	17	50	30–33,300	0.993	4.3	11	76	74	−17
BPAF	37	112	100–33,300	0.995	11.0	10	80	73	4
BPF	24	73	30–33,300	0.994	8.1	3	90	92	−19
BPS	79	239	100–33,300	1.000	14.1	10	95	93	−26
Benzophenone	17	50	30–33,300	0.987	5.0	2	101	105	7
Caffeine	689	2089	5000–33,330	0.995	10.3	21	39	29	−78
Carbamazepine	12	38	30–33,300	0.991	2.9	9	80	94	−17
Ciprofloxacin	59	179	100–33,300	0.999	4.9	6	65	82	−15
Clarithromycin	2	6	30–33,300	0.999	12.2	6	80	92	−16
Diclofenac	55	165	100–33,300	0.992	4.0	14	77	73	−12
Dimethomorph	3	9	30–33,300	1.000	4.7	10	81	97	−13
E1	52	158	100–33,300	1.000	5.2	11	85	84	−4
E2	35	105	30–33,300	0.999	7.1	10	73	76	−1
EE2	56	171	100–33,300	1.000	5.6	15	99	98	−12
Erythromycin	460	1395	5000–33,330	0.841	9.9	16	54	11	−99
Ibuprofen	92	279	100–33,300	1.000	8.4	14	83	78	−20
Methylparaben	30	91	30–33,300	0.998	3.7	14	72	80	12
Naproxen	31	94	30–33,300	0.991	6.4	9	87	83	−12
Propylparaben	4	11	30–33,300	0.995	6.2	15	78	79	8
Progesterone	56	170	100–33,300	0.998	14.2	8	100	94	8
Testosterone	26	78	30–33,300	1.000	4.3	7	87	96	−11
Tonalide	4	12	30–33,300	0.988	10.5	11	69	71	−11
Triclocarban	50	151	100–33,300	0.990	5.8	14	90	97	7
Triclosan	52	156	100–33,300	0.998	3.5	15	60	42	16

[a] Spike at 500 ng L^{-1}.
[b] Spike at 10000 ng L^{-1}.

desorption time of 5 min with 4 mL of ACN:MeOH (1:1) with 0.1 % FA.

3.4. Method validation

The optimized PTTANH-D-μ-SPE-LC-MS/MS protocol was validated using spiked-treated urban wastewater. The validation parameters (Table 2) included recovery (%), reproducibility (RSD%), repeatability (RSD%), linearity (R^2), LODs and LOQs (ng L^{-1}).

With the exception of amoxicillin, caffeine, erythromycin and triclosan, recovery, tested at two spiking concentrations (500 and 10000 ng L^{-1}), was between 70 %–120 %. Although recoveries for triclosan were below the accepted limit (60 and 42 %), it did show good repeatability and was included for further validation, while amoxicillin, caffeine, and erythromycin were excluded from the analysis.

The repeatability and reproducibility were determined as the RSD of intra-day and inter-day recovery experiments, resulting in average values of 6.9 % and 11 %, respectively. The linearity (Fig. S7), assessed from the calibration curves obtained by spiking analytes in wastewater at five different concentrations (100, 500, 5000, 10000, and 33000 ng L^{-1}), resulted in R^2 values higher than 0.99 for all the analytes. LODs and LOQs were between 2–92 ng L^{-1} and 6–279 ng L^{-1}, respectively. The matrix effect was in the range ±30 % for all the analytes.

The validation results highlight the promising applicability of the developed PTTANH-D-μ-SPE-LC-MS/MS protocol for wastewater analysis for most compounds. A comparison with published methods (Table 3) demonstrates that PTTANH-D-μ-SPE-LC-MS/MS offers comparable recovery rates with lower amounts of sorbent (40 mg compared to conventional SPE cartridges, which typically use 150 or 200 mg), sample (12 ml compared to 50–150 mL) and typically less organic solvent making it both cost-effective and more environmentally friendly. When compared to an in-house SPE-LC-MS/MS method, the present method used less sorbent material (reduced by 20 mg), showed improved LOQs for the majority of compounds, reduced extraction time by >50 % [Andreasidou, unpublished data]. Additionally, the procedure eliminates the need for preliminary filtration of the effluent samples, which is needed to prevent blocking of the SPE cartridge.

3.5. Treated wastewater analysis

To test the method, wastewater samples were collected (24-hour proportional sampling) from four different wastewater treatment plants (WWTPs) in Slovenia (namely DK, LJ, KR and NM) and were analyzed for 24 CECs (Table 4). Of the 24 target compounds, in total, 19 CEC were detected, e.g., 13 compounds LOD < x < LOQ and six >LOQ. Naproxen, a nonsteroidal anti-inflammatory drug, was detected in the highest concentrations, i.e., 6270 ng L^{-1} in wastewater obtained from Domžale-Kamnik WWTP, approximately three times higher than the highest concentrations reported in previous studies (2190 ng L^{-1}). In contrast, in Ljubljana (702 ng L^{-1}), concentrations were close to the reported median (576 ng L^{-1}) [65]. Tonalide, a non-biodegradable polycyclic musk used as a fragrance in cosmetics, personal care products, and household items such as detergents, was present at the lowest quantifiable concentrations in three different WWTPs (290–378 ng L^{-1}), which were close to median values reported in the literature (280–500 ng L^{-1}) [66].

In Kranj WWTP, only Diclofenac (nonsteroidal anti-inflammatory drug) was quantified (520 ng L^{-1}). In Novo Mesto WWTP, only Diclofenac and Tonalide were above the LOQ (470 and 290 ng L^{-1}, respectively). For those wastewater treatment plants, Diclofenac concentrations were similar to previous studies on Slovenian wastewater samples (average: 480 ng L^{-1}) [65], while for Domžale-Kamnik and Ljubljana, concentrations were between LOD and LOQ (55–165 ng L^{-1}). The presence of Diclofenac in effluents is in agreement with the literature since the compound presents medium to negative removal efficiencies [67]. Samples obtained from the Ljubljana wastewater treatment plant had the highest detection frequency in CEC, where Clarithromycin had the highest concentration (2570 ng L^{-1}), followed by Azithromycin (2440 ng L^{-1}) and BPA (965 ng L^{-1}). Previous studies in Slovenia reported similar concentrations for BPA (971 ng L^{-1}), which showed the highest detection frequency among bisphenols [65].

Table 4
Results for CECs in wastewater effluent after PTTANH-D-μ-SPE-LC–MS/MS analysis.

CEC	[ng L^{-1}]			
	DK	LJ	KR	NM
Acetamiprid	n.d.	n.d.	n.d.	n.d.
Azithromycin	<LOQ	2440	<LOQ	n.d.
BPA	n.d.	965	n.d.	n.d.
BPAF	<LOQ	<LOQ	<LOQ	<LOQ
BPF	n.d.	n.d.	n.d.	n.d.
BPS	n.d.	<LOQ	n.d.	n.d.
Benzophenone	<LOQ	<LOQ	<LOQ	<LOQ
Carbamazepine	<LOQ	<LOQ	<LOQ	<LOQ
Ciprofloxacin	n.d.	n.d.	n.d.	n.d.
Clarithromycin	<LOQ	2570	n.d.	n.d.
Diclofenac	<LOQ	<LOQ	520	470
Dimethomorph	n.d.	n.d.	n.d.	n.d.
E1	n.d.	n.d.	n.d.	n.d.
E2	n.d.	n.d.	n.d.	n.d.
EE2	n.d.	n.d.	n.d.	n.d.
Ibuprofen	<LOQ	n.d.	n.d.	n.d.
Methylparaben	<LOQ	<LOQ	<LOQ	<LOQ
Naproxen	6270	702	n.d.	n.d.
Propylparaben	n.d.	n.d.	n.d.	n.d.
Progesterone	n.d.	n.d.	n.d.	n.d.
Testosterone	n.d.	n.d.	n.d.	n.d.
Tonalide	378	<LOQ	<LOQ	290
Triclocarban	n.d.	n.d.	n.d.	n.d.
Triclosan	n.d.	n.d.	n.d.	n.d.

Table 3
Comparison of PTTANH-D-μ-SPE-LC-MS/MS method performance with other methods for determination of organic contaminants in wastewater.

Analytes	Sample volume [mL]	Sample Preparation	Sorbent (amount)	Analysis	Extraction Time [min]	Solvent [mL][a]	LOQ [ng L^{-1}]	RSD %	Reference
CECs	50	SPE	Oasis ® HLB (60 mg)	LC-MS/MS	> 60	5	47–2396	8	*Unpublished
Antimicrobials	40	SPE	Oasis® WCX (150 mg)	LC-MS/MS	> 40	15	20–18,100	5	[61]
Drugs of abuse	140	SPE	Oasis ® HLB (500 mg)	LC-MS/MS	> 1440	120	100–48,800	11	[62]
CECs	50	SPE	Oasis ® HLB (200 mg)	LC-MS/MS	NA	18	0.2–13	8	[63]
Psychoactive substances	50	SPE	PolyClean ® 2H (200 mg)	LC-MS/MS	> 80	14	0.9–50	7	[64]
CECs	12	D-μ-SPE	PTTANH (40 mg)	LC-MS/MS	25	4	6–279	7	Present Study

[a] Total consumption of organic solvent.
* Andreasidou et al., unpublished data.

Clarithromycin and Azithromycin are prominent macrolide antibiotics commonly used for the treatment of diseases such as bronchitis, and both are known to have low removal rates in conventional WWTPs [68]. Thus, the sampling period, February of 2024, can be connected to the elevated concentrations due to possible higher antibiotic consumption rates. The sampling period may also explain higher concentrations compared to the levels reported in the literature since it typically includes low temperatures, which can negatively affect removal [67,69]. Compounds detected but not quantified included BPAF, BPS, Benzophenone, Carbamazepine, Ibuprofen and Methylparaben, which are all CECs with high detection frequency and are known for being poorly biodegradable in the activated sludge processes [65].

3.6. Evaluation of method sustainability

The SPMS evaluation yielded a total score of 6.0 out of 10, indicating an 'acceptable' assessment of the method's sustainability (Fig. S8). The various parameters influencing the final score are outlined with individual scores and color coding, where green represents the best performance, followed by yellow, orange, and red. Other solid-phase extraction techniques for liquid samples scored as follows: 2.84 for conventional SPE, 5.16 for magnetic D-μ-SPE using an alternative polymer, 6.21 for D-μ-SPE with a metal–organic framework, and 6.95 for SPME using an alternative polymer [40]. The proposed method achieved the best score for "amount of extractant" (green, 20/20) since the amount of sorbent was below 0.1 g. Four parameters were classified as yellow, including sample volume, nature of extractant, energy consumption and production of waste. For energy consumption, the score of the metric was influenced since shaking and centrifuge were utilized, favored by the selection of room temperature as the working condition. The parameters of extraction time and number of steps were classified as orange since the total time was between 15 and 60 min, and the number of steps was six. The score was lowered by the additional steps after extraction since evaporation with nitrogen was introduced.

The AGREEprep analysis produced a score of 0.42 out of 1 (Fig. S9), compared to automated SPE-LC-MS (0.52). The overall score remains below ideal due to the lack of automation and the ex-situ protocol, which resulted in a score of zero since sample preparation was conducted in the laboratory. Additionally, the volume of chemicals and materials used, along with the use of LC-MS/MS, employing organic solvents as mobile phases, contributed to a lower score [41].

4. Conclusions

In this study, a novel polymeric sorbent, PTTANH, was successfully synthesized, characterized, and optimized for the extraction of 27 contaminants of emerging concern (CECs) from treated urban wastewater using the dispersive micro-solid phase extraction (D-μ-SPE) procedure. Following optimization, the method was validated for 24 CECs, achieving limits of detection (LOD) and limits of quantification (LOQ) that are comparable to those reported for SPE in the published literature while using a lower amount of sorbent and sample volume. The method also performed well according to SPMS in comparison to similar methods, demonstrating reduced use of organic solvents and minimal sorbent requirements. When applied to real wastewater effluents, proof of concept was provided, revealing that specific CECs were detected above the LOD, while six CECs were quantified above the LOQ across all collected samples. The finding highlighted the variability in concentrations between different wastewater treatment plants. Notably, these findings underscore the advantages of the proposed protocol, particularly the use of copolymers, which allow for decreased amounts of sorbent, sample volume, and organic solvent, resulting in an eco-friendly and cost-effective extraction process. In future work, the copolymer will be functionalized with different chemical groups to enhance recovery rates, expand the range of extractable organic pollutants and lower LOQs. This ability can also be applied to tailor the sorbent for applications in other challenging matrices.

CRediT authorship contribution statement

Eirini Andreasidou: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lorenzo Martello:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David Heath:** Writing – review & editing, Data curation. **Dimitrios N. Bikiaris:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Dimitra A. Lambropoulou:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Ester Heath:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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3.2 Uptake of CECs in Tomatoes Irrigated With Treated Wastewater: Differences Between Hydroponically and Field-grown Tomatoes

3.2.1 Contaminant uptake in wastewater irrigated tomatoes

The paper "Contaminant uptake in wastewater irrigated tomatoes", by A. Kovačič, E. Andreasidou, A. Brus, A. Vehar, D. Potočnik, M. Jagodic Hudobivnik, D. Heath, M. Pintar, N. Kacjan Maršič, N. Ogrinc, U. Blaznik and E. Heath, was published in Journal of Hazardous Materials in April 2023 (doi.org/10.1016/j.jhazmat.2023.130964). The study was performed in collaboration with the Biotechnical Faculty of the University of Ljubljana, Slovenia, under the supervision of Prof. Dr E. Heath. In this work, I contributed to the literature review, scientific evaluation of the data and manuscript preparation.

As mentioned within the thesis, treated wastewater reuse for irrigation is gaining popularity as a solution to water scarcity issues. However, the insufficient removal of CECs from wastewater effluent may introduce risks associated with their uptake by crops. Moreover, from the literature, it is apparent that uptake studies typically evaluate a limited number of contaminants and usually neglect comparisons between different cultivation systems to evaluate the impact of growing systems on contaminant uptake. The study investigated the uptake of 14 CECs and 27 potentially toxic elements (PTEs) in tomatoes grown in soil (lysimeters) and soil-less media (hydroponically). Samples were analysed using LC-MS/MS (for 14 CECs) and ICP-MS (for 27 PTEs). The CECs were quantified in fruits irrigated with spiked potable water (hydroponics) and spiked wastewater (lysimeters), including BPS, 2,4-BPF, and naproxen, with BPS having the highest concentration (0.034–0.134 µg/kg f.w.). The levels of all three compounds were statistically more significant in tomatoes grown hydroponically (<LOQ - 0.137 µg/kg f.w.) than in soil (<LOQ - 0.083 µg/kg f.w.). Differences were also identified in the elemental composition between tomatoes grown hydroponically or in soil and tomatoes irrigated with wastewater and potable water. To the author's knowledge, this study is the first to address the uptake of BPA alternatives, such as BPS and BPF, in tomato fruits following irrigation with wastewater. Based on CEC uptake and elemental composition, no health risk was detected from consuming tomatoes grown hydroponically or in soil.

This paper fulfils two aims of the thesis: it compares the same set of CECs in two different growing experiments with distinct cultivation methods. It also evaluates dietary exposure following the consumption of tomatoes grown using alternative methods. The outcome of this study, conducted in 2020, was crucial for future experimental design, which was applied in the studies described further, including an expanded list of CECs monitored and additional treatments.

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Research Article

Contaminant uptake in wastewater irrigated tomatoes

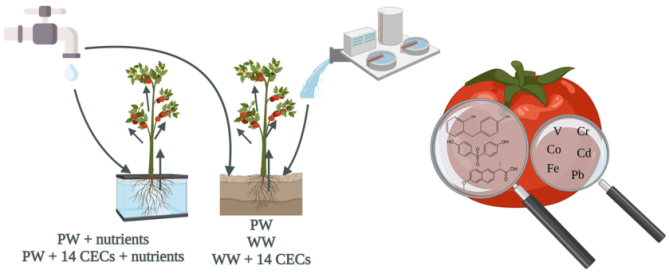
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HIGHLIGHTS

- Uptake study of 14 CECs and 27 elements in tomatoes grown in soil/soil-less media.
- Bisphenol S, 2,4 bisphenol F, and naproxen were detected in fruits.
- CECs presence and growing condition affect the elemental composition of tomatoes.
- Contaminants at determined levels showed low dietary chronic exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

As population growth and climate change add to the problem of water scarcity in many regions, the argument for using treated wastewater for irrigation is becoming increasingly compelling, which makes understanding the risks associated with the uptake of harmful chemicals by crops crucial. In this study, the uptake of 14 chemicals of emerging concern (CECs) and 27 potentially toxic elements (PTEs) was studied in tomatoes grown in soil-less (hydroponically) and soil (lysimeters) media irrigated with potable and treated wastewater using LC-MS/MS and ICP-MS. Bisphenol S, 2,4 bisphenol F, and naproxen were detected in fruits irrigated with spiked potable water and wastewater under both conditions, with BPS having the highest concentration (0.034–0.134 μg kg^{-1} f. w.). The levels of all three compounds were statistically more significant in tomatoes grown hydroponically (<LOQ - 0.137 μg kg^{-1} f. w.) than in soil (<LOQ - 0.083 μg kg^{-1} f. w.). Their elemental composition shows differences between tomatoes grown hydroponically or in soil and tomatoes irrigated with wastewater and potable water. Contaminants at determined levels showed low dietary chronic exposure. When the health-based guidance values for the studied CECs are determined, results from this study will be helpful for risk assessors.

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1. Introduction

Worldwide, many regions are experiencing an increase in water scarcity [27]. Even in countries with abundant water resources, uneven distribution can lead to water shortages, particularly during drier months [17]. For example, in the Mediterranean region, it is estimated that *"20% of the population lives under constant water stress and in summer, over 50% of the population is affected by water stress"* [11]. Agriculture remains the leading consumer of available freshwater, representing more than 70% of total usage. Thus, alternative water resources must be sought to complement conventional freshwater sources [33]. For instance, treated wastewater as a non-conventional water resource is already utilised globally for irrigated edible crops, e.g., cereals or leafy, root and fruit vegetables [29]. This practice yields certain benefits apart from saving freshwater by providing valuable nutrients, organic matter, and essential elements for crops [7].

Currently, the EU also favours utilising wastewater as an additional method to preserve available water resources and promote a circular economy. It also acknowledges that treated wastewater represents a reliable water supply with the potential benefits of utilising treated wastewater endorsed through a broad framework, including regulation (EU) 2020/741, which defines the minimum requirements for water reuse in agriculture [36]. This framework outlines the current European-wide minimum standards, including *E. Coli* levels, biochemical oxygen demand, total suspended solids and turbidity, with different concentrations depending on reclaimed water quality class. Many current conventional Wastewater Treatment Plants (WWTPs) are also redefining themselves as Water Resource Recovery Facilities (WRRFs) with the implementation of technical innovations, not only focusing on treated water but also on the production of nutrients, energy and other valuable bio-based materials from wastewater [23].

Despite this, doubts remain among many experts since it is known that conventional water treatment technologies are not able to completely remove Chemicals of Emerging Concern (CECs) or Potentially Toxic Elements (PTEs) [2,28]. The utilisation of reclaimed wastewater for irrigation may contribute to the introduction of CEC and PTEs in agroecosystems and their crop uptake [15,37]. Thus, an excess supply of macronutrients, CECs and trace elements in reclaimed water could affect plant growth and crop quality [24]. The bioaccumulation of CECs and PTEs in edible plants parts can also pose a health risk due to their subsequent introduction into the food chain [1,9,4,31]. For these reasons, a proper risk assessment is needed when considering using treated wastewater for irrigation.

Various vegetables have been studied under controlled conditions, including greenhouse experiments, field trials, and hydroponic systems [24,32,38]. However, there is still a significant knowledge gap regarding the uptake of PTEs and CECs in vegetables when using treated municipal wastewater. For example, most existing studies focus on either elements or small groups of organic contaminants, the most common being pharmaceuticals (e.g., carbamazepine, diclofenac, and ibuprofen), and only look at a single growing system [25,35,10]. Moreover, current research typically fails to consider the potential interactions between PTEs and CECs, and how they affect each other's accumulation and uptake in vegetables.

This paper examines the uptake of fourteen organic contaminants, including pharmaceuticals, hormones, and psychoactive substances, with some of them, e.g., BPA alternatives, being studied for the first time. It also assesses the occurrence of twenty-seven elements in tomatoes irrigated with treated wastewater. The tomato plant (*Solanum lycopersicum L. Rally*) was chosen as its roots, stems, leaves, and edible fruits make it an ideal research plant, and they are one of the most widely cultivated and consumed vegetables globally. We also compared two growing systems – soil (lysimeters) and soil-less (hydroponics) media – to explore the impact of different growing regimes and contaminant and element levels. A reliable and robust ultrasound-assisted extraction method was developed and optimised to facilitate this. Finally, dietary exposure and health risk assessments were estimated for the studied PTE and CECs. The findings thus provide a systematic insight into the uptake of PTE and CECs, offering valuable information for setting regulations regarding wastewater reuse in agriculture.

2. Materials and methods

2.1. Reagents and chemicals

General information about the investigated CECs is presented in the Supplementary information (Table SI-1). Bisphenol A (BPA; > 97%) was purchased from Merck (Darmstadt, Germany), isomers of bisphenol F (22BPF; 98%, 24BPF; 98% and 44BPF; > 98%), bisphenol S (BPS; > 98%), caffeine (CAF; 99%), ibuprofen (IB; > 98%), naproxen (NP; 98%), ketoprofen (KP; > 98%), diclofenac (DF; 97%), were purchased from Sigma-Aldrich (Steinheim, Germany), whereas estrone (E1; 99%), 17β-estradiol (β-E2; 98%), 17α-ethynyl estradiol (EE2; 98%) were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). Isotopically labelled surrogate standards: BPA (BPA-d_{16}, 98%), CAF ($^{13}C_3$-caffeine, 99%), DF ($^{13}C_6$-DF, 99.9%) were purchased from Sigma-Aldrich (Steinheim, Germany), IB (IB-d_3; 99.4%) from C/D/N Isotopes Inc. (Quebec, Canada), 17β-estradiol ($^{13}C_2$-βE2) from Camprto Scientific (Berlin, Germany) and BPS ($^{13}C_{12}$-BPS) and 44BPF ($^{13}C_{12}$-44BPF) from CanSyn Chem. Corp. (Toronto, Canada). All analytical grade solvents were obtained from J. T. Baker (Deventer, Netherlands). Formic acid (FA; 99%) and ammonium fluoride (NH_4F, 99.8%) were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Ultrapure water (18.0 MΩ cm at 25 °C) was prepared using the Milli-Q water purification system (Merck Millipore, Darmstadt, Germany).

Stock solutions (≈ 1.0 mg mL^{-1}) for CECs were prepared by dissolving each compound in methanol, and working standards (10, 100 and 1000 ng mL^{-1}) were prepared by appropriate serial dilutions of the stock solutions. Similarly, an internal standard solution (1.0 μg mL^{-1}) was prepared from a solution (1.0 mg mL^{-1}) of isotopically labelled compounds (BPA-d_{16}, $^{13}C_3$-caffeine, $^{13}C_6$-DF, IB-d_3, $^{13}C_2$-βE2, $^{13}C_{12}$-BPS and $^{13}C_{12}$-44BPF). All standards were prepared in methanol and stored in amber glass vials at 4 °C. Samples were filtered through 0.2 μm regenerated cellulose membrane syringe filters (Phenomenex, Torrance, USA).

For the elemental analysis, the additional reagents were obtained: nitric acid (HNO_3, 67 – 69% Superpure – for trace analysis) purchased from CARLO ERBA (Val de Reuil, France), a NIST 3113 Mercury (Hg) standard solution, Periodic table mix 1 for ICP (TraceCERT®, 33 elements) and a mixture of single elements: K, S, P, Mg, Mo, Sn and Sb (TraceCERT®) which were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Mixtures containing the single elements: Na (Certipur®) and U were purchased from MERCK (Darmstadt, Germany) and Honeywell, Fluka (Seelze, Germany), respectively. Reference materials: SRM 1573a (Tomato Leaves), SRM 1547 (Peach Leaves) and SRM 1570a (Spinach Leaves) were acquired from NIST (National Institute of Standards and Technology) (Gaithersburg, MD, USA).

The stock solution for the calibration curve of elemental analysis was prepared by dissolving a known weight of each compound in 5% HNO_3 to give the appropriate concentrations, i.e., for Hg from 0.1 ng g^{-1} up to 10 ng g^{-1} (0.1, 0.5, 1, 5 and 10 ng g^{-1}); for "mix 1 for ICP" from 0.1 ng g^{-1} up to 250 ng g^{-1} (0.1, 0.5, 1, 5, 10, 50, 100 and 250 ng g^{-1}). To achieve a higher calibration, single element standards Na, Ca, K, S, P and Mg (10,000, 5000, 2000 and 1000 ng g^{-1}) and Mo, Sn, Sb and U (10,000 and 1000 ng g^{-1}) were mixed with the stock solution. Measured concentrations were 0.1, 0.5, 1, 10 and 100 ng g^{-1}.

2.2. Experimental design and treatments

The study consists of two experiments using hydroponic tubs (Exp. 1) and lysimeters (Exp. 2) performed over one growing season in 2020.

Tomato *Solanum lycopersicum L. Rally* was grown in a greenhouse under controlled agronomic conditions until seedlings (with 5–6 leaves; eight weeks after sowing) were ready to be transferred to the experiments (Fig. 1a and b). Wastewater for plant irrigation was obtained from a local wastewater treatment plant in Ajdovščina, Slovenia, which treats municipal and industrial wastewater for a population of approximately 42,000 (8400 m^3/day).

Exp.1: Hydroponics

Seedlings were transplanted to 60-L covered hydroponic tubs in late June 2020. Tubs (120 cm × 60 cm) were covered with a sheet of Styrodur® (20 mm) in which six plants were fixed in prepared holes (φ50 mm) using inert polyester foam. The mean day and night temperatures were recorded hourly using a USB data logger (DL-120TH; Voltcraft, Hirschau, Germany). The daily mean temperature was between 16 °C and 29 °C and remained above 14 °C during the night. The relative humidity was between 56% and 98%.

In total, the experiment (Fig. 1a) included five tubs, each containing six plants: i) two tubs which served as controls, where plants were exposed to standard Hoagland Arnon nutrient solution prepared using potable water, containing the following ionic composition: 14.0 mM NO_3^-, 1.0 mM NH_4^+, 1.0 mM $H_2PO_4^-$, 6.0 mM K^+, 4.0 mM Ca^{2+}, 2.0 mM SO_4^{2-}, 2.0 mM Mg^{2+}, 10 μM Mn^{2+}, 5.0 μM Zn^{2+}, 30 μM B^{3+},15 μM Fe^{3+}, 0.75 μM Cu^{2+}, and 0.5 μM Mo^{6+}. The pH of the nutrient solution was adjusted to pH 6 by adding 74% H_2SO_4; electrical conductivity was 2 dS m^{-1}. The nutrient solution was pumped from the storage tub at a flow rate of 4 L h^{-1} using a drip irrigation system with one emitter per plant. The nutrient solution was circulated continuously and supplemented after a 50% drop with freshly prepared nutrient solution. Controls (two tubs, each containing six plants) were grown under CEC-free conditions (HPW), while three tubs (six plants each) contained potable water with added fertiliser and spiked with CEC at 0.1 mg L^{-1} (HSPW).

Exp.2: Lysimeter experiments

Seedlings were transferred to a lysimeter (WRRF in Ajdovščina; 45.8764 N, 13.9064 E, 106 m a.s.l.) on 19/5/2020. Ajdovščina has a

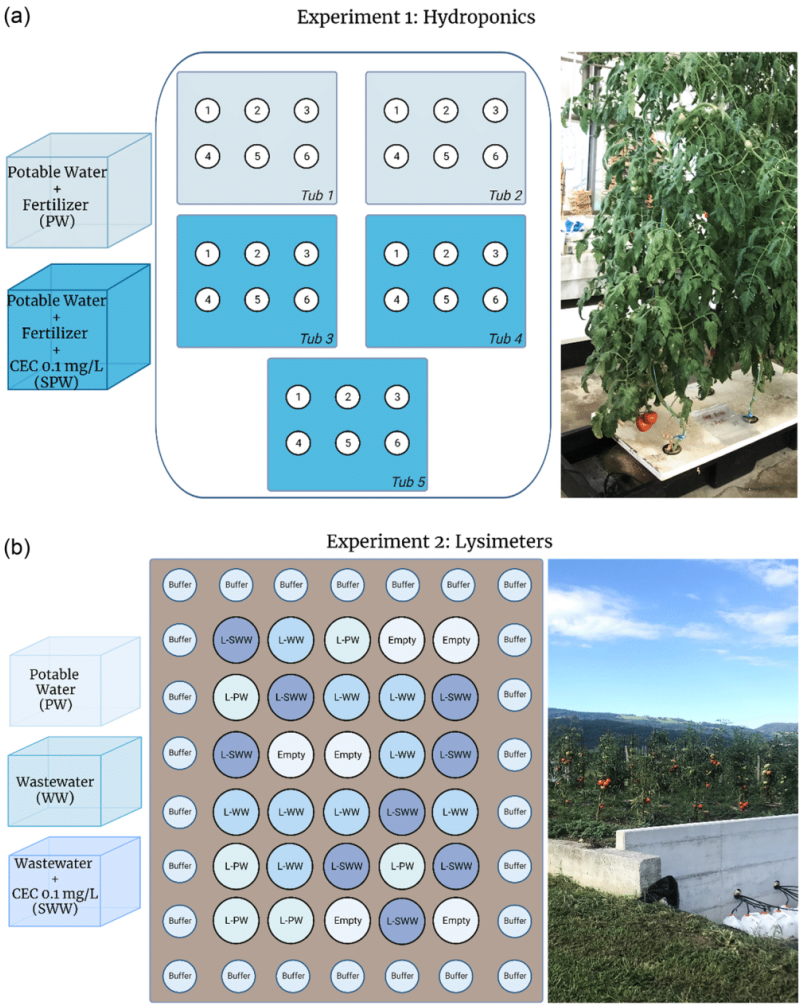


Fig. 1. a. Hydroponics experimental design (Exp.1). **b.** Lysimeter experimental design (Exp.2).

Mediterranean climate. The average air temperature from May to September 2020, measured at the nearest meteorological station Bilje (45.8964 N, 13.6245 E, 55 m a.s.l.), 21.7 km of areal distance from Ajdovščina, was 17.1, 20.3, 23.0, 23.2 and 19 °C. The sum of monthly precipitation measured at the nearest precipitation station Zalošče (45.8875 N, 13.7476 E, 75 m a.s.l.), 12.7 km of areal distance from Ajdovščina, was 108.4, 193.5, 118.0, 145.1, and 339.7 mm.

The tomatoes were grown under three sets (Fig. 1b) of conditions: i) six lysimeters served as a control, where plants were irrigated with potable water (LPW); nine lysimeters irrigated with wastewater from the WWTP (LWW), and nine lysimeters were irrigated with wastewater spiked with CEC at 0.1 mg L^{-1} (LSWW). Tomatoes in lysimeters were surrounded by a buffer zone, providing equal exposure to sun and wind. The media was freshly prepared on day zero and again at 2.5 months. Each lysimeter (40 cm in diameter, 100 cm and positioned 100 cm apart) contained one plant and local soil, i.e., Eutric brown soil on flysch (Table SI-3). The lysimeters were covered with a 2 mm thick plastic cover to prevent evaporation from the soil and rainwater from entering the lysimeters. Irrigation was achieved using an automated drip system (2 L h^{-1}). Plants were watered at 0.5 L per plant per day between 19/6–23/6, 1 L per day between 25/6–16/7 and 2 L every second day between 16/7–10/9. The total amount of irrigation water was 36 L.

All plants were fertilised at transplanting with 5 g N (KAN, 39%), 6.6 g P (Superphosphate 0:26:0; 26% P), and 17 g K (K_2SO_4; 50% K), corresponding to 70, 60, and 300 kg of nutrients per hectare, respectively. Additionally, the plants were fertilised with 5 g N (KAN, 39% N) three times at one-month intervals.

2.3. Sample collection and preparation

Tomato fruits were hand-harvested at the fully ripe stage. For the hydroponic experiments, the harvesting season was divided into three periods: (1st) 24/8–10/9, (2nd) 14/9–22/9 and (3rd) 28/9–9/10. Similarly, for lysimeter experiments, the first harvest was performed on 4/8, the second on 28/8 and the third on 11/9/2020.

During each harvesting, the ripened fruits were collected, washed with water, blotted dry and stored whole in plastic bags at − 20 °C. Frozen samples (in 60/600 g PE containers) were cut into pieces, freeze-dried and ground to a powder. Composite samples were prepared as follows: 1) all harvested fruits per plant (three or two plants selected) and per treatment (COM), 2) all harvested fruits per harvest (1st, 2nd and 3rd harvest, S1-S3) per treatment, 3) all harvested fruits per treatment from three or two plants per treatment (plants from tubs or lysimeters; P1-P3).

For determining CECs, each composite sample was prepared and analysed in triplicate. Briefly, 0.25 g of freeze-dried composite sample was weighed in a 10 mL centrifuge tube and spiked with the isotope-labelled internal standard mixture (V_{add} = 25 µL; c = 1 µg mL^{-1}). Ultrapure water (1 mL) was added, and the sample was vortexed (30 s) and shaken overnight (IKA KS 4000, 200 mot rpm). Afterwards, a 1:1 mixture of ethyl acetate:acetonitrile (4 mL) was added, and the mixture was sonicated (20 min) and centrifuged at 9000 rpm for 20 min. The supernatant was transferred to a 10 mL glass vial, and the residue was again suspended in 4 mL of ethyl acetate:acetonitrile (1:1) and further sonicated and centrifuged. The solvent from the combined extracts was removed using N_2 (40 °C). Once dry, the extracts were reconstituted in methanol:ultrapure water (0.5 mL, 1:1), centrifuged (9000 rpm, 20 min), filtered (0.2 µm regenerated cellulose membrane filters) and stored at −20 °C.

Samples of growth media spiked with CECs (100 mL) were analysed based on Kovačič et al. [18]. Briefly, 1 mL aliquot of water sample was diluted with 2 mL of ultrapure water, spiked with the internal standard mixture (30 µL, 1 µg mL^{-1}), and acidified (pH = 3) with 0.1 mL HCl_{aq} (c = 0.12 M). The samples were then loaded onto Oasis Prime HLB SPE cartridges (60 mg sorbent, 3 mL) at 2 mL min^{-1} using a Vacuum Manifold, washed with 1 mL of 10% $MeOH_{aq}$ and dried under vacuum (30 min). The elution step was performed using 5% formic acid in ethyl acetate (3 × 0.6 mL). Dried extracts (N_2, 40 °C) were reconstituted in a 1:1 solution of methanol:ultrapure water (1.0 mL), filtered through (0.2 µm) and stored at − 20 °C.

The samples for elemental analysis were prepared by weighing a known amount (0.1 g) of the freeze-dried tomato composite into pre-cleaned Teflon digestion vials to which 1 mL of 67 – 69% HNO_3 was added. The samples were digested using the ULTRAWAVE, Single Reactor Chamber Microwave Digestion System (MILESTONE, Italy). The program was as follows: heating to 240 °C for 20 min and held for 15 min at max 100 bar and max power: 1500 W. The digests were then diluted to 10 mL using Milli-Q water. Before analysis, samples were diluted with 5% HNO_3 in a ratio of 1:5 and 1:10.

2.4. Determination of CECs in tomato fruits

All samples were analysed using an UHPLC-Qtrap (Shimadzu Nexera X2 UHPLC, Kyoto, Japan) coupled to a linear ion trap quadrupole 4500 QqLIT-MS/MS (Sciex, Framingham, MA, USA). The separation was achieved at 40 °C using a Poroshell 120 EC-C18 column (100 × 3 mm; 2.7 µm particle size, Agilent Technologies, USA). The mobile phase was ultrapure water with 1 mM NH_4F and methanol with 1 mM NH_4F at a flow rate of 0.3 mL min^{-1}. The analyser was operated under electrospray ionisation (ESI) in negative mode with the following settings: curtain gas: 35 psi; collision gas: medium; source temperature: 550 °C; ion spray voltage: − 4.5 kV; ion source gas 1: 35 psi; ion source gas 2: 20 psi. The injection volume was 5 µL. The mobile phase gradient was as follows: 0 min, 70% B; 0–2 min, 80% B; 2–4 min, 85% B; 4–7 min, 100% B; 7.50 min; 100% B; 7.50–11.50 min; 70% B. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Transitions were optimised for the compound-dependent parameters (scheduled MRM), including declustering potential (DP), collision energy (CE), collision exit potential (CXP) and entrance potential (EP). Retention times and MS/MS optimised conditions are summarised in Table SI-2. Chromatograms were analysed using Analyst v1.6.3.

The method was validated for linearity, range, trueness, precision, the lowest limit of detection (LOD) and quantification (LOQ), analyte recovery, matrix effect and robustness following Eurachem guidelines [22]. The matrix-matched method was validated for four tomato-based matrices: three different tomato varieties collected from the supermarket (cherry, plump and beef tomato) and their pooled sample. All validation parameters were measured at low and high calibration levels, 2.5 and 15 ng g^{-1} for 13 CECs and 15 and 150 ng g^{-1} for BPA, respectively. Quality control samples (low calibrant) were included after every 20th injection. Procedural blanks (n = 4) samples were prepared and systematically included in each batch of analyses, while solvent blanks were analysed to evaluate potential carry-over. All samples were blank-corrected.

2.5. Elemental analysis of tomato fruits

The elemental analysis was performed as described by Potočnik et al. [30]. Briefly, B, Na, Mg, Al, P, S, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Cs, Ba, Hg, and Pb were analysed after digestion using an Agilent 8800 triple quadrupole inductively coupled plasma mass spectrometer, QQQ-ICP-MS (Agilent, USA). Internal standards (Y, Rh, Sc, and Gd) were added online in all modes, and external calibration was used to quantify the results. Blank samples and selected reference materials: SRM 1573a (Tomato Leaves), SRM 1547 (Peach Leaves) and SRM 1570a (Spinach Leaves) were added within each batch of samples. The LOD was calculated as three times the standard deviation of the blank samples and was for macro elements Mg 20, S 150, P 20, K 175 and Ca 1000 ng g^{-1} f.w., for microelements, B 18.5, Na 45, Al 12.5, Mn 0.5, Fe 8.5, Ni 0.75, Cu 0.75, Zn 9.0, Rb 0.25, Sr 0.5, Mo 0.25, Ba 0.25 ng g^{-1} f.w. and for trace elements V 0.02, Cr 0.35, Co 0.05, As 0.04, Se 0.25, Ag 0.02, Cd 0.01, Cs 0.05, Hg 0.015, Pb 2.0 ng g^{-1} f.w.

2.6. Dietary exposure and health risk assessment

Dietary exposure of the detected CECs and PTEs was estimated for children (toddlers) and adults by combining data on CEC and PTE concentration in composite samples of fresh tomatoes (Table SI-5) with the corresponding consumption levels from the EFSA Comprehensive European Food Consumption Database [34]. These are the most complete and detailed data currently available at the EU level. Only surveys appropriate for calculating chronic exposure were selected to assess repeated exposure (21 surveys for toddlers and 39 for adults). Dietary exposure in fresh tomatoes was assessed deterministically; average exposure was based on detected concentration and average consumption data, while high exposure was based on detected concentration and high consumption (97.5-percentile) data.

A temporary health-based guidance value has been derived only for BPS (DNEL 200 μg (kg day)$^{-1}$). Its ongoing assessment may change as BPS is suspected to affect human reproductive and hormonal systems [12]. The derived no-effect level (DNEL) is the level of exposure above which a human should not be exposed to a substance and was compared with the dietary exposure estimates for BPS.

The potential non-carcinogenic risk to human health resulting from consuming PTEs in tomatoes was assessed using the hazard quotient (HQ) approach calculated according to Eq. (1).

$$HQ = \frac{DE}{RfD} \quad (1)$$

DE is the dietary exposure (μg (kg day)$^{-1}$), and RfD is the reference dose (μg (kg day)$^{-1}$), Table SI-6). An HQ < 1 indicates the absence of significant non-carcinogenic health risk, whereas an HQ > 1 denotes a possibility of adverse health effects. The DE was calculated as follows:

$$DE = C_m \times C_d \quad (2)$$

where C_m (μg g^{-1} f.w.) is the concentration of PTE in fresh samples and C_d is the consumption of fresh tomatoes, where data are different on an individual level (toddlers and adults). In case the latest reference dose was available as a tolerable daily intake level (μg day^{-1}), an average weight of a toddler of 12 kg and an adult of 70 kg was assumed [34].

Since the concentrations of PTEs were measured in freeze-dried samples as ng g^{-1} d.w., all units were converted to ng g^{-1} f.w. (Eq. 3).

$$c_{f.w.} = \frac{c_{d.w.} \times (100 - W)}{100} \quad (3)$$

Here $c_{f.w.}$ is the concentration of PTEs based on fresh weight, $c_{d.w.}$ concentration based on dry weight and W [%] content of water in tomato, which is 95%.

2.7. Data processing and analysis

Data processing and visualisation were performed using R-based software (version 3.6.1), SigmaPlot 14 (Systat Software GmbH) and SIMCA (Sartorius). All data sets were checked for normal distribution using the Shapiro-Wilk test and for homoscedasticity of the variances using Levene's test or Brown-Forsythe test. The influence of compound type, growing conditions and harvesting time on the uptake of CECs was assessed using the Wilcoxon Ran-Sum or Kruskal-Wallis test at the significance level of $p \leq 0.05$, Principal Component Analysis (PCA) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA). A significant difference in elemental concentrations of control samples (HPW, LPW) and hydroponics samples (HPW, HSPW) was assessed using the t-test or Mann-Whitney U test, while in the case of lysimeter samples, it was assessed using One-way ANOVA or Kruskal-Wallis test.

3. Results and discussion

3.1. Method optimisation and validation

3.1.1. Optimisation of sample preparation for CECs extraction from tomatoes

The mixture of standards and internal standards solution was added in methanol to 0.25 g of lyophilised tomato and covered with 1 mL of different solvents (methanol, ultrapure water, acetonitrile). The sample was then thoroughly mixed and shaken in the dark at room temperature until all of the solvent had evaporated (12 hrs), ensuring the homogeneity of the added compounds in the analysed tomato sample. For ultrasound-assisted extraction of CECs from tomato fruit samples, different solvent combinations of ethyl acetate, methanol, acetonitrile, and the addition of formic acid, including different ratios and volumes (3–10 mL), were tested. For this purpose, a 50 ng g^{-1} concentration of each target CEC was added to 0.25 g of fruit sample. Ethyl acetate/acetonitrile (1/1) gave the highest extraction efficiency (42–127%) for all CECs, and thus, it was selected for further testing. Different solvents (ultrapure water, methanol, acetonitrile, 0.25–1 mL) for spiking tomato samples with labelled and unlabelled CECs were also tested. The supernatant was centrifuged and processed as described in Section 2.3.

Ultrasound-assisted extraction (internal standards covered with 1 mL in ultrapure water, two times 4 mL of ethyl acetate:acetonitrile = 1:1) of dry tomato fruit samples was also compared with QuEChERS extraction of the fresh tomato fruit. The obtained analyte recovery and method precision using QuEChERS were lower, and the matrix effect higher (RE=30–110%, method repeatability RSD=3–33%, ME= ± 10–3000%) when compared to the optimised ultrasound-assisted extraction method (RE=65–92%, method repeatability RSD = <15%, ME= ± 1–86%).

3.1.2. Method validation

Method validation was performed on four tomato-based matrices 1) beef tomato, 2) plump tomato, 3) cherry tomato and 4) pooled tomato matrix. Accordingly, all samples were blank-corrected. Sensitivity was evaluated in terms of LOD and LOQ, calculated as three times and ten times the standard deviation of the baseline (standard addition) divided by the slope of the calibration curves, respectively. Using the LOQ as the lowest calibration standard, LOQs were 0.5 or 1.0 ng g^{-1} (0.025 or 0.05 μg kg^{-1} f.w.) for 13 CECs and 40 ng g^{-1} (2.0 μg kg^{-1} f.w.) for BPA. The linearity of the method was obtained using spiked matrix-matched samples at different calibration points across the working range.

The calibration range for the analysis consisted of seven/eight calibration points (0.5/1–25/50 ng g^{-1} for 13 CECs and 40–500 ng g^{-1} for BPA) using the LOQ as the lowest calibration level, whereas the concentration of each of the internal standards remained fixed (25 ng g^{-1}). Each calibrant was prepared in triplicate. The coefficient of determination (R^2) was > 0.99 for all tested CEC, except for EE2 ($R^2 = 0.98$) when using a linear calibration curve. The analyte recovery is reported as the ratio between peak areas of analyte/internal standard spiked before and after extraction, whereas internal standard spiked after sample extraction is used to cover instrument signal variations. The mean recoveries were 64.7–91.5% and 55.6–94.0% at the low and high levels. The matrix effect (± 1–86%) was based on a comparison between the analyte peak area normalised with the internal standard when the analyte mixture was added to tomato extract compared to when added to the final reconstituted solvent (methanol:ultrapure water=1:1). Precision was expressed in terms of method repeatability, calculated as the relative standard deviation (RSD) of three parallel samples ($n = 3$), and instrumental repeatability, determined as the RSD of three consecutive injections of the same sample ($n = 3$). The RSDs of the calibrants were $< 15\%$. Instrument precision was $< 10\%$, except for naproxen at the higher level (19.7%). Trueness was expressed as [(experimental value - spiked value)/spiked value] ($n = 3$) and was less than 15% at both levels. The method's robustness was tested by

comparing the slope (k) of four blank corrected calibration curves. The calculated RSD of k_1, k_2, k_3 and k_4 was less than 20%, except for ketoprofen (25%), which also had the highest variations in ME between different tomato-type matrices. The developed method is robust for any tomato variety and can be used to quantify the tested CECs. The uptake of investigated CEC in this study was quantified using a matrix-matched calibration line from pooled tomato samples. All validation results are summarised in Table 1. No potential carry-over was observed within analysed batches, and the RSD of quality control samples was below 10%.

3.2. CECs in the irrigation water

The amounts of individual CECs in the spiked irrigation media after 1.5 months varied between 50 and 100 μg L^{-1}, except for E1 (<50 μg L^{-1}). After 2.5 months, BPA, caffeine, naproxen, ketoprofen, and ibuprofen remained above 50% of the initial theoretical concentration (c_i = 60–80 μg L^{-1}), which may be a result of degradation processes. Water samples were also taken from hydroponic tubs before and after refilling. The concentration of CEC before refilling was (except for naproxen) < 10 μg L^{-1}, in line with the concentration determined in irrigation media after refilling. Consequently, 1 m^3 irrigation media was freshly prepared after 2.5 months and analysed after four months of field experiments. Most CECs were > 50 μg L^{-1} in the water from the reservoir (m^3) and hydroponic tubs. The concentrations of CECs in all controls (without CECs) were <LODs.

3.3. Uptake of CECs in tomato fruit

All concentrations (Table SI-4) are given on a fresh weight basis (μg kg^{-1} f. w.), considering the composition of a ripe tomato: 95% water and 5% dry matter [16]. The RSDs of composite samples were < 20% (n = 3), confirming the homogeneity of the samples. Interestingly, out of the 14 compounds selected in this study, BPS, 24BPF, and naproxen were detected in tomato fruits grown in spiked media under both experiments (Fig. 2). No significant difference in the amount of CECs in tomatoes between different harvests (p-value = 0.754) was observed. A Wilcoxon Ran-Sum test showed a significant difference (p-value = 3.36E-13) in the uptake of CECs between tomatoes grown hydroponically or in soil, with more significant uptake when grown hydroponically.

The PCA, performed on the whole data set, grouped samples into three groups 1) hydroponically grown tomatoes without CEC, 2) hydroponically grown tomatoes with CEC, and 3) soil-grown tomatoes (Fig. 3). The data set obtained from different growing conditions with added CEC was formed into two groups, hydroponically and tomatoes grown in lysimeters, using the OPLS-DA method (Fig. SI-2). Slight separation in OPLS-DA of non-spiked (LWW and LPW) from spiked (LSWW) conditions is also observed (Fig. SI-2), while no separation between lysimeters treated with non-spiked wastewater (LWW) and potable water (LPW) was observed (Fig. SI-3) suggesting that reusing treated wastewater can be considered a reliable water supply in agriculture. The concentrations of BPS were higher in tomatoes grown hydroponically (0.071–0.134 μg kg^{-1} f. w.). However, in soil-grown tomatoes, BPS was also above LOQ (0.034–0.070 μg kg-1 f. w.), while 24BPF (<LOQ-0.081 μg kg^{-1} f. w.) and naproxen (0.050–0.137 μg kg^{-1} f. w.) were present above LOQ only in the case of hydroponically grown tomatoes. The results of the Kruskal Wallis test show a difference (p-value = 6.09E-14) in the uptake between compounds. The average concentrations of CECs present in hydroponically and soil-grown tomatoes were 0.08 ± 0.03 μg kg^{-1} f. w. and 0.06 ± 0.01 μg kg^{-1} f. w., respectively.

The uptake of BPS and 24BPF has not been previously examined in the literature. However, in line with Bueno et al. [6], naproxen was ≤LOQ in tomato fruits irrigated with reclaimed water under field conditions with added CECs (1 μg L^{-1}). Contrary to our findings, the authors observed the uptake of caffeine and diclofenac. Most studies that

Table 1
Method performance of a robust analytical method for determining 14 CECs in tomato fruit sample, including method repeatability, instrumental repeatability, trueness, linearity, extraction efficiency, matrix effect, LOD, LOQ and robustness.

Compound	Method repeatability (RSD)		Instrumental repeatability (RSD)		Trueness (%)		Linearity			Extraction efficiency (%)		Matrix effect (%)		LOD	LOQ	LOD	LOQ	Robustness RSD
	LC	HC	LC	HC	LC	HC	R^2	[ng g^{-1} d.w.]*	[μg kg^{-1} f.w.]* *	LC	HC	LC	HC	[ng g^{-1} d.w.]*	[ng g^{-1} d.w.*]	[μg kg^{-1} f.w.]* *	[μg kg^{-1} f.w.]* *	k_1-k_4
BPS	14.6	6.8	2.0	0.5	13.6	1.0	0.99	0.5–25	0.025–1.25	66.6	66.1	3.0	3.0	0.04	0.5	0.0020	0.025	4.4
BPA	10.3	3.5	3.5	3.5	9.1	6.7	0.99	40–500	2–25	94.0	91.5	22.4	37.1	4.1	40.0	0.21	2.0	16.5
22BPF	12.9	15.1	5.9	6.1	6.5	4.4	0.99	0.5–25	0.025–1.25	89.3	81.4	86.0	87.4	0.003	0.5	0.0002	0.025	20.5
24BPF	10.5	12.9	5.8	5.5	11.7	4.9	0.99	0.5–25	0.025–1.25	83.6	78.3	29.6	34.7	0.004	0.5	0.0002	0.025	21.1
44BPF	13.6	8.2	5.5	6.9	8.4	8.3	0.99	0.5–25	0.025–1.25	84.0	76.1	9.4	2.0	0.02	0.5	0.0010	0.025	5.2
Caffeine	9.3	12.1	4.9	9.8	12.7	6.0	0.99	0.5–50	0.025–2.25	79.9	85.2	83.6	4.7	0.02	0.5	0.0010	0.025	3.5
Ketoprofen	11.8	14.0	0.7	3.0	13.9	2.4	0.99	1.0–50	0.05–2.25	72.9	88.6	80.6	27.9	0.27	2.5	0.014	0.05	24.9
Ibuprofen	13.8	7.4	2.2	6.8	3.7	2.0	0.99	1.0–25	0.05–1.25	76.5	70.3	84.6	6.5	0.24	1.0	0.012	0.050	6.1
Naproxen	7.5	10.2	19.3	2.0	9.6	0.9	0.99	0.5–25	0.025–1.25	80.6	82.2	75.4	46.2	0.02	0.5	0.0010	0.025	7.3
E1	6.5	5.8	5.8	3.8	3.7	0.8	0.99	0.5–25	0.025–1.25	81.7	88.0	1.4	7.3	0.008	0.5	0.0004	0.025	7.2
β-E2	12.8	6.9	2.7	4.5	41.5	3.2	0.99	0.5–25	0.025–1.25	80.7	83.7	8.2	5.0	0.08	0.5	0.0040	0.025	3.8
EE2	11.7	6.9	6.8	5.6	4.2	11.9	0.98	1.0–50	0.05–2.25	79.3	87.7	27.8	10.9	0.39	1.0	0.020	0.050	4.1
E3	8.2	8.5	9.3	5.5	14.0	8.7	0.99	0.5–25	0.05–1.25	55.6	64.7	7.2	3.5	0.10	0.5	0.0050	0.025	2.9
Diclofenac	6.5	7.8	1.6	1.6	9.1	1.0	0.99	0.5–25	0.05–1.25	84.0	81.0	83.5	6.1	0.05	0.5	0.0025	0.025	3.0

* Concentration in ng g^{-1} dry weight [ng g^{-1} d.w.], * *Concentration in μg kg^{-1} fresh weight [μg kg^{-1} f.w.]

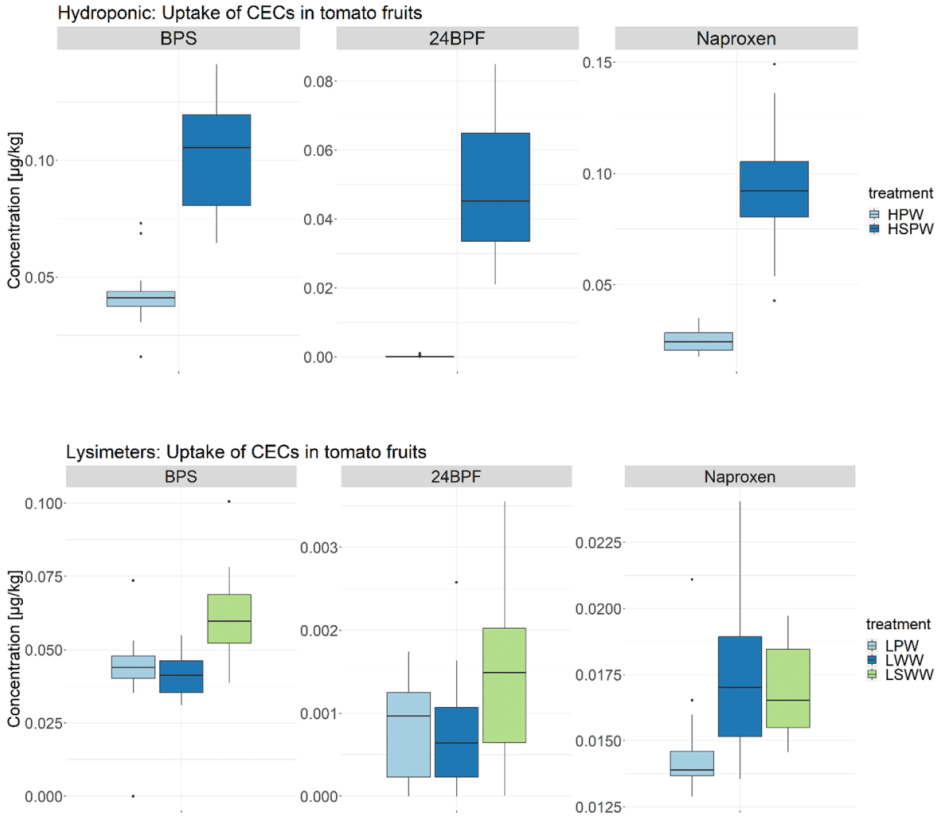


Fig. 2. Levels of CECs (µg kg^{-1} f. w.) in composite samples of hydroponically (HPW, HSPW) or soil-grown tomatoes (LPW, LWW, LSWW) under different conditions (treated with potable or wastewater in the absence or presence of CECs).

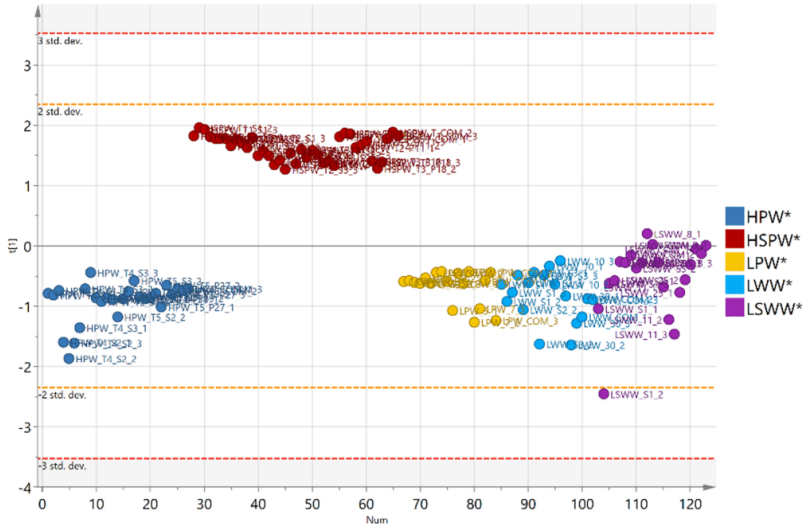


Fig. 3. Principal Component Analysis (PCA) of the whole data set (detected concentration of CECs from the composite tomato samples). Score plot PC1 vs PC2.

investigated caffeine uptake do report its presence in tomato fruits [25, 3]; however, this was not the case in our study. A possible explanation could be different studied concentrations and compositions of compounds added in growing media and field conditions (e.g., soil type). The uptake of diclofenac was also not observed in the study by Christou et al. [10], where they performed hydroponic cultivation using water with added pharmaceuticals. In the case of soil irrigation and prolonged WW irrigation, diclofenac was determined in tomato fruits 11.63 μg kg^{-1} f.w. Christou et al. [8]. Unlike Lu et al. [19], who studied hydroponically grown tomatoes with the addition of CECs (c_i = 50 μg L^{-1}) in wastewater and Margenat et al. [24], who studied tomatoes grown in peri-urban agriculture irrigated with no addition of CECs in reclaimed water, we did not observe the uptake of BPA nor 44BPF, respectively.

Given that the irrigated (lysimeters) and nutrient (hydroponics) medium were spiked with compounds at the same concentration level, it is interesting that only BPS, 24BPF and naproxen were determined in tomato fruits in both experiments. The absence of most of the added compounds in irrigation media in tomato fruits can be explained by the greater water flow (translocation) to leaves, leading to a higher accumulation of CECs in leaves than in the fruit, as observed by Martinez-Piernas et al. [25]. Secondly, CECs taken up by the plant can be metabolised to phase I metabolites (e.g., hydroxylation), as well as forming phase II metabolites, e.g., conjugation of the parent compound or phase II metabolites with glucose, glucuronic acid, malonic acid [26]. The metabolisation of the parent compounds supports our observation, e.g., the absence of parent compounds in tomato fruits.

3.4. Occurrence of elements in tomato fruit

The results of mean elemental concentrations in tomatoes from different growing conditions are presented in Fig. 4, including macro elements: K, Ca, Mg, P and S; microelements: Na, Rb, Fe, Zn, B, Cu, Mn, Al, Mo, Ba, Ni and Sr and trace elements: Cr, Pb, Se, Cd, Cs, Co, V, As, Ag and Hg. Within recent literature, a wide range of elemental concentrations in tomatoes has been reported that depend on variety, substrate, climate and irrigation-fertilisation strategies. Our results of HPW indicate a similar concentration range to the study performed by Gundersen et al. [14] in Sager et al. [21] in Austria, except for Mo, Ni, Cd, Cr, and Pb and Al, Fe and Rb, respectively (Table SI-5). Giorgia et al. [13] had lower macro and micro elements concentrations in Sicily but higher or comparable concentrations of trace elements. Concentrations of microelements in Morocco were much lower than in our study, while Cd was comparable and Pb higher [20]. Tomatoes from Salvador had higher concentrations of potentially toxic elements: Al, Co, Cr, Hg and V [5]. The concentrations in tomatoes irrigated with treated wastewater are comparable to or lower than those in the literature, where the concentrations for microelements ranged from 0.095 mg kg^{-1} f.w. (Mo) to 30 mg kg^{-1} f.w. (Fe) (Fig. 4), and for the trace elements from 0.454 μg kg^{-1} f.w. (Hg) to 0.361 mg kg^{-1} f.w. (Pb) (Table SI-6).

Statistical analyses (Table SI-7) showed that all the elements between HPW and LPW tomatoes are significantly different, except Se, Rb and Hg. Concentrations of Na, Mg, P, S, K and Mo were significantly higher in HPW tomatoes, indicating their uptake from the applied fertiliser. In comparison, concentrations of Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Ag, Cd, Cs, Ba, and Pb were significantly higher in LPW,

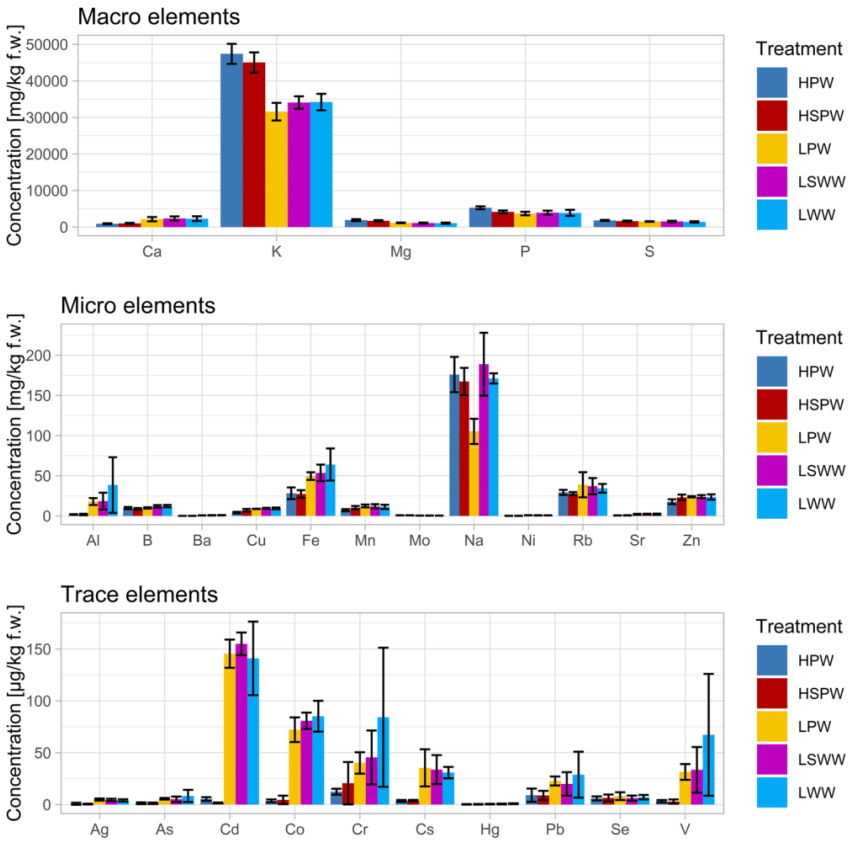


Fig. 4. Mean concentrations of macro, micro and trace elements in different treatments.

indicating their higher concentrations in soil and their uptake into the tomato fruits. The most remarkable level of 27 times higher in LPW than HPW was observed for Cd. A similar observation was also noted in the study performed by Gundersen et al. [14]. Possible explanations are 1) a more accessible form of Cd can be present in the soil, 2) more intensive microbiological activity in the soil can promote uptake of Cd in the plant, and 4) the root system in the soil is bulkier and allows higher utilisation of Cd from the substrate. The separation of the HPW and LPW tomatoes was also confirmed with OPLS-DA analysis (Fig. SI-4). Hydroponics tomato comparison showed significantly higher Mg, P, S and Cd concentrations in HPW tomatoes.

In comparison, concentrations of Mn, Cu and Zn were significantly higher in HSPW, which could be the consequence of the altered uptake of elements by added CECs, i.e., reducing the uptake of the first group of elements and increasing the uptake of the second group. The separation of the HPW and HSPW tomato samples by OPLS-DA can be seen in Fig. SI-5. Comparing tomato samples grown in lysimeters (Fig. SI-6), only B and Na were significantly higher in LWW tomato samples, and no elements were significantly higher in LPW samples, indicating higher uptake of B and Na from the treated wastewater. Surprisingly, there were no significantly different concentrations of elements between LWW and LSWW tomato samples (Fig. SI-7). Therefore, there is no evidence that CECs addition affects the uptake of elements from treated wastewater. The PCA (Fig. 5) performed on the whole data set of measured elements in tomato fruit grouped the samples grown in HPW, HSPW and LPW, which was also confirmed with OPLS-DA (SI-2.2) but did not separate tomatoes grown in LWW and LSWW. There were two outliers in the LWW group, named LWW_S1_COMP and LWW_30_COMP, which are the composite sample from the first sampling and the composite sample by plant 30. Both have significantly higher Al, V, and Cr and significantly lower concentrations of P and Zn than other LWW samples.

3.5. Dietary exposure and health risk assessment

Average chronic exposure to the detected CECs (24BPF, BPS, naproxen) from consuming fresh hydroponically produced tomatoes for children (toddlers: 1–3 years) was 0.01–0.13 ng (kg bw day)$^{-1}$, 0.02–0.27 ng (kg bw day)$^{-1}$, 0.02–0.26 ng (kg bw day)$^{-1}$ with high exposure of 0.07–0.48 ng (kg bw day)$^{-1}$, 0.15–0.97 ng (kg bw day)$^{-1}$, 0.14–0.93 ng (kg bw day)$^{-1}$. The highest exposure for children was 0.97 ng (kg bw day)$^{-1}$ for BPS. Results for chronic exposure of adults were lower and average consumption of fresh tomatoes resulted in exposures of 0.01–0.08 ng (kg bw day)$^{-1}$, 0.01–0.15 ng (kg bw day)$^{-1}$, 0.01–0.15 ng (kg bw day)$^{-1}$. Exposure levels for high consumers were in the range of 0.05–0.26 ng (kg bw day)$^{-1}$, 0.09–0.51 ng (kg bw day)$^{-1}$, 0.09–0.50 ng (kg bw day)$^{-1}$.

The selected population groups are less exposed via tomatoes grown in soil than soil-less media, which is expected since the CEC concentrations are also lower. Only the concentration of BPS was measured above the LOQ. The average chronic exposure to BPS for children was 0.02–0.16 ng (kg bw day)$^{-1}$, and for adults, 0.01–0.09 ng (kg bw day)$^{-1}$. The highest estimated exposure of BPS was 0.59 ng (kg bw day)$^{-1}$ for toddlers as high consumers of fresh tomatoes. By comparing the highest exposure estimates of BPS with the currently derived no-effect level of 200 μg (kg bw day)$^{-1}$, there is no health concern for any age group. However, such an assessment has a provisional status. The obtained exposure results will be helpful in risk characterisation when the health-based guidance values for the studied CECs are determined.

Regulation (EC) 1881/2006 of the European Commission has set maximum levels for certain toxic elements in vegetables. For example, the maximum level of 0.05 mg kg^{-1} of Cd and 0.10 mg kg^{-1} for Pb were established. Our results indicate the highest estimated daily intake (EDI) of 16.1 ng (kg_{bw} day)$^{-1}$ for Cd in LSWW and 3.01 ng (kg_{bw} day)$^{-1}$ for Pd in LWW (Table SI-9) that are negligible concerning daily intake budgets. Further, average and high chronic exposures to detected PTEs from fresh tomatoes grown in all five different treatments and hazard quotients for toddlers and adults are given in Table SI-9 and Table SI-10. The data show that all the calculated hazard quotients for each PTE in each treatment were < 1, and the hazard index was < 1 for each treatment, indicating the absence of significant non-carcinogenic health risk in the case of consuming tomatoes from all five different treatments.

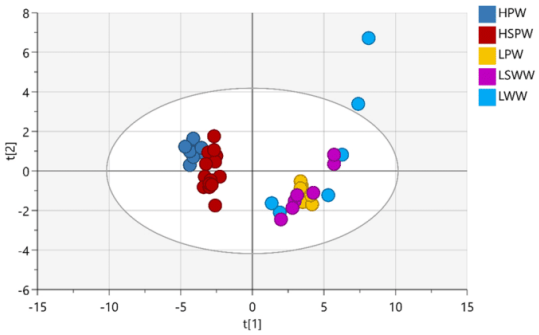


Fig. 5. Principal Component Analysis (PCA) results for the whole data set of elements in tomato fruit (determined concentrations from the composite tomato samples). Scores Scatter Plot t[1] vs t[2].

4. Conclusion

The present study provides new evidence on the levels of CECs in tomato fruits produced under soil and soil-less growing conditions using treated wastewater, as well as the influence of those chemicals on PETs occurrence. Bisphenol S, 24BPF and naproxen out of the 14 spiked CECs were detected with higher uptake in the case of hydroponically grown tomatoes. Importantly, we showed that the elemental composition in tomatoes was affected by the addition of CECs. Based on CEC uptake and elemental composition, no health risk from consuming tomatoes grown hydroponically or in soil was detected. However, our data also revealed how different irrigation regimes affect the uptake of chemicals. The results of this study add to the argument that, if properly monitored, reusing treated wastewater for irrigation can be a safe approach in agriculture and can support the decisions of policymakers and stakeholders in creating future legislative frameworks related to sustainable water resource management in agriculture. Nevertheless, more studies need to be performed for a definite answer on the safety of wastewater reuse for irrigation, providing more information about CECs, including the formation of metabolites and transformation products, possible synergistic effects and different agricultural practices.

Credit authorship contribution statement

Ana Kovačič: Conceptualization, Investigation, Formal analysis, Data curation, Methodology, Visualization, Writing – original draft. **Eirini Andreasidou:** Writing – original draft. **Anže Brus:** Formal analysis, Data curation. **Anja Vehar:** Formal analysis, Data curation, Writing – original draft. **Doris Potočnik:** Formal analysis, Data curation, Writing – original draft. **Marta Jagodic Hudobivnik:** Formal analysis. **David Heath:** Methodology, Conceptualization, Writing – review & editing. **Marina Pintar:** Supervision, Conceptualization, Methodology, Writing – review & editing. **Nina Kacjan Maršič**: Supervision, Conceptualization, Methodology, Writing – review & editing. **Nives Ogrinc:** Supervision, Writing – review & editing. **Urška Blaznik**: Formal analysis, Data curation, Writing – original draft. **Ester Heath:** Supervision, Conceptualization, Writing – review & editing, Project administration, Resources, Funding acquisition.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

Acknowledgments

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Novelty statement

The use of treated wastewater (TWW) for agricultural production is compelling, particularly as the supply of clean freshwater decreases, leading to water scarcity in many regions. Thus, understanding the risks associated with potentially introducing harmful chemicals into crops from TWW irrigation is crucial to protect health and the environment. The present study provides novel evidence on the uptake of 14 chemicals of emerging concern and 27 potentially toxic elements in tomato fruits produced in soil and soil-less conditions irrigated with potable and treated wastewater. The results add to the argument that the reuse of treated wastewater for irrigation can be a safe approach in agriculture and provide essential information for supporting the decisions of policymakers and stakeholders regarding treated water reuse.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.130964.

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3.2.2 Uptake of emerging contaminants in tomato plants: A field study on treated wastewater reuse

The paper titled: "Uptake of Emerging Contaminants in Tomato Plants: A Field Study on Treated Wastewater Reuse", by E. Andreasidou, A. Kovačič, L. Manzano-Sánchez, D. Heath, T. Kosjek, M. Pintar, N. Kacjan Maršič, U. Blaznik, A.R. Fernández-Alba, M.D. Hernando and E. Heath was published in Environment International in November 2025 (doi.org/10.1016/j.envint.2025.109916). I was responsible for experimental design, experimental work, sampling, method optimisation, method validation and data analysis. I was also responsible for data interpretation, visualisation and writing of the manuscript.

As stressed before, reusing TWW for agricultural irrigation can potentially introduce CECs into the food chain. This field-scale study was based on the knowledge gained from our previous experiment (3.2.1). Specifically, the study looked at the uptake, translocation and bioaccumulation of 27 CECs in tomatoes irrigated under four regimes: potable water (PW), treated wastewater (TWW), and both aqueous media spiked with CECs (1 mg/L). Results showed that CECs were less stable in TWW than in PW, which influenced their plant uptake and bioavailability. Under regular TWW irrigation, bioaccumulation in edible tissues was minimal, with only carbamazepine detected above the LOQ (22 ng/g).

Spiked treatments enabled the quantification of six CECs, specifically acetamiprid, azithromycin, BPS, carbamazepine, clarithromycin, and dimethomorph, in tomato fruits. Meanwhile, up to 17 compounds were detected in roots, followed by leaves and stems. Despite the introduction of TWW and/or CEC, fruit yield was not affected across treatments. Dietary exposure assessment indicated that CEC concentrations in tomatoes irrigated with TWW posed no immediate health risk, even for vulnerable populations such as toddlers. BPS exceeded the selected tolerable daily intake under TWW spiked with CECs, which was up to 1000 times higher than concentrations typically found in TWW. However, soil analysis revealed the persistence of compounds (azithromycin and triclocarban) up to 30 months after irrigation, with risk quotients exceeding environmental safety thresholds.

This paper fulfils multiple aims of the thesis, as it includes uptake and translocation estimation based on a single distinct growing experiment for wastewater reuse under field conditions. The study includes results on CECs concentrations in potable water, wastewater, tomato fruits, plant parts and soil, according to which dietary intake of consumers and environmental risk of CECs were calculated. Overall, the findings of this study suggest that the consumption of tomatoes irrigated with TWW poses a limited human health risk. Still, the accumulation of CECs in soil can have negative environmental impacts. However, to fully understand the risks associated with wastewater reuse, additional factors should be considered, including a broader range of CECs based on suspect screening, as well as metabolites and transformation products.

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Full length article

Uptake of emerging contaminants in tomato plants: a field study on treated wastewater reuse

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ABSTRACT

Reusing treated wastewater (TWW) for agricultural irrigation offers a sustainable option to reduce water scarcity; however, it also raises concerns about potential contamination of the food chain. This study evaluated the uptake and translocation of 27 contaminants of emerging concern (CECs) in tomatoes grown under field conditions. The experiment consisted of four drip irrigation regimes: (1) potable water (PW), (2) TWW, (3) PW with addition of CECs (1 mg/L), and (4) TWW with addition of CECs (1 mg/L). Results show that CECs were less stable in TWW than in PW, influencing their uptake. Tomato fruits also showed minimal bioaccumulation under non-spiked conditions, with only carbamazepine above LOQ when irrigated with TWW (22 ng/g). In spiked media, acetamiprid, azithromycin, bisphenol S, carbamazepine, clarithromycin, and dimethomorph were above LOQ in tomato fruits, while up to 17 CECs were quantified in roots > leaves > stems. Fruit yield also remained unaffected. Dietary exposure estimates were below the risk threshold for tomatoes irrigated with TWW. Soil analysis revealed CECs (azithromycin, triclocarban) exceeding safety thresholds up to 30 months after irrigation. In summary, while targeted CECs in tomato fruits pose no immediate health risk, their accumulation in soil highlights the need for long-term monitoring.

1. Introduction

The growing pressure on global freshwater resources due to urbanisation and intensified agricultural activities has made water scarcity an urgent issue (FAO, 2021). At least 11 % of Europeans are affected, while in the Mediterranean basin, up to 70 % of the population faces seasonal summer water shortages due to pressures from agriculture, tourism, and public demand (European Environment Agency, 2025). Reusing municipal treated wastewater (TWW) could help ease the problem, while offering environmental, economic, and social benefits. While its agricultural use remains concentrated mainly in southern Europe, e.g., Spain, Italy, and Cyprus, TWW has the potential to alleviate water stress by up to 10% (European Environment Agency, 2021; European Investment Bank, 2022). Additionally, TWW is a source of nutrients that can partially offset the need for synthetic fertilisers (Ofori et al., 2021).

A significant barrier in reusing TWW for agriculture is the presence of micropollutants, including contaminants of emerging concern (CECs), which could pose a risk to human health if they enter the food chain. Contaminants of emerging concern include residues of pharmaceutical and personal care products (PPCPs), hormones, and industrial chemicals that are not comprehensively regulated (Shi et al., 2022). At the policy level, the EU Regulation (EU) 2020/741 establishes harmonised minimum water quality and monitoring requirements to ensure the safe use of reclaimed water for agricultural irrigation (EU Parliament Council, 2020). The current EU legislation not only sets limits for microbiological parameters (e.g., *E. coli*, and in specific cases *Legionella* spp. and

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intestinal nematodes) and physicochemical parameters (e.g., BOD, TSS, turbidity) but also demands a comprehensive risk assessment as part of a water reuse risk management plan, where additional requirements may be necessary concerning heavy metals, pesticides, disinfection by-products, pharmaceuticals, anti-microbial resistance and other emerging contaminants, including micro pollutants and micro plastics. However, harmonised protocols for monitoring CECs regarding TWW reuse and associated risks are lacking (Carter et al., 2025).

The efficiency of wastewater treatment plants (WWTPs) in removing CECs depends on several factors, including plant size, treatment configuration, the composition of incoming wastewater, and the physicochemical properties of the CECs themselves (Golovko et al., 2021; Ofrydopoulou et al., 2022). Accordingly, the revised Urban Wastewater Treatment Directive (EU) 2024/3019 aims to strengthen treatment requirements. In addition to other requirements, the directive lists 12 model CECs, of which six must be reduced by more than 80%; otherwise, additional quaternary treatment must be introduced. This implementation is expected to improve wastewater effluent quality by increasing CECs removal, thereby reducing associated human and environmental risks. However, the implementation of quaternary treatment will be phased in by 2045 for WWTPs exceeding 150,000 population equivalents (PE), while smaller WWTPs will follow (European Union, 2024).

Studies have shown that CECs can be taken up by plants and potentially translocated to edible tissues (Christou et al., 2024). For example, CECs, including carbamazepine and diclofenac, have been determined in tomatoes, carrots, and leafy vegetables irrigated with TWW (Ben Mordechay et al., 2021; Wu et al., 2014). These findings indicate a direct link between irrigation practices and human exposure. However, their uptake depends on their physicochemical properties (e.g., solubility, hydrophobicity, polarity, and molecular weight), soil properties (e.g., organic matter content and pH), plant species and irrigation method (Christou et al., 2019; Fernandes et al., 2024).

Regarding the risk of consuming produce grown using TWW, Specker et al., (2025) found that although CECs are present in crops irrigated with TWW, the associated human health risks remain limited. Moreover, studies differ in their applied methodologies (Earl et al., 2024) and consumption rates, commonly excluding vulnerable population groups, such as infants or toddlers (Ben Mordechay et al., 2022; Martínez-Piernas et al., 2019; Ponce-Robles et al., 2022). Studies often overlook the identification of CEC transformation products formed during wastewater treatment or plant biotransformation, which may represent an additional source of risk (Martínez-Piernas et al., 2021; Santos et al., 2025). Furthermore, studies often employ inconsistent methods for reporting concentrations and distributions, which limit cross-study comparability and risk assessment (Fernandes et al., 2024). Despite this, Paltiel et al. (2016) found that individuals consuming vegetables grown with treated wastewater had detectable levels of carbamazepine and its metabolites in their urine, suggesting exposure through dietary intake.

Given the growing interest in TWW reuse in agriculture, further field studies are needed to evaluate the risks associated with its use. This study examines the uptake, translocation and accumulation of 27 model CECs, commonly present in TWW (Andreasidou et al., 2025; Česen et al., 2019; Verlicchi et al., 2023), including selected pesticides (2), pharmaceuticals (9), personal care product ingredients (6), hormones (5), stimulants (1) and industrial chemicals (4). Tomato (*Solanum lycopersicum* L. cv. Rally) was chosen as a model plant because it is one of the most commonly grown vegetables in the EU and is consumed whole, raw and processed (Eurostat, 2024). Unlike previous studies (Ben Mordechay et al., 2018; Bueno et al., 2022; Wu et al., 2014), four irrigation treatments were included: (1) potable water (PW), (2) treated wastewater (TWW), (3) PW+CEC, and (4) TWW+CEC, under controlled field conditions (lysimeters), to examine the effect of matrix composition (1 and 2; 3 and 4) and spiking (1 and 3; 2 and 4). Since uptake is concentration-dependent, a higher spike (1 mg/L) was used than in other studies (Pan and Chu, 2017; Shi et al., 2024) to ensure detectable levels in the plants, allowing the assessment of CEC translocation and bioaccumulation that would not be possible if concentrations were below the limit of detection. In addition, the study also addressed dietary exposure risks and the impact of TWW reuse on soil quality. The overall aim is to contribute to the development of safer wastewater reuse practices.

2. Materials and methods

2.1. Reagents and chemicals

Analytical standard of high purity (> 97%) of acetamiprid, azithromycin, benzophenone, bisphenol F (BPF), bisphenol AF (BPAF), bisphenol S (BPS), caffeine, carbamazepine, ciprofloxacin, clarithromycin, diclofenac sodium salt, dimethomorph, erythromycin, estrone (E1), 17α-ethynylestradiol (EE2), ibuprofen, methylparaben, naproxen, progesterone, propylparaben, testosterone, tonalide, triclocarban and triclosan were purchased from Sigma-Aldrich (Steinheim, Germany), whereas, bisphenol A (BPA), was purchased from Merck (Darmstadt, Germany), amoxicillin trihydrate was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and β-estradiol (E2) was purchased from Thermo Fisher Scientific (Waltham, MA USA). Additional compound information is given in the Supplementary Information (Table S1).

Labelled standards for BPA-d_{16}, $^{13}C_3$-caffeine, carbamazepine-d_{10}, clothianidin-d_3, $^{13}C_6$-diclofenac, ibuprofen-d_3, methyl 4-hydroxybenzoate-(ring-$^{13}C_6$), and propyl 4-hydroxybenzoate-(ring-$^{13}C_6$) were purchased from Sigma Aldrich (Steinheim, Germany). Oxybenzone-d_5 was purchased from C/D/N Isotopes Inc. (Quebec, Canada), $^{13}C_{12}$-triclosan from Cambridge Isotope Laboratories (Tewksbury, MA, USA), $^{13}C_2$-β-estradiol from Campro Scientific (Berlin, Germany), $^{13}C_{12}$-BPS and $^{13}C_{12}$-4,4-BPF from CanSyn Chem. Corp. (Toronto, Canada).

All analytical grade solvents were obtained from J.T. Baker (Deventer, Netherlands), including methanol (MeOH), acetonitrile (MeCN) and LC-MS grade water. Formic acid (FA) and ammonium fluoride (NH_4F) were purchased from Sigma Aldrich (St. Louis, USA). Ultrapure water (18.2 MΩ·cm at 25 °C) was prepared using the Milli-Q water purification system (Merck Millipore, Darmstadt, Germany). Ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA) was purchased from Kemika d.d. (Zagreb, Croatia).

Stock solutions (1 mg/mL) were prepared by dissolving each compound in MeOH. Working standards (10, 100 and 1000 ng/mL) were prepared by serial dilutions of the stock solutions in MeOH. Similarly, an internal standard solution (1 μg/mL) in MeOH was prepared from solutions of individual isotopically labelled compounds. All standards were stored in amber glass vials at 4 °C. Samples were filtered before analysis through regenerated cellulose membrane filter vials (Phenomenex, Torrance, USA) with a 0.2 μm pore size.

2.2. Experimental design and treatments

The experiment was conducted outdoors during the 2022 growing season at the lysimeter facility of the University of Ljubljana's Living Lab, located at the Ajdovščina WWTP in the Vipava Valley, Slovenia (45.8764° N, 13.9064° E, 106 m.a.s.L.), which is a biological treatment plant with denitrification. It treats approximately 8,400 m^3/day of wastewater, comprising 30% municipal and 70% industrial sources and is designed to handle an organic load equivalent to 42,000 population equivalents (PE). The use of the lysimeter enabled application of individual irrigation treatments, while simulating field conditions (Fig. 1). Tomato seedlings (*Solanum lycopersicum* L. cv. Rally) were grown in a greenhouse under controlled environmental conditions for 8 weeks, during which each plant developed 5–6 leaves. One seedling was then transplanted into each lysimeter cell. Both PW and TWW were obtained from the wastewater treatment plant.

Each lysimeter cell was 40 cm in diameter and 100 cm in depth, and

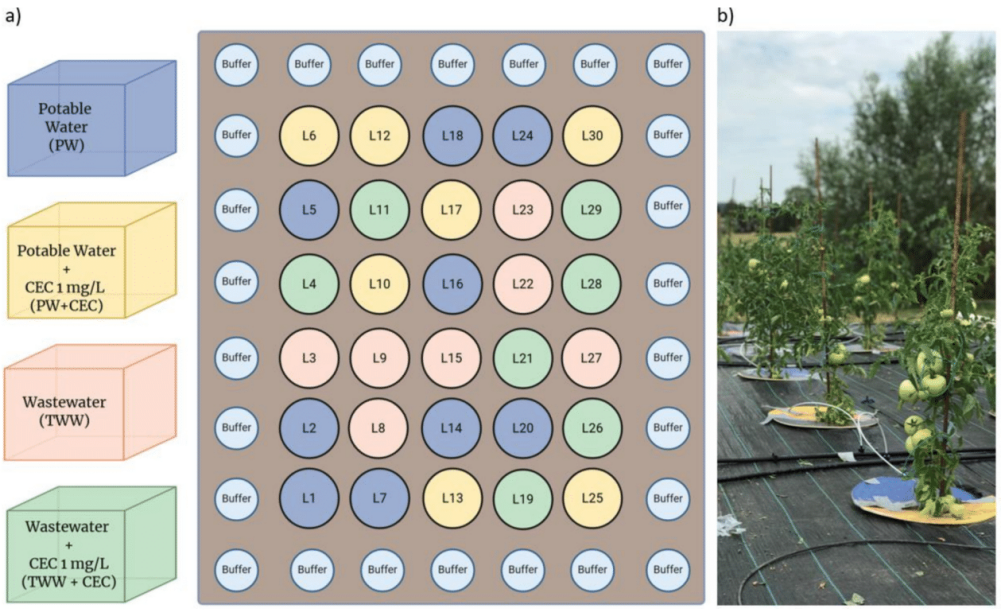


Fig. 1. a). Randomised planting scheme for lysimeters with surrounding buffer-zone plants. b). Picture of lysimeters.

was positioned 100 cm apart. The soil in each lysimeter cell or planting unit was a eutric brown soil on flysch. Soil properties are given in Table S2. A buffer zone comprising a single row of tomato plants was planted around the perimeter of the lysimeter to protect test plants and provide comparable exposure to the elements (e.g., sun and wind). The buffer zone was irrigated with potable water. Seedlings were transferred to the field on 02/06/2022. The lysimeter was covered with a 2 mm-thick plastic cover to ensure that each plant received the same amount of irrigation media. Before planting, all lysimeters were fertilised with 5 g N per plant (CAN, 27% N), 6.6 g P_2O_5 per plant (Superphosphate 0:26:0; 26% P), and 28 g K_2O per plant (K_2SO_4; 50% K), resulting in 50, 66, and 280 kg of nutrients per hectare, respectively, at a planting density of 1 plant per 1 m^2. During the vegetation period, nitrogen was applied three additional times (at the end of June, July, and August) at a rate of 5 g N per plant (CAN, 27% N).

Tomatoes were grown under four irrigation conditions: C1: potable water (PW) as the control (n = 9 plants); C2: treated wastewater (TWW) (n = 7 plants); C3: potable water spiked with CECs (PW+CEC, 1 mg/L) (n = 7 plants); and C4: treated wastewater spiked with CECs (TWW+CEC, 1 mg/L) (n = 7 plants) using drip irrigation.

The potable water used in the experiment originated from the Hubelj spring that provides drinking water to the municipality of Ajdovščina, Slovenia. In this experiment, potable water was used as the control, while treated wastewater was applied to simulate treated wastewater reuse (da Silva et al., 2025). For the spiked samples, the concentrations of added CECs (1 mg/L) were far higher than those typically found in treated wastewater, which can range from LOQ (ng/L) up to µg/L. This elevated spiking was intentionally applied to increase the likelihood of detecting their uptake and translocation in tomato tissues, as performed in other similar studies (Denora et al., 2023), and since uptake is known to be concentration-dependent (Zeng et al., 2022). Additionally, a 2020 study found that spiking at 0.1 mg/L resulted in minimal uptake (Kovačič et al., 2023). All irrigation media were stored in the dark in 1 m^3 plastic containers covered with Styrofoam. Fresh irrigation solutions were prepared on day zero and every three weeks for the 11-week duration of the experiment. Irrigation was automatically controlled based on soil water measurements from HydraProbe probes (Stevens, Inc., Portland, Oregon, USA) installed at 15 cm and 45 cm within the lysimeter cells, providing sufficient water to the plants while preventing leachate.

2.3. Sample collection

Tomato fruits were hand-collected when fully ripe. The first sampling event was conducted on 14 July 2022, followed by eight additional samplings divided into three sampling periods (S1, S2, and S3). The final sampling was on 12/9/2022. During each sampling, ripe fruits were collected in plastic bags, transferred to the laboratory, weighed to evaluate yield for different treatments and stored whole at −20 °C. Frozen fruits were cut into pieces, weighed, freeze-dried and ground. Based on measurements before and after freeze-drying, the average water content of tomato fruits was calculated (95% water, 5% dry matter). To reduce the number of samples to a manageable amount, composite samples were prepared for each treatment, as (1) fruits per plant (four plants selected) and (2) fruits per sampling period.

At the end of the experiment, plant parts were air-dried, separated into leaves, roots, and stems, then ground using a stainless-steel blade mixer and sieved (2,000 µm mesh). Samples of irrigation media (10 mL) were collected on day zero, followed by weekly sampling. The containers were refilled four times, with additional samples (1 L) taken during each refill and at the conclusion of the experiment. All samples were transported in cooled boxes at 4 °C and stored at −20 °C until analysis. After harvesting, the lysimeters were uncovered, and the soil was left exposed to the atmosphere. Soil samples (three replicates) were collected at the average root depth of each plant at the beginning (time 0), at the end of the experiment (Month 3) and two years after the completion of the experiments (Month 30). After the experiment, the soil was left fallow. Soil samples (approximately 100 g) were dried, ground, sieved (mesh size: 2,000 µm) and stored in polypropylene containers.

2.4. Sample preparation

For wastewater and potable water, a 50 mL aliquot of each sample was spiked with a mixture of isotope-labelled internal standards (25 µL, 1 µg/mL), filtered (GF-4, 1.4 µm, Macherey-Nagel, Germany), followed by the addition of Na_2EDTA solution in Milli-Q to reach a final concentration of 0.2% (2 mL, 50 mg/mL). The samples were extracted using Oasis Prime HLB cartridges (60 mg, 3 cc). After loading, the sorbent was washed with 3 mL of 10% MeOH (v/v) and dried under vacuum for 30 min. The analytes of interest were eluted using 1.8 mL MeOH:MeCN (1:1) (v/v) with 0.1% FA (3 aliquots of 0.6 mL). The eluates were dried with nitrogen (N_2) at 40 °C, reconstituted in a 1:1 solution of MeOH:Milli-Q (0.4 mL), filtered (Verex vials (0.2 µm)) and stored at −20 °C. Macronutrients, including plant-available P (as P_2O_5), K (as K_2O), Ca (as CaO), Mg, and N, were determined in irrigation media according to the methods described in SI-2.

For spiked liquid samples (PW+CEC, TWW+CEC), a 1 mL aliquot was spiked with a mixture of isotope-labelled internal standards (25 µL, 1 µg/mL), followed by the addition of Na_2EDTA solution in MQ (100 µL, 20 mg/mL). The samples were extracted using Oasis Prime HLB 96-well plates (30 mg). After loading, the sorbent was washed with 0.5 mL of 10 % MeOH in Milli-Q (v/v) and dried under vacuum for 30 min. The analytes of interest were eluted using 1 mL MeOH:MeCN (1:1) (v/v) with 0.1% FA (2 aliquots of 0.5 mL), and eluates were treated as per wastewater and potable water (50 mL).

To determine CECs in tomato fruits, 0.25 g of freeze-dried composite sample was weighed into a 15 mL centrifuge tube and spiked with 20 µL of isotope-labelled internal standards (1 µg/mL). Acetonitrile (1 mL) was added, and the sample was vortexed for 30 s and shaken overnight (200 rpm). Then, 4 mL MeOH with 0.1% FA was added, and the mixture was sonicated at 30 °C for 20 min (Ultrasonic Cleaner, 550D, VWR, Vienna, Austria), followed by centrifugation at 9,000 rpm for 20 min. The supernatant was transferred to a 200 mL vial, and the residue was re-extracted twice with 4 mL of MeOH/0.1 % FA, each time sonicated and centrifuged. The combined extracts were diluted with 90 mL Milli-Q water and loaded onto Oasis Prime HLB cartridges (500 mg, 6 cc). After washing with 10 mL 10% MeOH and drying under vacuum for 30 min, analytes were eluted with 6 mL MeOH:MeCN (1:1) with 0.1% FA in three 2 mL aliquots. Eluates were dried under nitrogen at 40 °C, reconstituted in 0.4 mL MeOH:Milli-Q (1:1), filtered (0.2 µm RC membrane), and stored at –20 °C before analysis.

For leaves, roots, and stems, 1 g of dried composite sample was weighed into a 50 mL centrifuge tube and spiked with 50 µL of isotope-labelled internal standards (1 µg/mL). Ten millilitres of Milli-Q water was added, followed by vortexing for 10 s. Then, 10 mL of MeCN with 5% FA was added, and the mixture was shaken in an axial agitator (speed: 3.5 m/s, Agytax®, Cirta Lab. S.L., Spain) for 5 min. Subsequently, 4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate were added. The mixture was vortexed for 10 s, shaken for 5 min, and centrifuged for 5 min at 3,700 rpm. The extract was then processed using an automated PAL® RTC X-Y-Z autosampler with prefilled µSPE cartridges (45 mg sorbent comprising $MgSO_4$, PSA, C18, and GCB in a 20:12:12:1 ratio). Cartridges were preconditioned with 100 µL acetonitrile before loading 200 µL of extract at 2 µL/s. The cleaned extract was collected in 2 mL vials, and the cartridges were eluted with 100 µL of acetonitrile containing 5% FA. The injection needle was washed with three cycles of 50:50 MeCN:Milli-Q between injections to avoid cross-contamination. Each run lasted 12 min. Cleaned extracts were transferred into LC vial inserts and stored at −20 °C until analysis.

For determining CECs in soil, 1 g of dried sample was weighed into a 15 mL centrifuge tube and spiked with a mixture of isotope-labelled internal standards (25 µL, 1 µg/mL). Acetonitrile (1 mL) was added, and the sample was vortexed (30 s) and shaken overnight (IKA KS 4000, 200 rpm). Afterwards, MeOH with 0.1% FA (4 mL) was added, and the mixture was sonicated (10 min, 30 °C) and centrifuged at 9,000 rpm for 10 min. The supernatant was transferred to a 20 mL glass vial, and the residue was suspended in 4 mL of MeOH containing 0.1% FA, then sonicated and centrifuged for two additional cycles. The solvent from the combined extracts was dried under nitrogen (N_2) at 40 °C, reconstituted in a 1:1 solution of MeOH:Milli-Q (0.4 mL), filtered through a Verex vial (0.2 µm), and stored at −20 °C.

2.5. LC-MS/MS analysis

Samples were analysed using a UHPLC (Shimadzu Nexera X2 UHPLC, Kyoto, Japan) coupled to a linear ion trap quadrupole 4500 QqLIT-MS/MS (Sciex, Framingham, MA, USA). Separation was achieved on a Poroshell 120 EC-C18 column (100 × 3 mm; 2.7 µm particle size; Agilent Technologies, USA) in the LC column oven compartment at ambient temperature (21 °C). The 4500 QTRAP analyser was operated under electrospray ionisation (ESI) in both modes (positive and negative). For the positive mode, the mobile phases were acetonitrile (A) and 0.1 % formic acid in ultrapure water (B). In contrast, for the negative mode, the mobile phases were 0.1 % NH_4F in methanol (A) and 0.1 % NH_4F in ultrapure water (B). Full details are given elsewhere (Andreasidou et al., 2025). Data were analysed using Analyst v1.6.3. For roots, leaves and stems, the method was transferred to a UHPLC coupled to a linear ion trap quadrupole 6500 QqLIT-MS/MS (Sciex, Framingham, MA, USA). Data analysis was performed using SCIEX OS Software.

The method was validated in accordance with EU guidelines, assessing linearity, accuracy, recovery, matrix effects, LOD, and LOQ (European Commission DG-SANTE, 2021). The validation results for each matrix are provided in the supplementary information (SI-2, Tables S3-9).

2.6. Bioconcentration and translocation factors

The bioconcentration factor (BCF) and translocation factor (TCF) were calculated to assess the uptake and internal distribution of CECs in tomato plants. Concentrations of compounds in tomato fruits and plant parts used for the calculations were in µg/kg dry weight (d.w.). The BCF was estimated as the ratio of the concentration of each compound in tomato fruit to the corresponding concentration in the irrigation water, as shown in Eq. (1).

$$BCF\ (L/kg) = \frac{Concentration\ in\ fruit\ (\mu g/kg)}{Concentration\ in\ irrigation\ water\ (\mu g/L)} \quad (1)$$

This approach aligns with previous studies and assumes efficient water delivery and minimal loss through the drip irrigation system, which typically achieves an efficiency of over 90% (Bueno et al., 2022). However, this calculation does not account for the potential accumulation of contaminants in the soil over repeated irrigation events, which may lead to an underestimation of actual exposure and consequently affect BCF/RCF interpretation. The same approach was used to estimate the Root Concentration Factor (RCF) of tomato plants using Eq. (2).

$$RCF\ (L/kg) = \frac{Concentration\ in\ roots\ (\mu g/kg)}{Concentration\ in\ irrigation\ water\ (\mu g/L)} \quad (2)$$

Similarly, TCF was used to quantify the internal distribution of compounds across plant compartments, including leaf-to-root, stem-to-root, and fruit-to-root translocation (Eq. (3)).

$$TCF\ = \frac{Concentration\ in\ fruit,\ leaf\ or\ stem\ (\mu g/kg)}{Concentration\ in\ roots(\mu g/kg)} \quad (3)$$

A TCF < 1 indicates that most of the compound remains in roots, whereas a TCF > 1 suggests the translocation and potential accumulation in the rest of the tissues (Fučík et al., 2024).

2.7. Human health risk assessment

Dietary exposure to the detected CECs was estimated for children (toddlers), adults, and adolescents by combining data from composite samples (transformed to fresh weight, assuming the evaluated tomato fruits contained 95% water) with the corresponding consumption levels from the EFSA Comprehensive European Food Consumption Database (EFSA, 2025). The consumption data were selected based on the Exposure hierarchy up to Level 7 (Vegetables and vegetable products, Fruiting vegetables, Solanacea, Tomatoes and similar Tomatoes) for the Slovenian Population (Table S10). The default body weight values suggested by EFSA were used for further calculations. Dietary exposure to fresh tomatoes was assessed deterministically; average exposure was based on the detected concentration and average consumption data, while high exposure was based on the detected concentration and high consumption (97.5-percentile) data. The estimated daily intake (EDI) (mg/kg per body weight (bw) per day) was calculated according to Eq. (4).

$$Dietary\ exposure\ (mg/kg\ bw/day) = \frac{Concentration\ of\ CEC\ (mg/kg\ fresh\ weight) \times Food\ Consumption\ \left(\frac{kg}{day}\right)}{Body\ weight\ (kg)} \quad (4)$$

The results for each compound were compared with Acceptable Daily Intake (ADI) and Tolerable Daily Intake (TDI) values collected from the literature (Table S11).

2.8. Environmental risk assessment

Predicted no-effect concentration (PNEC) values for soil were compared with measured environmental concentrations (MEC) in different periods to assess the potential environmental risk associated with CECs in soil. The assessment was based on established regulatory methodologies, following the guidance from the European Chemicals Agency (ECHA) (European Chemicals Agency, 2008).

Due to the absence of toxicity studies on terrestrial soil organisms for the selected compounds, PNEC values for soil were derived from aquatic toxicity data using the equilibrium partitioning method (EPM). Specifically, available PNEC values for freshwater organisms (µg/L) were obtained from the Norman Ecotoxicology Database, which includes the Lowest PNEC values based on QSAR models or experimentally obtained values, suitable for screening and prioritisation purposes ("NORMAN Ecotoxicology Database," 2025).

$PNEC_{soil}$ (µg/kg) was calculated from the predicted no-effect concentrations in the aquatic environment ($PNEC_{water}$) value (µg/L) multiplied by the soil–water distribution coefficient (K_{oc}) (L/kg) of each selected compound, according to Eq. (5) and provided in Table S12.

$$PNECsoil\left(\frac{\mu g}{kg}\right) = \left(0.1176 + 0.01764 \times Koc\left(\frac{L}{kg}\right)\right) \times PNECwater\left(\frac{\mu g}{L}\right) \quad (5)$$

The model is based on equilibrium assumptions and incorporates default bulk density and volume fraction parameters (European Chemicals Agency, 2012). However, it does not consider specific soil physicochemical properties, which can affect contaminant behaviour. This methodology for $PNEC_{soil}$ is used for rough screening when terrestrial toxicity data are unavailable and is particularly applicable to substances with log K_{ow} values between 3 and 6. The equilibrium partitioning method may result in either overestimation or underestimation of actual toxicity to soil invertebrates, but it is considered acceptable as a conservative approximation (Van Beelen et al., 2003).

For each compound, the risk quotient (RQ) was calculated as the ratio of the measured environmental concentration (MEC) to the predicted no-effect concentration (PNEC), according to Eq. (6):

$$RQ = \frac{MECsoil\left(\frac{\mu g}{kg}\ d.w.\right)}{PNECsoil\left(\frac{\mu g}{kg}\ d.w.\right)} \quad (6)$$

A factor of ten was applied to the MEC/PNEC for chemicals with a log $K_{ow} > 5$, as a safety factor to accommodate the uncertainty of the equilibrium partitioning method for CECs with high log K_{ow} (European Centre for Ecotoxicology and Toxicology of Chemicals, 2004).

The risk quotient is interpreted using standard thresholds: values between 0.01 and 0.1 indicate a low risk under the tested conditions, values between 0.1 and 1 suggest a medium risk, and values greater than 1 indicate a high environmental risk (Hernando et al., 2006).

2.9. Data processing and analysis

Data processing, statistical analysis, and visualisation for CECs were performed in R (version 4.3.3) (R Core Team, 2023). For all matrices, values below the LOQ were treated as missing and imputed using random values between zero and the matrix and treatment-specific LOQ, since it incorporates the uncertainty of measurements below the quantification limit and maintains variability within the dataset without introducing systematic bias. Concentration values were $\log_{10}$-transformed to correct for skewness and meet assumptions of parametric tests. Normality was tested using the Shapiro-Wilk test; the $\log_{10}$-transformed data met the assumption of normal distribution. Independent two-sample t-tests were used to compare treatments, with effect sizes (Cohen's d) calculated to assess the magnitude of the observed differences. Compounds were considered significantly different if $p \leq 0.05$ and $|d| > 1$. Descriptive statistics and log-scale boxplots were used to explore variability within each matrix. Principal Component Analysis (PCA) was applied to log-transformed and Pareto-scaled data to explore overall multivariate patterns and sample clustering. Loadings were further examined to assess which compounds contributed most to the observed variance.

3. Results and discussion

The levels of CECs were evaluated in irrigation water, plant parts (fruits, leaves, roots, stems) and soil across the experimental system. The comparisons focused on treatments between control (PW) and TWW, and environmental concentrations versus spiked concentrations within the same matrix type (PW+CEC and TWW+CEC). Results are discussed in terms of measured concentrations, statistical differences, and multivariate patterns (PCA, loadings). All concentrations are presented based on dry weight.

3.1. Irrigation water

During the three-month experiment, the irrigation reservoirs were refilled five times. The consumption of irrigation media was 4,700 L for PW (for nine plants), 3,200 L for TWW, 3,400 L for PW+CEC, and 2,600

L for TWW+CEC (for seven plants in each treatment). Since irrigation was automatically controlled based on soil water measurements, differences in total irrigation volumes might be associated with variations in soil water retention and evapotranspiration across treatments, as treated wastewater can increase soil water-holding capacity and reduce plant irrigation demand (Hashem and Qi, 2021).

Nutrient concentrations were measured across different samples (mg/L) and varied across the four irrigation treatments (PW, TWW, PW+CEC, TWW+CEC) (Table S13 and S14). Total nitrogen levels did not differ significantly among the irrigation media, and phosphorus concentrations were uniform across treatments. Although significant differences were observed in potassium and calcium between specific treatments, no firm conclusions can be drawn due to variability in the irrigation media (refilled with fresh media) used during the experiment.

The analysis of CECs in PW and TWW is given in Table S15. Levels of CECs were low in PW (five compounds: 3.8–18 ng/L), close to the respective LOQs, consistent with previously reported values (Tröger et al., 2021; Wang et al., 2022). In wastewater (Fig. 2), 24 compounds were quantified (220–3,060 ng/L), with the highest mean concentration observed for dimethomorph (3,064 ng/L), followed by benzophenone (2,500 ng/L) and erythromycin (2,400 ng/L). Other compounds with elevated concentrations included carbamazepine, BPAF, and azithromycin, all of which exceeded 1,200 ng/L. Dimethomorph is a common fungicide, and its presence may be attributed to intensive viticultural activities in the Vipava River Valley, where it is commonly applied to control fungal diseases in grapevines. Its persistence and potential runoff into the wastewater system could explain its prevalence in the effluent (Roškarič et al., 2023). The presence of erythromycin, carbamazepine, benzophenone, and BPAF likely results from limited removal efficiency due to treatment loads exceeding the wastewater treatment plant's capacity, especially during the spring and summer (Radavelli et al., 2024).

Samples of spiked matrices (PW+CEC and TWW+CEC) were collected weekly (n = 16) to evaluate the presence of CECs and their stability under the selected storage conditions (Figs. 3 and 4).

Independent two-sample t-tests were performed to assess differences in CECs between PW+CEC and TWW+CEC. Seventeen compounds showed significantly higher concentrations in PW+CEC than in TWW+CEC, indicating higher stability when spiked in potable water. The compounds 17α-ethynylestradiol, ibuprofen, carbamazepine, progesterone, BPAF and BPS were significantly different ($p < 0.05$) with effect sizes (Cohen's d) exceeding 1. This separation is also evident in PCA (Fig. S1), where samples irrigated with spiked potable water (PW+CEC) and treated wastewater (TWW+CEC) formed two clusters along the first principal component (PC1), which explains 55.5% of the total variance. The PCA loadings plot (Fig. S2) further supports this, with compounds such as carbamazepine, BPS, BPAF, testosterone, and naproxen showing high positive loadings on PC1, suggesting a stronger association with PW+CEC samples. These differences can be attributed to matrix complexity, including the presence of microorganisms, as well as transformation (degradation, oxidation, hydrolysis) and adsorption to suspended solids in TWW (Baker and Kasprzyk-Hordern, 2011). Iakovides et al., (2025) confirmed that storing TWW can alter the content of micropollutants and the fate of CECs in agricultural systems.

3.2. Tomato yield

Regarding yield (Table S16), no statistically significant differences were observed across the four irrigation treatments (Fig. 5). Pairwise comparisons between each treatment also revealed no significant differences. All p-values exceeded 0.05, and none of the treatment comparisons met the predefined effect size threshold (|Cohen's d| > 1). The data suggest that neither the use of treated wastewater nor the presence of CECs had an adverse effect on tomato yield.

When nutrient concentrations were matched by season and correlated with yield, no statistically significant differences were observed ($p > 0.05$), although individual nutrients showed associations with yield during specific sampling periods. In sampling period 2, all nutrients

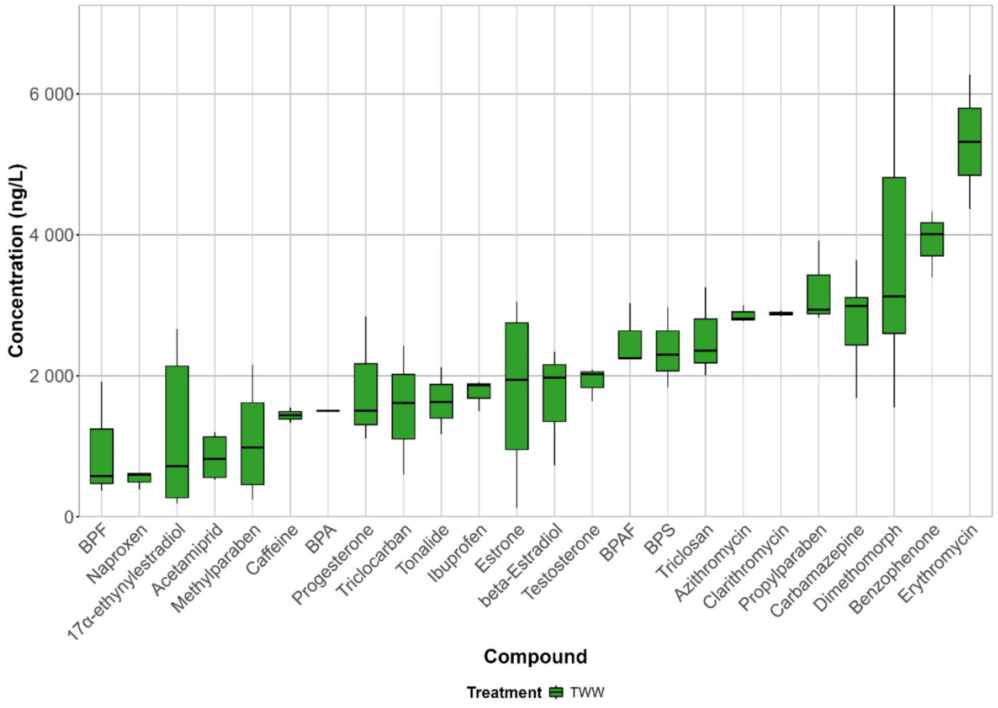


Fig. 2. Boxplots showing CECs concentrations (ng/L) measured in treated wastewater across five sampling periods.

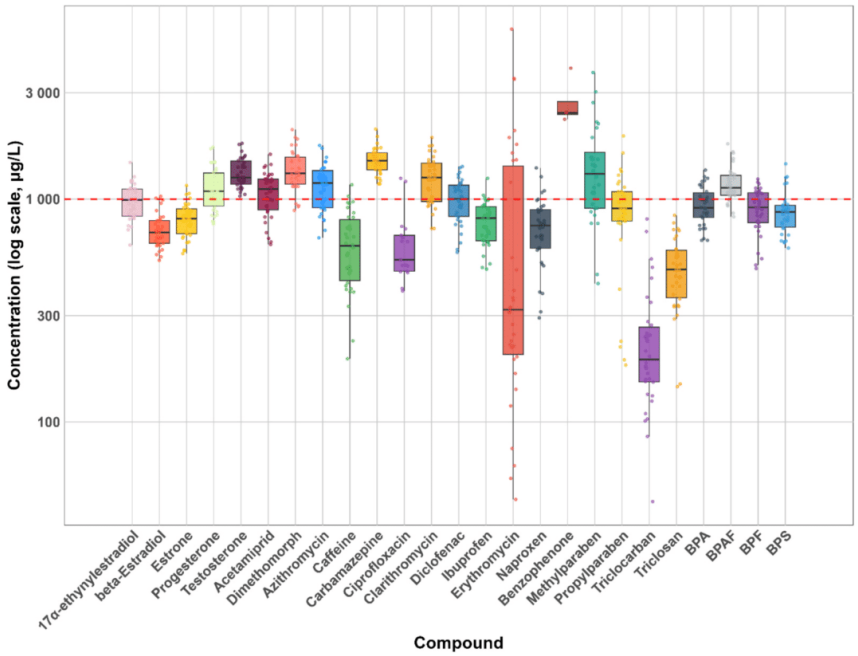


Fig. 3. Boxplots showing the weekly levels of CECs in spiked (1000 µg/L, red dashed line) potable water (n = 16).

showed a weak positive (N, P, Ca, K) or negative (Mg) correlation, while correlations in periods 1 and 3 were negligible (Fig. S3). These effects were not statistically significant, indicating that nutrient concentrations in the irrigation water did not affect yield. This lack of significance is likely due to the equal application of fertilisers across all plants, which ensured consistent nutrient availability across treatments and minimised confounding effects.

3.3. Bioaccumulation of CECs in tomato fruit

For tomatoes irrigated with PW (control) and TWW, limited accumulation was observed in TWW, with carbamazepine being the only compound above its LOQ (10.8–22.0 ng/g). In contrast, spiked treatments showed higher levels of CECs up to 1,070 ng/g (Fig. 6 and Table S17). In PW+CEC, dimethomorph, acetamiprid and carbamazepine were consistently quantified across all replicates, with mean levels of 65.9, 133 and 892 ng/g, respectively. In TWW+CEC, dimethomorph (61.2 ng/g), acetamiprid (108 ng/g) and carbamazepine (618 ng/g) were quantified in lower concentrations. Additionally, azithromycin (15.6 ng/g), clarithromycin (18.1 ng/g), and BPS (28.8 ng/g) were also quantified, and since they were present in at least one composite sample (Fig. S4), accumulation was considered positive. However, no statistically significant differences were observed between per-plant and per-sampling period composites for any quantified compounds ($p > 0.05$ for all comparisons). Although individual and mean values did vary, they were not significant when accounting for within-group variability. This result confirms that the sampling strategy did not significantly affect CECs profiles, indicating that per-plant samples, which serve as biological replicates in this study, provide a reliable basis for treatment comparisons.

PCA (Fig. S5) revealed a shift in sample distribution, with spiked treatments (PW+CEC, TWW+CEC) shifted toward positive PC1, whereas non-spiked treatments (PW, TWW) clustered around the centre of PC1. Although the 95% confidence ellipse for TWW+CEC was broad and overlapped with those of the non-spiked groups, the group centroids showed separation. Loadings highlighted carbamazepine, dimethomorph, and acetamiprid as major contributors to this variance (Fig. S6). The higher levels of CECs in tomato fruits irrigated with PW+CEC compared to TWW+CEC are likely due to their greater stability in the potable water matrix (Section 3.1).

Carbamazepine was the only compound above the LOQ in tomatoes irrigated with TWW (22.4 ng/g). Notably, it is among the most frequently detected compounds in tomato uptake studies, with reported concentrations ranging from 0.19 up to 46 ng/g (average of 5 ng/g) under field conditions (García-Valverde et al., 2024; Margenat et al., 2019; Martínez-Piernas et al., 2019). In the present study, carbamazepine levels exceeded the average reported in previous experiments, highlighting the potential influence of irrigation scheme, water quality, and soil properties. Carbamazepine, with a log K_{ow} of 2.45 and a pKa of 13.9, exists predominantly in its neutral molecular form at environmental pH, which facilitates its uptake and translocation in plants (Section 3.4).

Caffeine, with a log K_{ow} of −0.07, was reported in tomato fruits ranging from < LOQ (Riemenschneider et al., 2016; Sunyer-Caldú et al., 2023; Wu et al., 2014) to 32 ng/g (García-Valverde et al., 2023; Goldstein et al., 2014). In the present study, caffeine was below the limit of detection in all treatments, possibly due to site-specific soil factors (Topp et al., 2006). Christou et al., (2017) reported that the accumulation of pharmaceuticals in tomatoes, including diclofenac, depended not only on their physicochemical properties and wastewater quality but also on the duration of irrigation since diclofenac was detected in tomato fruits after the second growing season of consecutive TWW application, but not when TWW irrigation was applied for the first single growing period. Other studies reported benzophenone, BPF, dimethomorph, methylparaben, naproxen and propylparaben in tomatoes (12.7–3,440 ng/g). However, the presence of specific CECs in tomatoes grown in peri-urban agriculture was not attributed solely to TWW irrigation and may be linked to the direct application of fungicide (dimethomorph) and to leaching from plastic pipelines (BPF) (Margenat et al., 2019; Sunyer-Caldú et al., 2023).

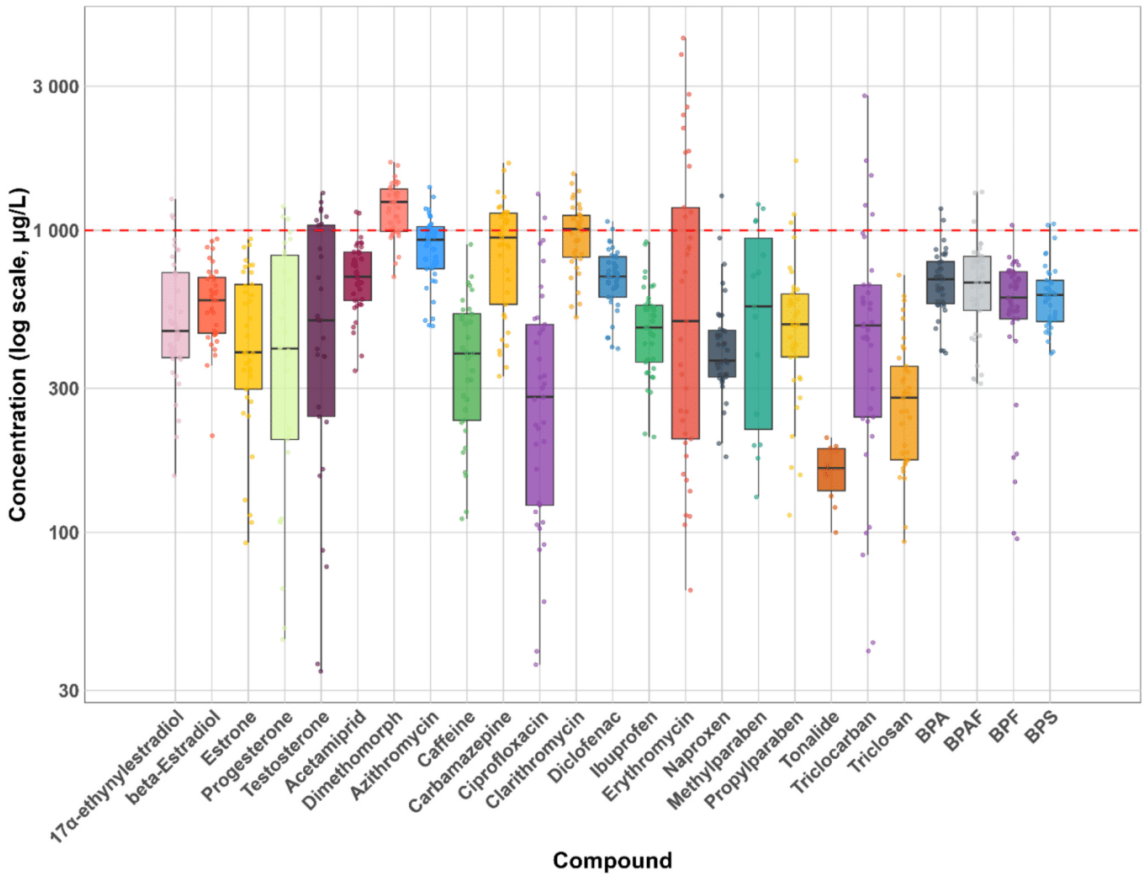


Fig. 4. Boxplots showing the weekly levels of CECs in spiked (1000 µg/L, red dashed line) treated wastewater (n = 16).

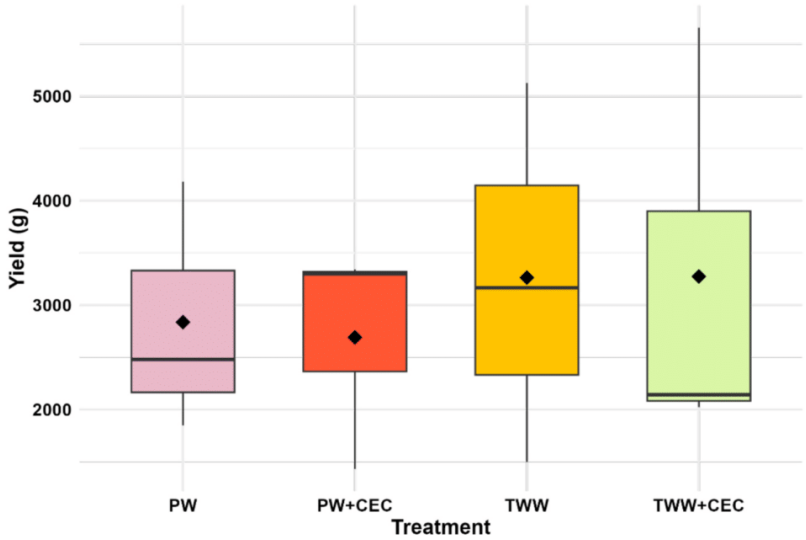


Fig. 5. Boxplots of average tomato yield (g) in each sampling period per treatment. Pairwise comparisons between each treatment were not significant ($p > 0.05$).

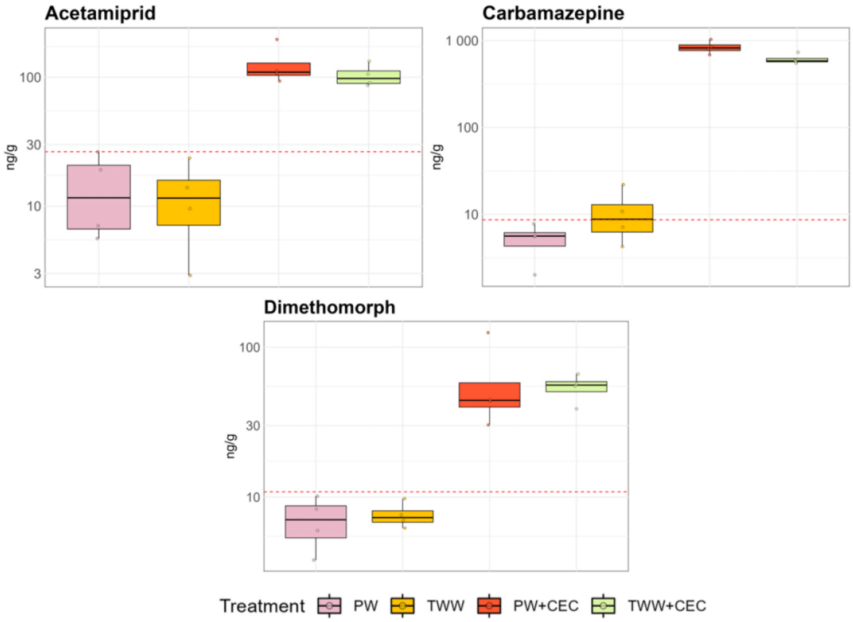


Fig. 6. Boxplots showing levels of CECs (ng/g) in tomato samples across all treatments. The dotted red line represents the compound-specific LOQ.

Only a few studies have examined spiked media, and differing concentrations make direct comparisons difficult. However, Denora et al., (2023) found both carbamazepine (89.2 ng/g) and clarithromycin (0.03 ng/g) in tomatoes irrigated with wastewater spiked with CECs (0.6 mg/L), unlike this study, where concentrations were 612 and 18.1 ng/g, respectively, at a similar spiking level (1 mg/L). Bigott et al., (2022), who studied the uptake of clarithromycin and ciprofloxacin in lettuce irrigated with spiked water and spiked wastewater (100 μg/L), reported the accumulation of clarithromycin (up to 100 ng/g) and ciprofloxacin (3.5 ng/g). In the present study, only clarithromycin was detected despite the high spiking concentration.

The accumulation of BPS (28.8 ng/g) in TWW+CEC is consistent with previous findings (Kovačič et al., 2023), which reported up to 1.4 ng/g under a similar experimental design and spiking level at 100 μg/L. Azithromycin, detected only in the case of TWW+CEC, is known to accumulate in spinach, radish and lettuce grown in soils amended with sludge (Kodešová et al., 2019; Sidhu et al., 2019). In the present study, acetamiprid and dimethomorph were above LOQ in tomato fruits irrigated with spiked wastewater. Interestingly, although dimethomorph was relatively high compared to other CECs in treated wastewater at 7,000 ng/L, it was not detected in tomatoes irrigated with TWW, as uptake is concentration-dependent. However, it may not be in sufficient amounts to exceed the LOQ in tomato fruit, compared to the much higher amount used in the spiked samples.

Variability between this and previous studies likely reflects the influence of factors such as wastewater composition, soil physicochemical properties, plant species and specific growing conditions (Christou et al., 2024; Gatta et al., 2025). Additionally, the physicochemical properties of CECs are important, as plant cell membranes limit the passage of ionic compounds. Nevertheless, weak bases such as azithromycin and clarithromycin were detected in TWW+CEC treated plants, likely due to varying pH levels across plant tissues (Picó et al., 2019; Trapp et al., 2023). A further limiting factor for assessing bioaccumulation is the ability to detect CECs, as compounds may be present at trace levels in fruit, below the detection limit (Nightingale et al., 2025).

3.4. Uptake and translocation of CECs in plant parts

None of the target compounds (Table S18) were quantified in the roots, stems or leaves of plants irrigated with PW and TWW. For spiked treatments, 17 compounds were quantified, with the highest number in roots (15), followed by leaves (10), fruits (6) and stems (5). Descriptive analysis suggests that uptake can be linked with CECs concentration in irrigation media, where CECs stability was higher in PW+CEC compared to TWW+CEC, as concentrations in different plant parts were generally higher under the PW+CEC treatment (Fig. S7) than in the TWW+CEC treatment (Fig. S8).

Carbamazepine exhibited the highest concentrations across all plant parts. In PW+CEC treatment, carbamazepine concentrations were 9,040 ng/g in roots, 2,750 ng/g in stems, and 14,600 ng/g in leaves, a similar pattern to that reported by Denora et al., (2023). In TWW+CEC-irrigated plants, values were lower, i.e., 8,200 ng/g in roots, 2,070 ng/g in stems, and 7,260 ng/g in leaves. In PW+CEC, concentrations followed a specific pattern from highest to lowest: roots > leaves > stems > fruits. However, this trend did not apply to acetamiprid, a systemic insecticide, which showed greater translocation to the leaves in both spiked treatments, i.e., 1,450 ng/g in PW+CEC and 1,760 ng/g in TWW+CEC, followed by the roots (1,210 and 1,290 ng/g) and stems (96.8 and 69.5 ng/g), respectively. Dimethomorph exhibited extensive translocation, with maximum concentrations of 25,700 ng/g in roots, 4,750 ng/g in leaves, and 731 ng/g in stems under PW+CEC treatment. Similar levels were observed in TWW+CEC, with slightly reduced leaf concentrations (roots: 27,400 ng/g; leaves: 3,670 ng/g; stems: 729 ng/g). The highest concentration observed overall was BPS in roots irrigated with PW+CEC (30,600 ng/g), which was nearly twice that in roots irrigated with TWW+CEC (15,600 ng/g). Bisphenol S and BPF were also present in all plant parts, while BPA was below the LOD in stems. Other compounds, such as estrone, β-estradiol, 17α-ethynylestradiol, testosterone, ibuprofen, diclofenac, propylparaben, triclosan, and tonalide, were found in the roots with limited translocation to the aerial parts. The roots showed the highest accumulation, likely due to their direct contact with irrigation water, while stems and leaves act as secondary reservoirs

for translocated CECs. Plants irrigated with TWW+CEC exhibited lower concentrations of most compounds across all tissues (Table S18), supporting the hypothesis that matrix composition and compound stability in irrigation water may influence uptake and accumulation. Overall, the general order of accumulation, in terms of average concentration (μg/kg) and compound detection frequency, was roots > leaves > stems > fruits, agreeing with Riemenschneider et al., (2016), although based on the total amount of CECs (μg), Bueno et al., (2022) reported fruits > stems/leaves > roots.

Analytes that are neutral or cationic at the irrigation water pH, such as carbamazepine and caffeine, can readily cross plant cell membranes, translocate via xylem-driven mass flow, and distribute effectively across various plant tissues (García-Valverde et al., 2023; Martínez-Piernas et al., 2019). This study showed that carbamazepine had the highest translocation in leaves, followed by roots, stems, and fruits, with results similar to those reported in other studies (Miller et al., 2016). In contrast, CECs predominantly in anionic form, such as diclofenac and ibuprofen, are often repelled by negatively charged plant cell membranes, resulting in limited translocation and preferential accumulation in the roots (Wu et al., 2013), as observed in the current study. Bueno et al., (2022) studied tomato plants irrigated with reclaimed water spiked with CECs (1 μg/L). They reported higher average concentrations of acetamiprid and caffeine in roots than in the present study. It is also possible that the original compounds underwent transformation in the soil or were metabolised within the plant, which may account for the observed differences in their measured concentrations within plants (Wei et al., 2023). However, metabolites were out of the scope of this study.

The BCF, RCF and TCF were calculated for each quantified compound. Carbamazepine showed the highest BCF across treatments (Table S19), with BCFs of 0.59 (PW+CEC), 0.69 (TWW+CEC), and 6.7 (TWW). Acetamiprid and dimethomorph followed with moderate BCFs (0.12–0.15 and 0.048–0.051, respectively). In contrast, clarithromycin, azithromycin, and BPS had BCFs below 0.03 in the TWW+CEC treatment, indicating limited transfer from water to fruit. The RCFs (Table S20) indicated that specific compounds preferentially accumulated in roots. BPS showed the highest values (34.7 in PW+CEC and 25.1 in TWW+CEC), suggesting strong root uptake with limited translocation, followed by dimethomorph (18.9–22.9), carbamazepine (6.04–9.2), and triclosan (4.2).

TCFs (Table S21) revealed compound-specific mobility patterns with generally low root-to-leaf values. Carbamazepine (1.6 in PW+CEC; 0.89 in TWW+CEC) and acetamiprid (1.2–1.4) had the highest translocation among the compounds assessed. In contrast, roots-to-stem TCFs were considerably lower for all compounds, with carbamazepine having the highest values (0.30 in PW+CEC and 0.25 in TWW+CEC), followed by acetamiprid (0.08 and 0.05). Low stem/root TCFs suggest limited distribution in stems, particularly for compounds such as BPS, BPF, and dimethomorph, for which TCFs were consistently < 0.05. Acetamiprid showed the highest fruit/root TCFs (0.11 and 0.08), followed by carbamazepine (0.10 and 0.08), while dimethomorph and BPS exhibited negligible values (< 0.003).

3.5. Accumulation in soil

The persistence of CECs in the soil was investigated by analysing composite soil samples from each irrigation treatment at time = 0, 3, and 30 months (Table S22). No compounds were found at the beginning of the experiment (t = 0) in any of the soil samples. When comparing months 3 and 30, although more compounds were present in the soil irrigated with TWW (Fig. S9), namely azithromycin and clarithromycin, compared to PW (BPAF and Triclocarban) (Fig. S10), there was no statistically significant difference between treatments (p values > 0.05).

Fifteen CECs were quantified in soil irrigated with spiked media (PW+CEC-Fig. 7 and TWW+CEC-Fig. 8). Although there was no significant difference in the concentration of CECs between treatments, the concentration of individual compounds varied according to their dissipation half-lives (DT_{50}) and organic carbon–water partition coefficients (Table S23). A comparison based on sampling time revealed compound-specific trends at months 3 and 30.

By month 30, only BPS, carbamazepine and dimethomorph showed a significant decrease ($p < 0.05$), while the others showed no change. Dimethomorph, with a moderate DT_{50} (10–61 days), declined over the 30 months, likely due to the amount of CECs introduced and specific soil properties. In contrast, carbamazepine, with a long half-life (DT_{50} up to 1,624 days), decreased by half between months 3 and 30.

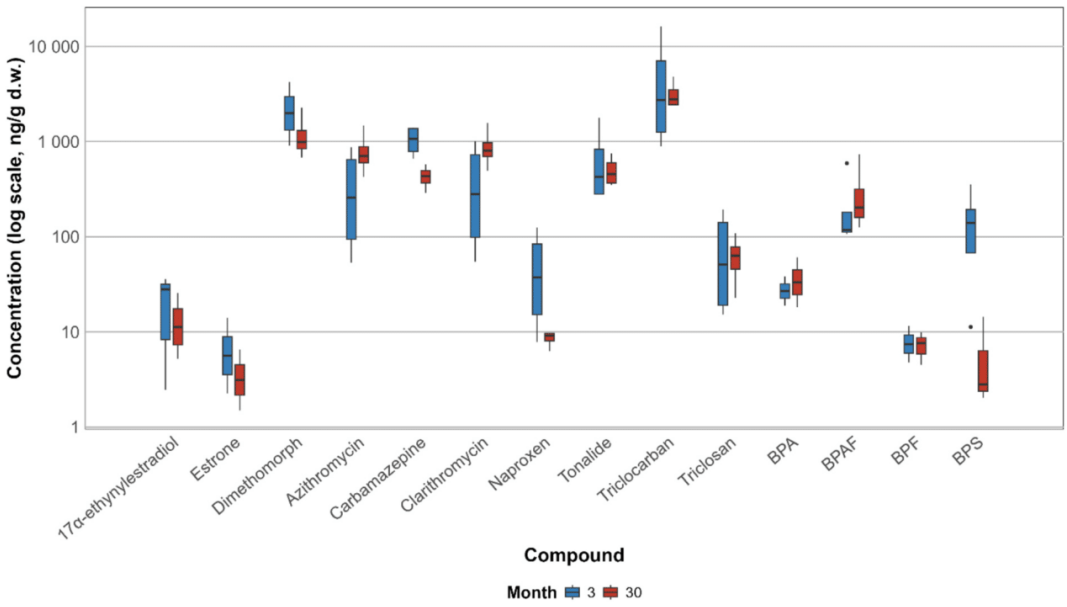


Fig. 7. Boxplots showing CECs concentrations at months 3 and 30 in soil irrigated with PW+CEC.

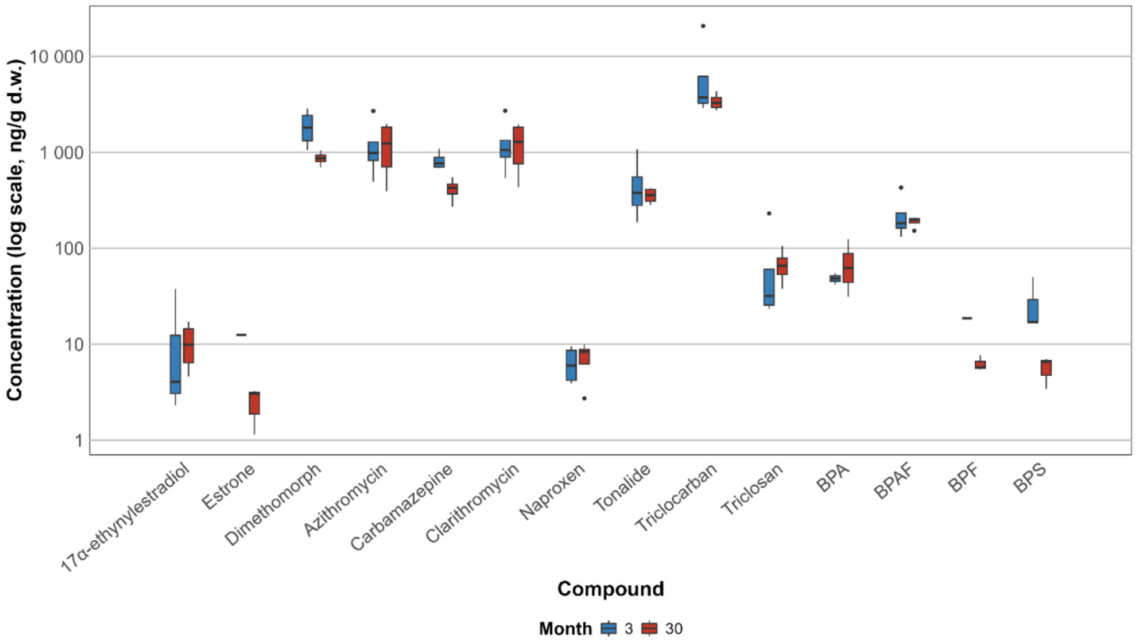


Fig. 8. Boxplots showing CECs concentrations at months 3 and 30 in soil irrigated with TWW+CEC.

Triclocarban persisted at high levels in soil irrigated with spiked media (Month 30, mean: 4,800 ng/g), indicating slower degradation than triclosan. This persistence aligns with previous findings and may be due to triclocarban's strong sorption to soil particles, which limits its bioavailability to degrading microorganisms (Wu et al., 2009). The finding is consistent with its physicochemical properties, as triclocarban is a hydrophobic antimicrobial (log K_{ow}: 4.34) with a relatively long half-life in soil (108 days) and a high Koc (12,000), indicating strong sorption and limited mobility compared to triclosan (log K_{ow}: 4.76, DT_{50} = 18 days, Koc: 3,500). Likewise, the macrolide antibiotics azithromycin and clarithromycin were detectable through month 30, consistent with their DT_{50} values (82–676 days, respectively).

Bisphenols were also detected at months 3 and 30; however, the average remained the same for BPA and BPAF, where BPAF is the most hydrophobic (log K_{ow}: 4.26) and immobile in soil, with a high Koc (760,000). In comparison, BPS, the most hydrophilic compound tested (log Kow: 1.65), is photodegradable, with variable DT_{50} (15–280), and exhibits a reduction in concentration from months 3 to 30 (Choi and Lee, 2017).

3.6. Dietary exposure to CECs and health risk assessment for tomato fruits

The average chronic exposure was calculated across population groups and consumption rates, using the maximum concentration of individual CECs quantified across treatments on a fresh-weight basis (Table S24). A comparison of ADI or TDI values with the estimated exposure levels for TWW indicates no risk to human health. Carbamazepine was the only compound quantified in tomatoes irrigated with TWW, with estimated exposures below the ADI of 0.16 μg/kg bw/day across all population groups. Even the most vulnerable group, toddlers, showed exposure levels of approximately 0.002 μg/kg bw/day, nearly half of the ADI.

Although the spiking scenarios (1 mg/L) are much higher than would be expected under real conditions, dietary exposure levels for acetamiprid, azithromycin, carbamazepine, clarithromycin, and dimethomorph were, although higher, still up to four orders of magnitude below their respective ADIs (Table S11). Only BPS exceeded the tolerable intake levels for all population groups based on the TDI for BPA, since no TDI exists for BPS. These results indicate that, despite the high spiking concentrations, the potential dietary risk from most of the CECs studied is minimal. Although risks are low, this assessment remains tentative as only 27 CECs were targeted. Further studies are required to characterise the presence, toxicity, and persistence of other emerging contaminants not included in the study, as well as CEC metabolites and transformation products and the potential additive or synergistic effects of chemical mixtures.

3.7. Environmental risk assessment for soil

To assess the environmental risks of CECs, RQs were calculated as the ratio of measured environmental concentrations (MEC) to predicted no-effect concentrations (PNEC) (Table S25), with RQ > 1 indicating high ecological risk. In soils irrigated with PW or TWW, BPAF was the only bisphenol above LOD, with RQs ranging from 0.048 to 0.448, indicating a low risk. Under TWW irrigation, RQ values exceeded the threshold for azithromycin, supporting concerns about antibiotic resistance, as antibiotics can stimulate the expression and persistence of resistance genes in microbial communities (Sidhu et al., 2021). Triclocarban also exceeded risk thresholds under both PW and TWW irrigation, even at month 30, underscoring its persistence and potential for bioaccumulation in soil (Khetan and Collins, 2007).

In the spiked treatments (PW+CEC and TWW+CEC), most compounds exceeded the risk thresholds at three and 30 months. At month three, 17α-ethynylestradiol had the highest RQ (2.7 million under PW+CEC), which remained unchanged at month 30, reflecting its potent endocrine-disrupting effects and extremely low PNEC. Triclocarban also showed persistently high RQs, reaching up to 1,000,000 under TWW+CEC at month three and consistently above 400,000 at month 30 (Higgins et al., 2011). Azithromycin presented RQs ranging from 27.8 to

88.6 across spiked scenarios, indicating sustained risk. BPA and BPAF also consistently exceeded the risk threshold in both spiked treatments, with BPA reaching 7.86 under TWW+CEC and BPAF peaking at 2.58 under PW+CEC at month 30. These findings highlight the persistence of several CECs in soil and reinforce the need for long-term monitoring strategies, including non-targeted analyses under realistic (TWW) and elevated (TWW+CEC) exposure scenarios.

4. Conclusions

This field-scale study evaluated the uptake and translocation of CECs in tomato plants irrigated with PW (control), TWW, and spiked (PW+CEC, TWW+CEC) scenarios. Findings confirm that under regular TWW irrigation, bioaccumulation of CECs into edible tissues was limited, with carbamazepine being the only CEC quantifiable in tomato fruits. In contrast, irrigating with spiked media at much higher levels resulted in higher concentrations across all plant parts, especially in the roots, where up to 17 CECs were quantified. In comparison, 6 CECs were also quantified in tomato fruit, although no effect on yield was observed. Importantly, dietary exposure risk assessment indicated no health risk, even for vulnerable groups such as toddlers, when tomatoes were irrigated with TWW. Only BPS, under unrealistic spiked conditions, exceeded the tolerable daily intake.

While the results reveal the limited accumulation of CECs in edible tomato tissues when irrigated with TWW, specific compounds persisted (up to 30 months) in the soil, with risk quotients exceeding safety thresholds for azithromycin and triclocarban, highlighting the importance of long-term soil monitoring to protect environmental and human health. However, it is important to acknowledge that this study focused on only 27 CECs. To fully understand the risks associated with wastewater reuse, future research should include a broader range of compounds through suspect screening and non-target analysis, including metabolites and transformation products.

Declaration of generative AI and AI-assisted technologies in the writing process

While preparing this manuscript, the authors utilised ChatGPT for translation and grammar correction. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109916.

Data availability

Data will be made available on request.

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3.2.3 Hydroponically grown tomatoes in treated wastewater: Uptake of CECs and impact on plant growth

The paper in preparation and entitled "Hydroponically grown tomatoes in treated wastewater: Uptake of CECs and impact on plant growth," authored by E. Andreasidou, A. Kovačič, L. Manzano-Sánchez, Š. Železnikar, D. Heath, M. Pintar, N. Kacjan Maršič, U. Blaznik, A.R. Fernández-Alba, M.D. Hernando and E. Heath will be submitted for publication in December 2025 in Environmental Research. Regarding the conduction of the experiment, I was responsible for experimental design, experimental work and sampling. I was also responsible for data analysis, visualisation and writing the original draft.

The study investigates the uptake, translocation, and bioaccumulation of 27 contaminants of emerging concern (CECs) in hydroponically grown tomato plants (*Solanum lycopersicum* L. cv. Rally) under different growth conditions, including nutrient solution (control), nutrient solution spiked with CECs (0.1 and 1 mg/L), treated wastewater, and treated wastewater spiked with CECs (1 mg/L). A stability experiment was also conducted to evaluate the persistence of target compounds in potable water and treated wastewater under different storage conditions (-20 °C, 4 °C, and 20 °C with/without light). Results demonstrated that most CECs remained stable for at least one week when stored at 4 °C or -20 °C, while degradation was more pronounced at room temperature.

In the hydroponic experiment, uptake by roots was substantial, with concentrations reaching up to 118 µg/g for specific compounds. Translocation to aerial parts was compound-specific and limited overall, with a distribution pattern of roots > leaves > stems > fruits. In fruits, between 6 and 12 CECs were quantified depending on the treatment condition. The calculated bioconcentration and translocation factors indicated that neutral and moderately hydrophobic compounds (e.g., carbamazepine, BPS) exhibited higher mobility, whereas anionic and hydrophobic ones (e.g., diclofenac, ibuprofen) accumulated mainly in roots. A phytotoxicity test revealed that high CEC concentrations and nutrient depletion inhibited plant growth. Although the tomatoes were not ripe, the dietary exposure assessment indicated negligible health risks for most CECs, except for bisphenols BPF and BPS, which exceeded the conservative tolerable daily intake values.

This study aimed to achieve two objectives of the thesis, namely to investigate the uptake and translocation of CECs in tomato plants grown hydroponically in treated wastewater and to assess the potential risks associated with their consumption. The novelty of this study lies in testing the stability of CECs under various storage conditions and evaluating their effects on plant growth in a hydroponic (soilless) system, encompassing four growth regimes that utilise either a nutrient solution or TWW. The conditions were selected to evaluate the effect of matrix composition and the effect of CECs addition (by comparing the nutrient solution with TWW, with or without spiking). The selected treatments were the same as those in the soil-grown experiment (lysimeters) to facilitate differences with conventional growing practices (3.2.2). The work is important when considering the reuse of wastewater in sustainable water management practices.

Hydroponically grown tomatoes in treated wastewater: Uptake of CECs and impact on plant growth

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1. Introduction

Freshwater scarcity is recognised as one of the main issues threatening food production, a problem exacerbated by climate change, population growth, and competing demands for limited water resources. Agriculture, which accounts for over 70% of global freshwater consumption, is particularly susceptible to drought [1], [2], [3]. As a result, there is a growing need to adopt sustainable water management strategies [4].

Among these strategies is the use of treated municipal wastewater (TWW) to supplement irrigation, a practice that shows considerable promise. TWW is already being implemented in regions of southern Europe (Spain, Italy and Cyprus) and the Middle East [5], [6]. Currently, 1 billion m^3 of treated wastewater is reused annually within the EU [7]. TWW is also a source of macronutrients, such as nitrogen and phosphorus, that support plant growth and can decrease reliance on synthetic fertilisers [8], [9].

Although the argument for wastewater reuse is well supported from a resource recovery perspective, its application in food crop irrigation could potentially introduce pathogens and chemical pollutants (potentially toxic elements, and contaminants of emerging concern, CECs), which have been detected in TWW effluent, into the food chain. [10]. Contaminants of emerging concern are a broad category of synthetic and naturally occurring compounds, including pharmaceuticals, personal care product ingredients, industrial chemicals, and hormones [11], [12].

At the policy level, the EU Regulation (EU) 2020/741 establishes harmonised minimum water quality and monitoring requirements to ensure the safe use of reclaimed water for agricultural irrigation, which only sets limits for microbiological parameters (e.g., E. coli, and, in specific cases, Legionella spp. and intestinal nematodes) and physicochemical parameters (e.g., BOD, TSS, turbidity) [13]. However, requirements for CECs may be considered necessary following a comprehensive risk assessment, although many are only partially removed by conventional wastewater treatment technologies [14], [15], with concentrations of CECs ranging from nanograms to micrograms per litre having been detected in effluents [16], [17].

Wastewater reuse can introduce these CECs into agro-ecosystems, where they can accumulate in soil and be taken up by plants. Previous studies have detected compounds such as carbamazepine, triclosan, and diclofenac in edible plant tissues following irrigation with reclaimed water [18], [19]. The introduction of CECs through wastewater can also impact agricultural soils, e.g., by altering soil/root microbiome important for optimum plant growth [20], [21]. Thus, understanding the fate of CECs in the soil-plant continuum is critical to ensure the safe reuse of wastewater for food production.

Hydroponics is a method of growing plants without soil, in a pure circulating nutrient solution system or a system with minimal substrate. Here, the substrate refers to the use of an artificial medium, such as sand, gravel, vermiculite, rockwool, perlite, peat moss, coir (the fibrous husk of the coconut), or sawdust, to provide mechanical support to plant roots, rather than soil. This technique offers more control over growing conditions, leading to faster growth and higher yield [22], [23].

Previous research using hydroponic systems has demonstrated the uptake of different CECs, including carbamazepine, diclofenac, and triclosan [24], [25], [26], with accumulation depending on compound, plant characteristics and environment [27]. Most studies focus on leafy vegetables, as they are widely cultivated (hydroponically), have short growth cycles, high transpiration rates and large surface areas that favour contaminant uptake, making them ideal model crops for experimental studies [28].

Studies on fruit-bearing crops, such as tomatoes, remain limited (Table 1). Additionally, most studies focus on a limited number of compounds, while overlooking the influence of complex contaminant mixtures that are typically present in treated wastewater. To the authors knowledge, human health risk assessment data are limited for hydroponically grown tomatoes. For example, among the published studies, only six investigate tomato plants, of which only three examine bioaccumulation in

fruits. Within these studies, Riemenschneider et al. [25] evaluated the uptake of carbamazepine in hydroponically grown tomatoes and quantified 11 transformation products. Lu et al. [29] studied the uptake of BPA and nonylphenol following foliar exposure and root exposure (hydroponics set-up). Although the estimated daily intake was calculated, assessment of risks to human health was beyond the scope of this study. Risk assessment was evaluated by Kovačič et al. [30], which showed low dietary chronic exposure for BPS, BPF, and naproxen following the consumption of tomatoes grown hydroponically in a spiked nutrient solution (0.1 mg/L).

Table 1: Literature on uptake studies of hydroponically grown tomatoes.

Compounds Studied	**Plants Studied**	**Plant Parts**	**Treatment**	**Compounds detected in tomato fruits**	**Refs**
Carbamazepine and 13 metabolites	Tomato	Roots, leaves, stems, fruits	Nutrient solution + spiking at 500 µg/L	Carbamazepine and 11 metabolites	[25]
Nonylphenol, BPA	Lettuce and tomato	Roots, leaves, stems, fruits	Nutrient solution + spiking at 50 µg/L	Nonylphenol, BPA	[29]
14 CECs (pharmaceuticals, hormones, industrial chemicals)	Tomato	Fruits	Nutrient solution + spiking at 100 µg/L	Bisphenol S, bisphenol F, naproxen	[30]
16 CECs (pharmaceuticals, personal care products, endocrine disrupting chemicals)	Carrot, lettuce, tomato	Roots, leaves	Nutrient solution + spiking at 1 µg/L	-	[31]
7 CECs (pharmaceuticals, personal care products, hormones, industrial chemicals, insect repellents)	Tomato, corn and wheat	Roots, leaves, stems	Nutrient solution + spiking at 4 mg/L	-	[32]
11 CECs (pharmaceuticals, PFAS)	Lettuce and tomato	Roots, stems	Nutrient solution + spiking at 5 µg/L	-	[33]

Often neglected during experiments is the stability of CECs during the storage period, since different processes, such as hydrolysis and sorption, can change the micropollutants content [34], [35]. Different storage conditions, light exposure and temperature can significantly influence the persistence of pharmaceuticals and personal care products in aqueous systems, altering the effective dose that reaches plant roots [36]. Evaluating the degradation and sorption losses is essential during uptake studies [37].

This study aims to close the identified knowledge gap by investigating the uptake, translocation, and bioaccumulation of 27 contaminants of emerging concern in hydroponically grown tomato plants (*Solanum lycopersicum L.* cv. Rally) under various treatments: (1) nutrient solution (control), (2) nutrient solution spiked with a mixture of CECs, (3) TWW, and (4) TWW spiked with a mixture of CECs. Growing plants this way offers controlled conditions for studying the uptake of CECs and evaluating the effect of CECs on plants, compared to soil-based systems, since soil-related effects (e.g., sorption and microbial degradation) in the rhizosphere are excluded [38], [39].

The study was designed to test two main effects: (i) the effect of matrix composition (nutrient solution and TWW, with and without spiking) and (ii) the effect of increasing CEC input concentration. Based on previous uptake studies, it is hypothesised that treated wastewater would lead to higher uptake of CECs than nutrient solution. The higher spiking level was therefore chosen to facilitate their detection

in different plant parts. In addition, a stability experiment was conducted to evaluate the fate of the studied CECs under different sample storage conditions (20 °C light/dark, 4 °C, and -20 °C). Finally, the potential human health risk associated with consuming hydroponically grown tomatoes was evaluated through a dietary exposure assessment.

2. Materials and Methods

2.1 Reagents and chemicals

Details on reagents and chemicals used are provided elsewhere [40]. All solvents (MeOH and acetonitrile, MeCN) were LC-MS grade. Ultrapure water (18.0 MΩ cm at 25 °C) was obtained using the Millipore Direct-Q purifying system (Merck Millipore, Darmstadt, Germany). Stock standard solutions and selected deuterated internal standards (ISTDs) for the selected 27 CECs (purity > 97%) were prepared by dissolving each compound in methanol (MeOH) at concentrations of 1 mg/mL. Working solutions were prepared by serial dilutions from the initial stock with methanol to achieve different concentration levels (10, 100, and 1000 ng/mL). Standards and working solutions were stored in the dark at -20 °C.

2.2 Experimental Design

2.2.1 Hydroponics Experiment

The entire experiment was conducted during the 2022 growing season under glass at the Biotechnical faculty, University of Ljubljana (Slovenia) from May 2022 (18/05/2022) for 58 days. Tomato seedlings (*Solanum lycopersicum L.*, cv. Rally) were first cultivated for a period of eight weeks, until each plant reached the 5–6 leaf developmental stage, before transplanting. Afterwards, seedlings were transplanted to 60-L covered hydroponic tubs in the greenhouse. Each tub (120 cm × 60 cm) was covered with a sheet of Styrodur® (20 mm) in which plants were fixed in prepared holes (⌀ 50 mm) using inert polyester foam. Plants were grown in a pure circulating nutrient solution system, without any other type of substrate present as a support, to avoid sorption of CECs present in the aqueous media onto the substrate [41]. In total, the experiment consisted of fifteen tubs, each containing four plants (Fig. 1). Three tubs were allocated to each treatment, resulting in a total of twelve plants per treatment. The selected treatments are presented in Table 2.

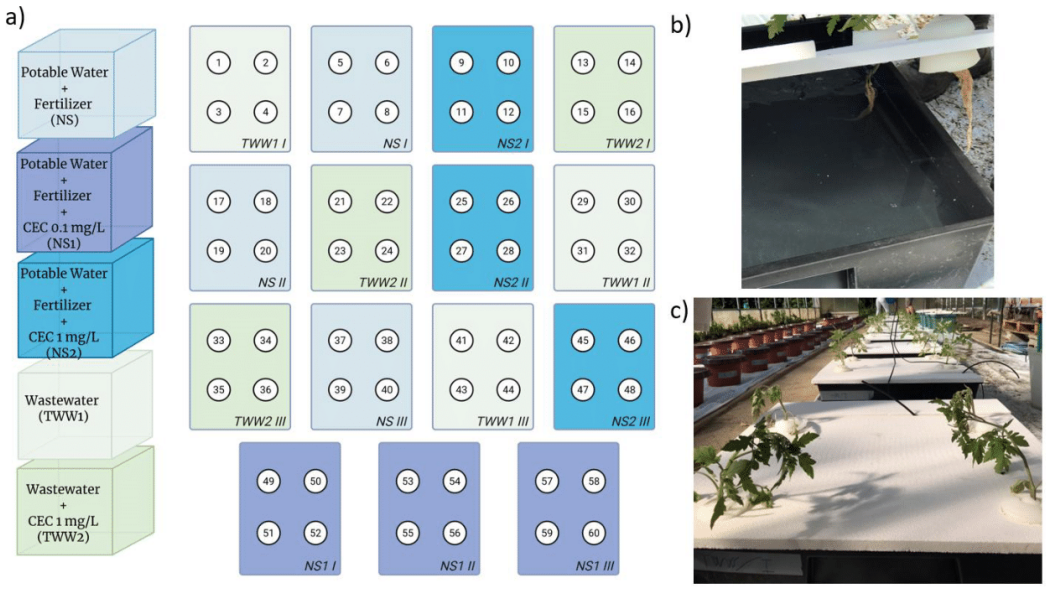


Figure 1: a) Hydroponics experimental design, b) hydroponic tub close-up, including the mixing pump and c) hydroponic plants set up (four plants per tub).

Table 2: Treatments included in the uptake experiment.

Abbreviation	Treatment	Addition of CEC (mg/L)
NS	Nutrient solution (Control)	-
NS1	Nutrient solution + CEC low	0.1
NS2	Nutrient solution + CEC high	1
TWW1	Treated wastewater	-
TWW2	Treated wastewater + CEC high	1

For the control, nutrient solution (NS), plants were exposed to the standard Hoagland Arnon solution prepared using potable water and following ionic composition: 14.0 mM NO_3^-, 1.0 mM NH_4^+, 1.0 mM $H_2PO_4^-$, 6.0 mM K^+, 4.0 mM Ca^{2+}, 2.0 mM SO_4^{2-}, 2.0 mM Mg^{2+}, 10 µM Mn^{2+}, 5.0 µM Zn^{2+}, 30 µM B^{3+},15 µM Fe^{3+}, 0.75 µM Cu^{2+}, and 0.5 µM Mo^{6+}. The pH was adjusted to pH 6 by adding 74% H_2SO_4. Electrical conductivity was 1 dS/m. Plants were exposed to standard Hoagland Arnon nutrient solution with the addition of CECs at 1 mg/L in NS2.

Treated wastewater (TWW1 & TWW2) was obtained from the Ajdovščina WWTP, Slovenia, a biological treatment plant with denitrification, designed for an organic load of ≤42,000 population equivalents (PE). The WWTP treats 8,400 m^3/day of wastewater, comprising 30% municipal and 70% industrial sources. TWW was used to evaluate its potential as a growing medium and investigate the uptake of CECs compared to the nutrient solution. Treatment TWW1, as well as TWW with the addition of CECs at 1 mg/L (TWW2), did not include any addition of nutrients. Since TWW contains nutrients, this approach was chosen to assess whether they are sufficient to support plant growth without supplementation, compared to potable water. For the preparation of spiked media (NS1, NS2, TWW2), 1,000 litres were spiked with 1 L of a stock solution of the selected CECs in ethanol (1 g/L). The media

were stored in 1m^3 plastic (HDPE) containers. The nutrient solution within the tubs was circulated continuously via a pump and refilled when the media in the tubs was reduced by 50%.

During the first 14 days, reduced plant growth was observed in treatments NS2, TWW1, and TWW2, characterised by a poor root system, leaf chlorosis, and necrosis. The high concentration of CEC (1 mg/L) was considered the primary factor contributing to poor growth. Therefore, an additional set of hydroponic tubs (NS1) was included, containing a nutrient solution spiked with CECs at a 10-fold lower concentration (0.1 mg/L); this concentration had been previously used without adversely affecting plant growth [30]. However, plants in these tubs still exhibited minimal growth. This observation suggests that, in addition to the presence of ethanol residues used to prepare the spike, reduced nutrient availability and high temperature could also have contributed to the poor growth observed [42].

2.2.2 Phytotoxic experiment on early-stage tomato plant development.

A smaller phytotoxicity experiment was conducted to investigate the source of growth inhibition observed during the main experiment and to assess the effect of varying CEC concentrations on plant growth (Fig. 2). Tomato plants (variety *Solanum lycopersicum L.* Rally) were initially grown in a greenhouse under controlled agronomic conditions until the seedlings developed 3–4 leaves, approximately four weeks after sowing. The seedling's roots were carefully cleaned of any compost residues before being transferred to a series of glass jars (800 mL) containing 600 mL of growth media. To ensure sufficient aeration, a small aquarium diffuser was placed in each jar and connected to an aquarium pump (Figure 2). Each jar was placed in a blacked-out beaker and covered with a black cloth. At the beginning of the experiment, each plant was acclimated for one week in a nutrient solution. After this period, only healthy plants were selected, and jars were refilled with fresh solution.



Figure 2: Set-up of phytotoxic experiment.

The experiment included a control treatment (C) exposed to nutrient solution, tomato plants exposed to different concentrations (1, 0.1, 0.01 mg/L) of the selected CEC spiked into the nutrient solution, and treated wastewater (Table 3). Each treatment included five replicates.

Table 3: Treatments included in the phytotoxic experiment.

Treatment		CEC Spiking conc. (mg/L)
C	Nutrient solution (control)	-
S1	Nutrient solution + CEC	0.01
S2	Nutrient solution + CEC	0.1
S3	Nutrient solution + CEC	1
S4	Treated wastewater	-
S5	Treated wastewater + Nutrients	-

The nutrient solution was prepared according to the Hoagland-Arnon method, which is the same as that used in the hydroponic experiment. The use of an organic solvent during spiking was eliminated by drying the spiking solution under nitrogen and then redissolving it in the nutrient solution. Growth parameters (plant mass and chlorophyll) were measured at time = 0 and then every 7 days until the end of the experiment (t = 22 days) and reported as weeks (0-3). Measurements of chlorophyll content were carried out using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan),

2.2.3 Stability

Testing of CEC stability was designed to mimic the storage conditions of aqueous media during uptake experiments as well as storage in the laboratory after sampling. This approach was used since, in standard reuse practices, TWW is transported and stored before application, which can alter the concentration of CECs [36]. The conditions tested included three temperatures (−20 °C, 4 °C, and room temperature), each under both darkness and light exposure (using a fluorescent Osram Fluora lamp), and six time intervals (0, 1, 3, 7, 14, and 27 days). Samples were prepared for each condition and time interval by spiking 1 mL of wastewater or potable water with a mixture of the selected CECs (1 mg/L). The test solutions were stored in Eppendorf tubes. Each sample was prepared in triplicate.

2.3 Sample collection, preparation and analysis

Due to the poor growth, the plants of treatments NS2, TWW1, and TWW2 were removed from the growth media after 38 days. Tomato fruits were also collected, although not fully ripe or of a marketable size. Plant parts were also collected and air-dried. The remaining treatments (NS, NS1) were removed from the growth media after 58 days, to achieve the exposure of NS1 plants for a similar period as NS2 (approximately 40 days). At both samplings, fruits were collected in plastic freezer bags, transferred to the laboratory and stored whole at -20 °C. Frozen samples were cut into pieces using ceramic knives, weighed, freeze-dried, and ground into a powder. Composite samples were prepared on a per-tub basis for treatments with higher fruit yield (NS, TWW1), whereas for treatments spiked with CECs, a single composite sample was prepared using all harvested fruits from each treatment. After drying, plant parts were separated into leaves, roots and stems. All parts were ground to a fine powder. Aqueous samples from storage containers (100 mL) were collected at 1, 38, and 58 days. Additionally, samples (10 mL) were collected before and after refilling the tubs to evaluate the presence of CECs.

Different sample preparation protocols were applied for the different samples. For wastewater and potable water, 50 mL were extracted using Oasis Prime HLB cartridges (60 mg, 3 cc, Waters Corporation, Milford, MA, USA), with a linear range of 80-8000 ng/L. For spiked liquid samples (wastewater and potable water containing CECs), a 1 mL aliquot was extracted using an Oasis Prime HLB 96-well SPE plate (30 mg sorbent per well; Waters Corporation, Milford, MA, USA). To determine CECs in tomato fruits, freeze-dried composite samples (0.25 g) were processed using solid-liquid extraction with sonication, followed by centrifugation. The extracts were purified using Oasis Prime HLB cartridges (500 mg, 6 cc). For leaves, roots, and stems, 1 g of the dried composite sample was extracted and cleaned up using QuEChERS and automatic µSPE. All extraction methods are defined in [43].

Samples were analysed using a UHPLC (Shimadzu Nexera X2 UHPLC, Kyoto, Japan) coupled to a linear ion trap quadrupole 4500 QqLIT-MS/MS (Sciex, Framingham, MA, USA) using a Poroshell 120 EC-C18 column. The 4500 QTRAP analyser was operated under electrospray ionisation (ESI) in both modes (positive and negative). For plant parts, the same method was transferred to a UHPLC coupled to a linear ion trap quadrupole 6500 QqLIT-MS/MS (Sciex, Framingham, MA, USA). Full details on the analysis are described elsewhere [44]. All methods were validated in accordance with EU guidelines (DG-SANTE) [45]. Data processing, statistical analysis, and visualisation for CECs were performed in R (version 4.3.3) [46].

2.4 Bioconcentration, root concentration and translocation factors

The bioconcentration factors (BCF) were calculated as the ratio of CEC concentration in fruits to the nominal concentration in the growth medium (Eq. (1)) [47].

$$BCF\ (L/kg) = \frac{Concentration\ in\ fruit\ (\mu g/kg)}{Concentration\ in\ irrigation\ water\ (\mu g/L)} \quad (1)$$

The root concentration factors (RCF) were calculated based on Eq. (2).

$$RCF\ (L/kg) = \frac{Concentration\ in\ roots\ (\mu g/kg)}{Concentration\ in\ \ irrigation\ water\ (\mu g/L)} \quad (2)$$

The translocation factor (TF) was calculated according to Eq. (3) to assess compound translocation from roots to aerial plant parts, including leaves, stems, and fruits, in cases where a compound was quantified in that specific compartment.

$$TF\ = \frac{Concentration\ in\ fruit, leaf\ or\ stem\ (\mu g/kg)}{Concentration\ in\ roots\ (\mu g/kg)} \quad (3)$$

2.5 Human health risk assessment

Dietary exposure was quantitatively assessed for three population groups, including children (toddlers), adolescents, and adults, by using respective body weight values suggested by EFSA. The calculation integrated the concentrations of tomato composite (expressed on a fresh weight basis, based on a water content of 95%) with food consumption data, which was acquired from the EFSA Comprehensive European Food Consumption Database (Table 4) [48].

Table 4: Consumption rates for tomatoes in Slovenia, acquired from EFSA Food consumption statistics.

Population Group (L2)	Body weight (kg)	Exposure hierarchy (L7)	Number of subjects	Number of consumers	Mean (kg/day)	Standard Deviation	97.5th percentile (kg/day)
Adults	70	Tomatoes	385	189	0.0297	51.73	0.17742
Adolescents	53	Tomatoes	484	198	0.0158	34.28	0.11646
Toddlers	12	Tomatoes	343	156	0.0079	16.45	0.05429

Data on consumption were selected for the Slovenian population, utilising the FoodEx2 exposure hierarchy up to level 7 (Vegetables and vegetable products to tomatoes). A deterministic approach was employed, with the mean dietary exposure derived using average consumption data, and high exposure scenarios were based on the 97.5th percentile of tomato consumption. The estimated daily intake (EDI), expressed in milligrams per kilogram body weight per day (mg/kg bw/day), was calculated according to Eq. (4):

$$Dietary\ exposure\ (mg/kg\ bw/day) = \frac{CEC\ (mg/kg\ FW) \times Food\ Consumption\ (kg/day)}{Body\ weight\ (kg)} \quad (4)$$

The resulting EDI values for individual compounds were subsequently compared with health-based reference values, including Acceptable Daily Intake (ADI), Tolerable Daily Intake (TDI), Occupational

Exposure Limits (OEL), Permitted Daily Exposure (PDE) and No Observed Adverse Effect Level (NOAEL) values retrieved from the scientific literature (Table 5).

Table 5: Health-based reference values for individual CEC.

Compound	µg/kg bw/day	Assessment	Ref.
Acetamiprid	5	ADI	[49]
Azithromycin	1.7	ADI	[50]
BPF	0.0002	TDI	[51]
BPS	0.0002	TDI	[51]
Caffeine	5700	ADI	[52]
Carbamazepine	0.16	ADI	[53]
Ciprofloxacin	0.15	ADI	[54]
Clarithromycin	1.4	OEL	[54]
Dimethomorph	50	ADI	[55]
Erythromycin	5	ADI	[54]
Naproxen	1.43	OEL	[54]
Propylparaben	2000	PDE	[56]

2.6 Data processing and analysis

Data processing, statistical analysis, and visualisation were carried out in R (version 4.3.3) (R Core Team, 2023). Values below the LOQ were imputed with random values between zero and the matrix- and treatment-specific LOQ to retain variability without bias. Concentrations were $\log_{10}$-transformed to correct for skewness, and normality was verified using the Shapiro–Wilk test. Depending on the data distribution, differences between treatments were assessed using either parametric tests (t-tests or one-way ANOVA followed by Tukey's HSD) or non-parametric alternatives (Kruskal-Wallis followed by Dunn's test with Holm adjustment). Effect sizes (Cohen's d, η^2, or ε^2) were calculated to evaluate the magnitude of differences, and treatments sharing the same significance letter were considered statistically similar.

For the stability experiment, raw area-ratio values were normalised to the Day-0 measurement of each compound and condition to obtain the percentage of signal remaining over time. To evaluate degradation, a linear regression model was fitted for each compound and each storage condition (4 °C, 20D, 20L, −20 °C). Sampling day was included as the predictor variable, allowing the regression slope to represent the degradation rate, namely how fast the compound loses signal as the storage time increases. Negative slopes indicated loss of signal over time and positive slopes indicated increase. Heteroscedasticity-consistent (HC3) standard errors were used to ensure robust estimation despite non-constant variance across days. Because each compound was measured under all four storage conditions, the set of slopes constituted a repeated-measures structure (one slope per compound per condition). Global differences in degradation rate across the four storage conditions were therefore assessed using the Friedman test, a non-parametric alternative to repeated-measures ANOVA. Where the Friedman test indicated significant differences, pairwise comparisons between storage conditions were performed using the Wilcoxon signed-rank test, with compounds treated as matched pairs. A significance level of $\alpha = 0.05$ was used for all tests, and Benjamini–Hochberg correction was applied to Wilcoxon post-hoc comparisons, with adjusted $p < 0.05$ considered statistically significant.

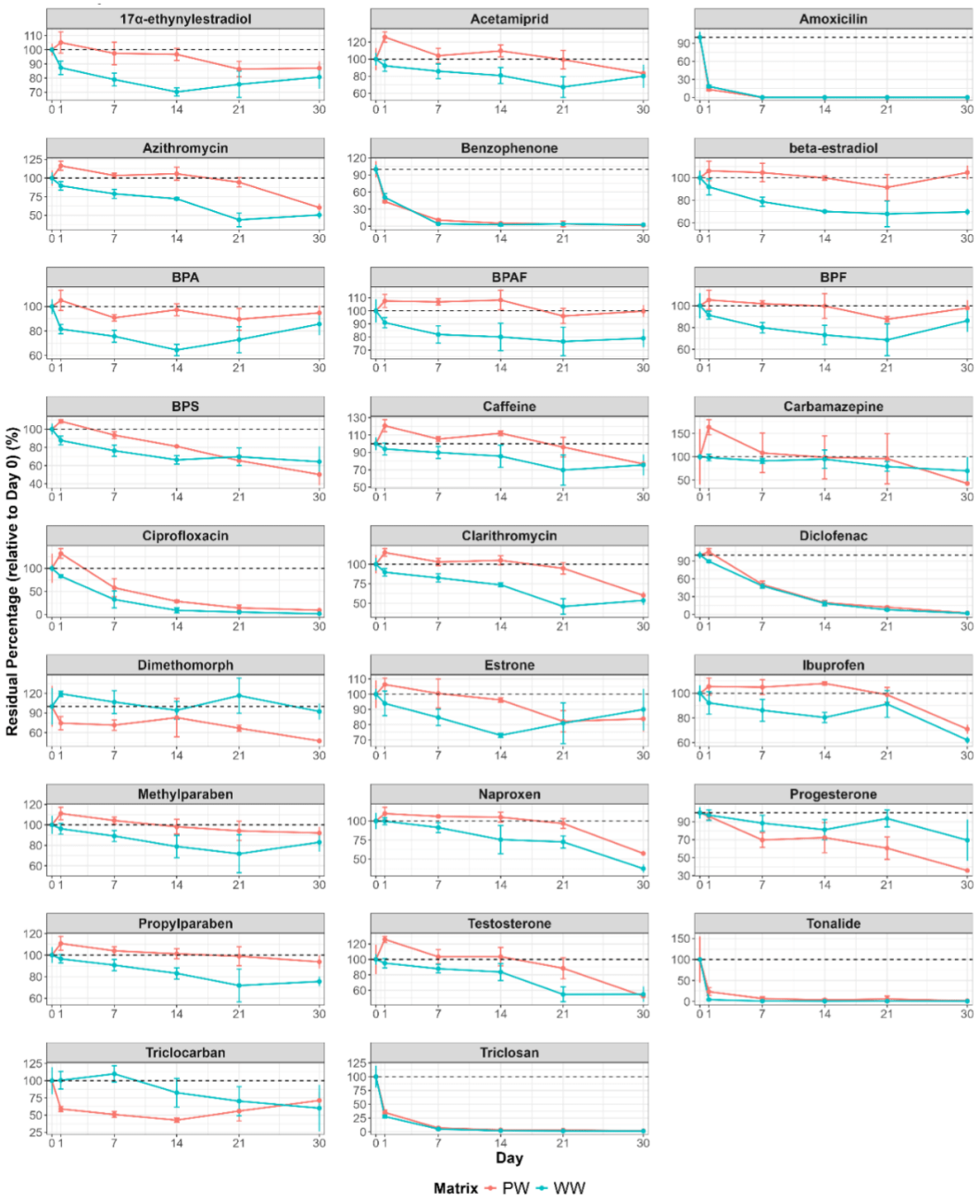
3. Results and Discussion

3.1 CECs in growth media and stability results

3.1.1 Stability of CECs

Compound stability was expressed as the percentage of the Day-0 area-ratio signal remaining at each subsequent sampling point. Erythromycin was excluded from the dataset because the signal of initial measurement was low, resulting in increased percentage changes over time.

For wastewater, storage at room temperature (21 °C) under light (Fig. 3) resulted in 18 out of 26 compounds 75% of their initial response was retained after 7 days, and 19 compounds remaining above 50% after 30 days. Under dark conditions (Fig. 4), 20 compounds remained above 75% after one week, and 19 remained above 50% after 30 days, indicating only partial photodegradation under light exposure, which was considered statistically significant. At 4 °C (Fig. 5), 21 compounds retained more than 75 % after one week and 19 compounds more than 50 % after 30 days. The highest stability was observed under frozen storage (-20 °C) (Fig. 6), where 23 compounds remained above 75% after 7 days and 24 compounds above 50% after 30 days.



287

288 Fig. 3: Stability of selected contaminants of emerging concern (CECs) over 30 days under 21 °C (light).
289 Results are expressed as residual percentage relative to Day 0 for potable water (PW) and wastewater
290 (WW) matrices. Error bars indicate standard deviations (n = 3).

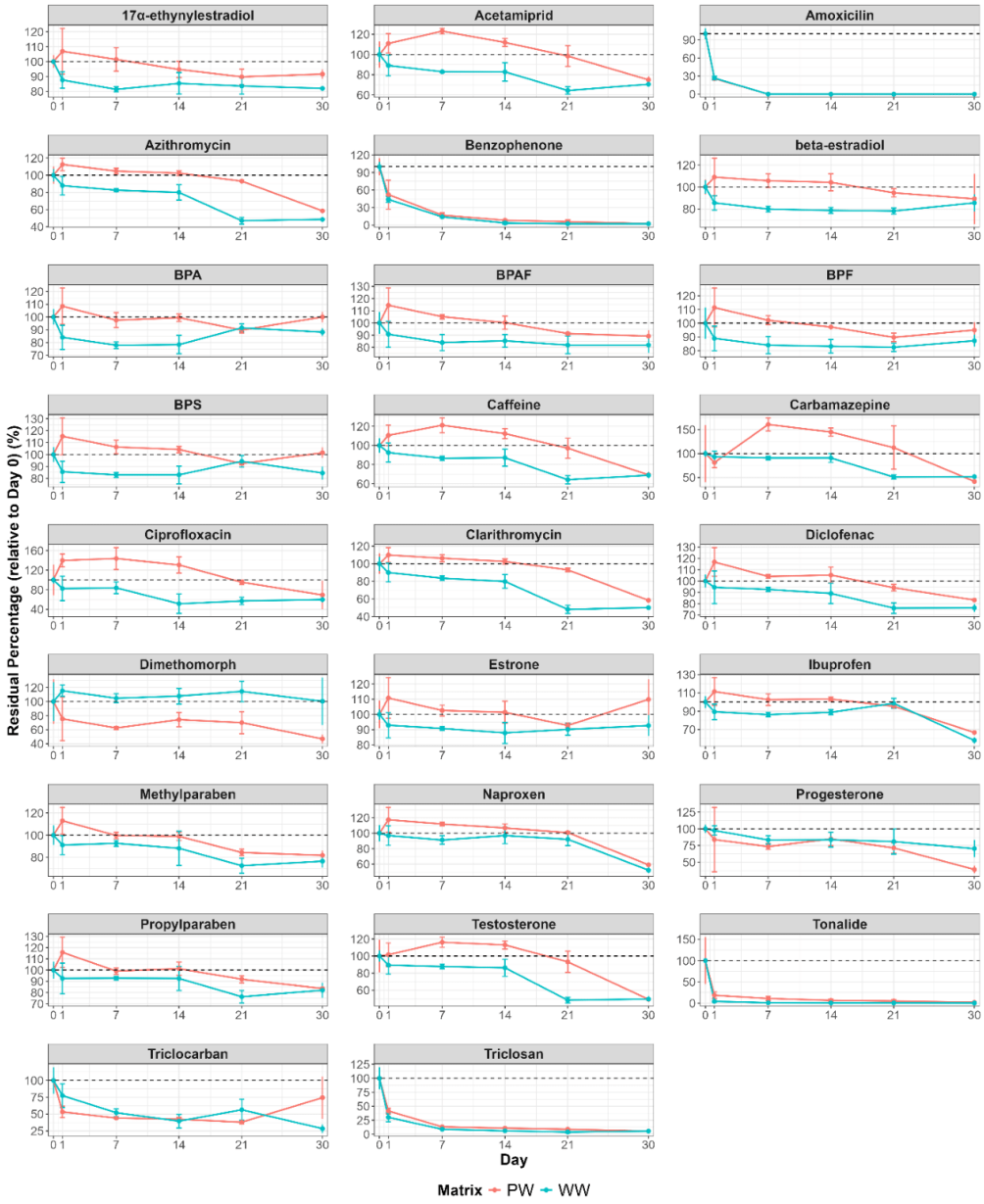
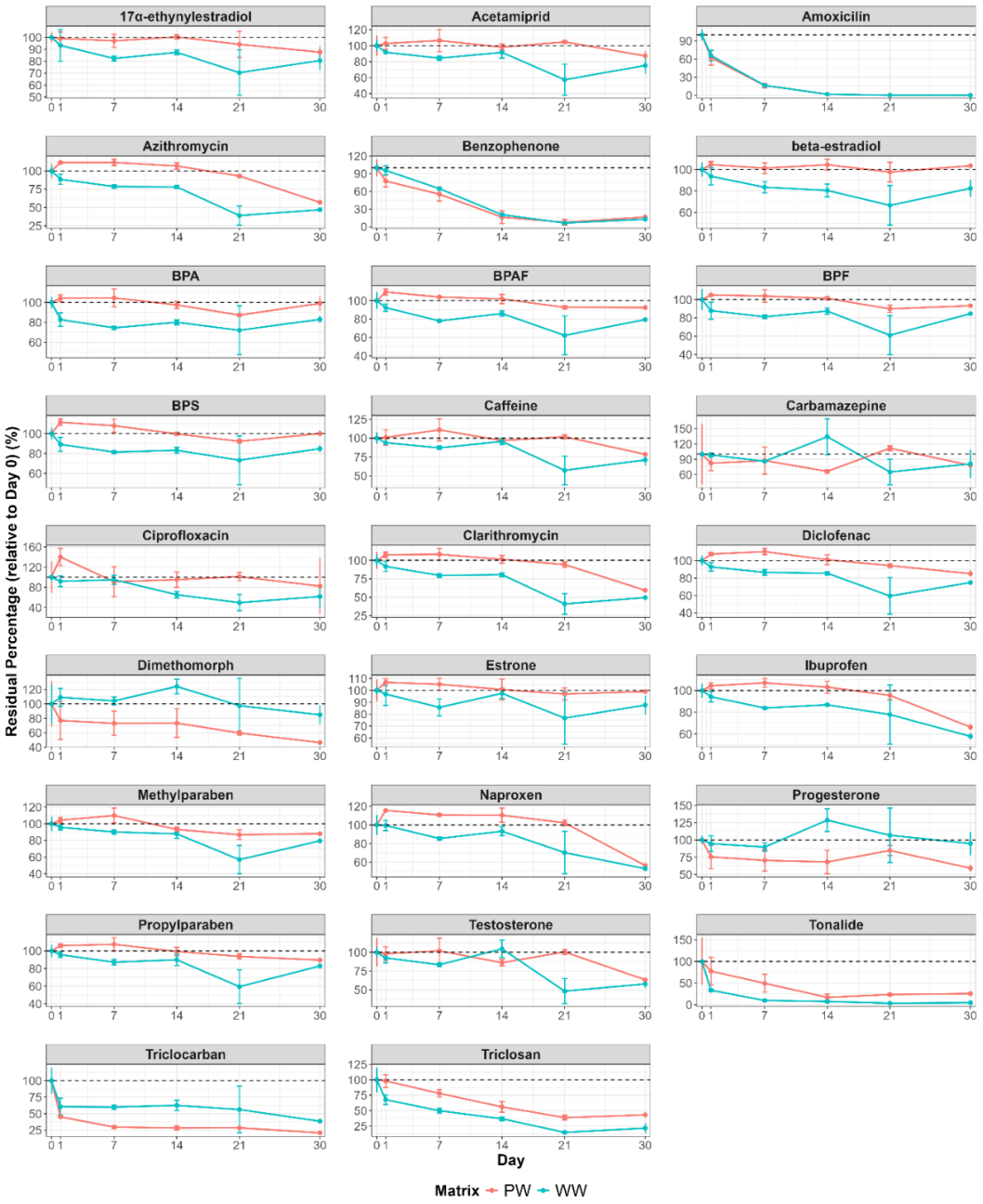


Fig. 4: Stability of selected contaminants of emerging concern (CECs) over 30 days under 21 °C (dark). Results are expressed as residual percentage relative to Day 0 for potable water (PW) and wastewater (WW) matrices. Error bars indicate standard deviations (n = 3).



295

296 Fig. 5: Stability of selected contaminants of emerging concern (CECs) over 30 days under 4 °C. Results
297 are expressed as residual percentage relative to Day 0 for potable water (PW) and wastewater (WW)
298 matrices. Error bars indicate standard deviations (n = 3).

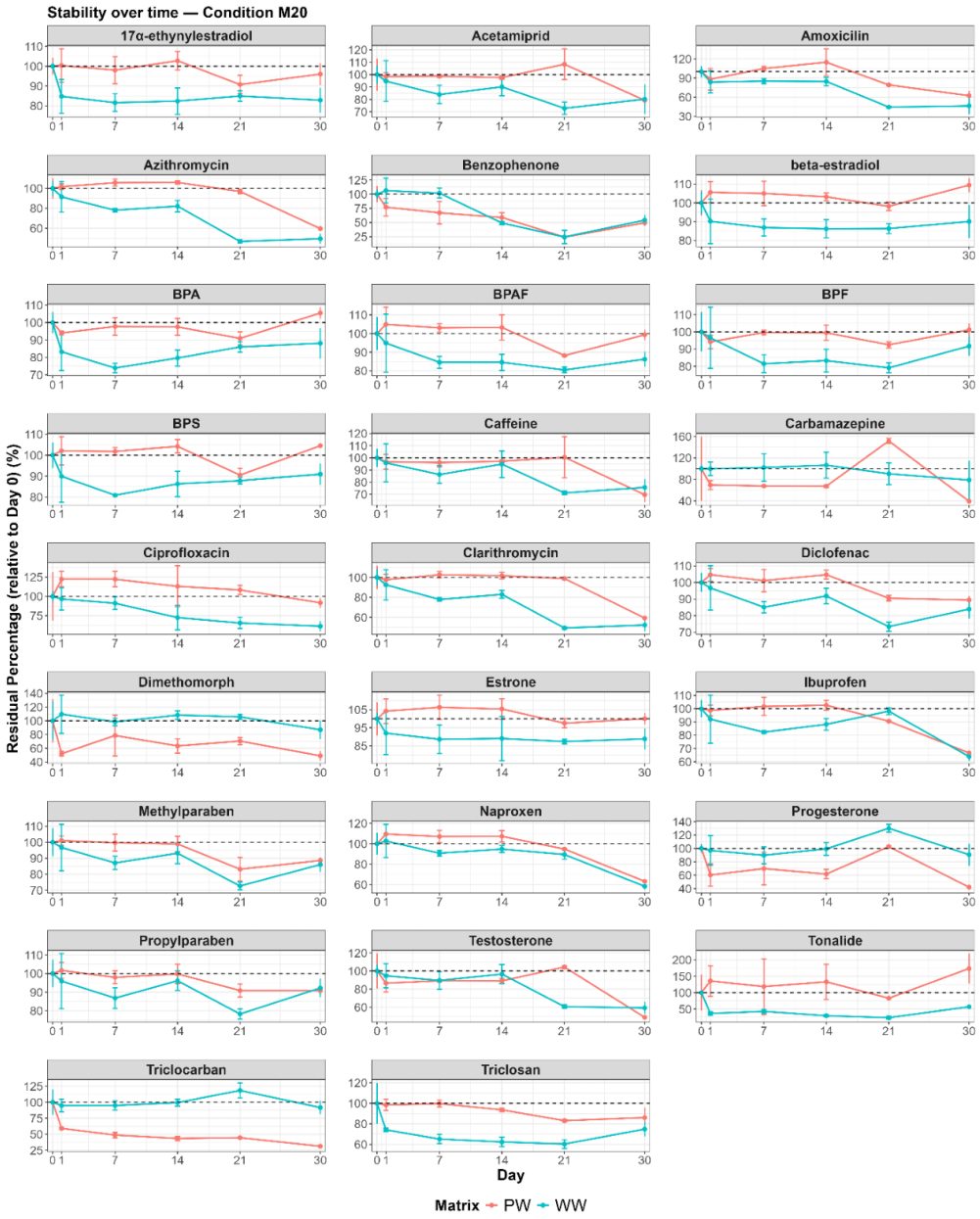


Fig. 6: Stability of selected contaminants of emerging concern (CECs) over 30 days under -20 °C. Results are expressed as residual percentage relative to Day 0 for potable water (PW) and wastewater (WW) matrices. Error bars indicate standard deviations (n = 3).

For potable water, 17 of 26 compounds stored at 21 °C under light remained above 75% after 7 days and 17 compounds above 50% after 30 days. Under dark conditions, 20 compounds remained above 80% after 7 days, and 19 remained above 50% after a month. At 4 °C, 20 compounds maintained more than 80 % of their initial concentration after one week, and 19 compounds exceeded 50 % after a month. The highest stability was achieved at -20 °C, where 22 compounds remained above 80% after 7 days and 20 compounds above 50% after the final sampling.

Across all tested conditions, compound stability strongly depended on chemical class, storage temperature, and matrix type. Antibiotics such as amoxicillin exhibited low stability, particularly in wastewater and at room temperature, confirming their known susceptibility to hydrolysis and microbial transformation in aqueous matrices [57]. Personal-care products like triclosan and triclocarban, and tonalide were also unstable in wastewater and light-exposed samples, reflecting their sensitivity to photo- and biodegradation [58], [59]. Bisphenols were overall stable, even in the wastewater matrix, which agrees with literature findings [60]. Overall, only -20 °C differed significantly from the other storage conditions, while 21 °C (light and dark) and 4 °C showed comparable stability. A comparison between PW and TWW showed no statistically significant difference, with matrix effects varying per compound.

3.1.2 CECs in growth media

The analysis of CECs in PW showed that concentrations were below LOD for all the tested compounds. In wastewater, 21 compounds were above the LOQ (37–1,840 ng/L), with the highest mean concentrations observed for BPAF (1,320 ng/L), followed by BPA (1,140 ng/L). Other compounds with elevated concentrations (>500 ng/L) included beta-estradiol, BPS, carbamazepine, estrone and naproxen. Samples of spiked matrices (NS1, NS2, TWW2) were collected before and after tub refills (sampling events, n=11) to evaluate CEC levels in the growth media (Fig. 7-9). In agreement with the stability results, the concentration ranges in TWW2 exhibited higher variability for most compounds compared to those in NS2. The differences in CECs content between the two media can be attributed to the different matrices, since the presence of microorganisms and suspended solids in TWW can result in sorption mechanisms and microbial activity [36], [61]. Other transformation processes that can occur and can be considered compound-dependent include degradation, oxidation, and hydrolysis.

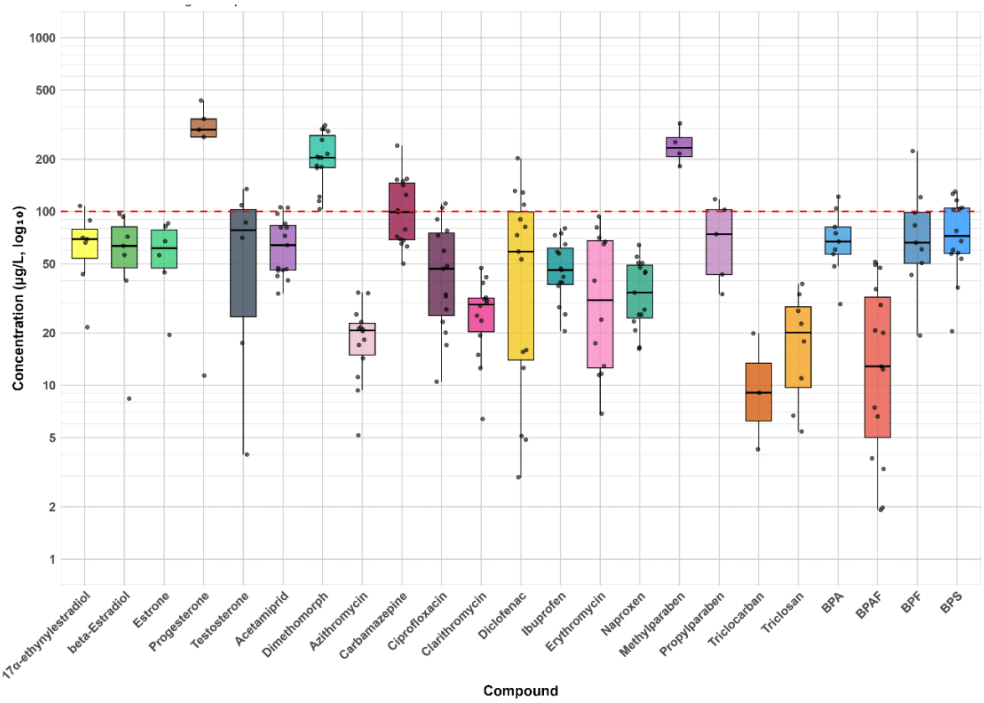


Fig. 7: Boxplots showing the weekly levels of NS1 in spiked (100 µg/L, red dashed line) potable water (n=11).

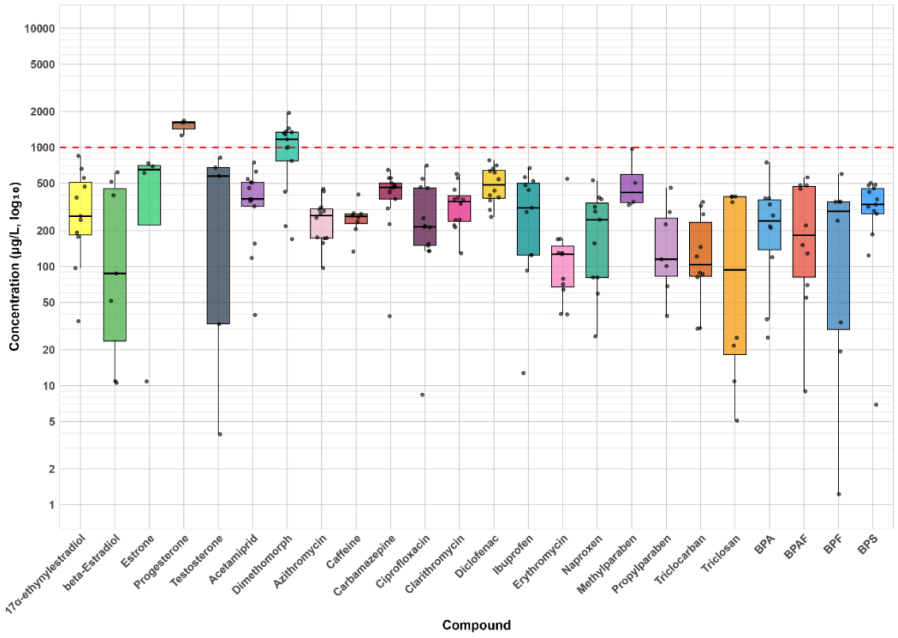


Fig. 8: Boxplots showing the weekly levels of NS2 in spiked (1000 µg/L, red dashed line) potable water (n=11).

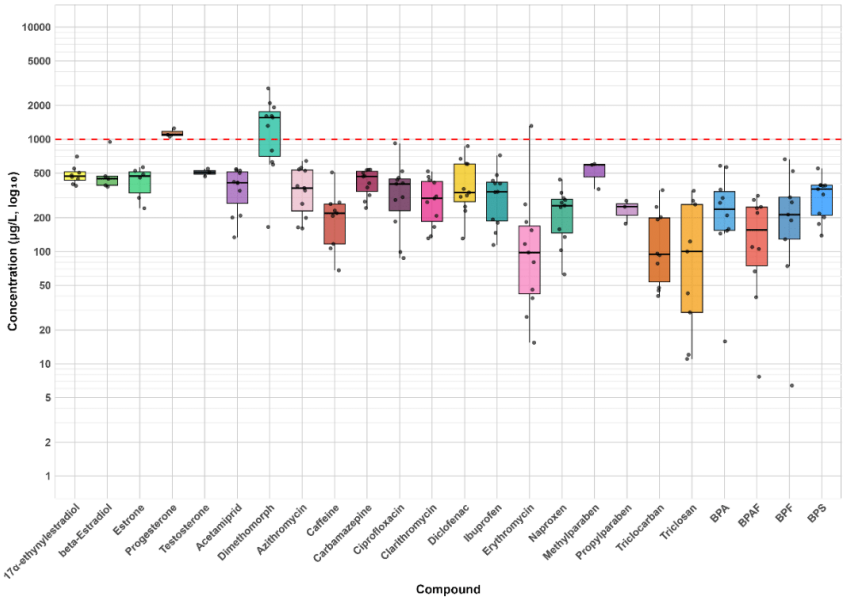


Fig. 9: Boxplots showing the weekly levels of TWW2 in spiked (1000 µg/L, red dashed line) potable water (n=11).

3.2 Uptake, translocation and bioaccumulation of CECs in tomato plants

In roots, contaminant accumulation varied between treatments. In NS (control), no compounds were quantified. In roots treated with NS1, 14 compounds were >LOQ, including bisphenols (BPA, BPF, BPS), hormones (17α-ethynylestradiol, beta-estradiol, estrone), pharmaceuticals (carbamazepine, diclofenac, ibuprofen), personal care product ingredients (propylparaben and triclosan) and pesticides (acetamiprid and dimethomorph), in the range 453 ng/g to 29,4 µg/g. In roots treated with NS2, three additional compounds were >LOQ, namely progesterone, testosterone and tonalide. The concentration range was 257 ng/g to 118 µg/g, suggesting that root uptake scales with increased exposure. In TWW1 (Fig. 10), root uptake was limited, since only four compounds were quantified (BPA, diclofenac, ibuprofen and triclosan) in the range 55.3-189 ng/g. For TWW2, the compounds quantified were the same as for NS2. However, concentrations varied, with specific compounds having higher concentrations (e.g., BPS: TWW2, 210 µg/g and NS2, 73.5 µg/g; ibuprofen: TWW2, 130 µg/g and NS2, 10.8 µg/g).

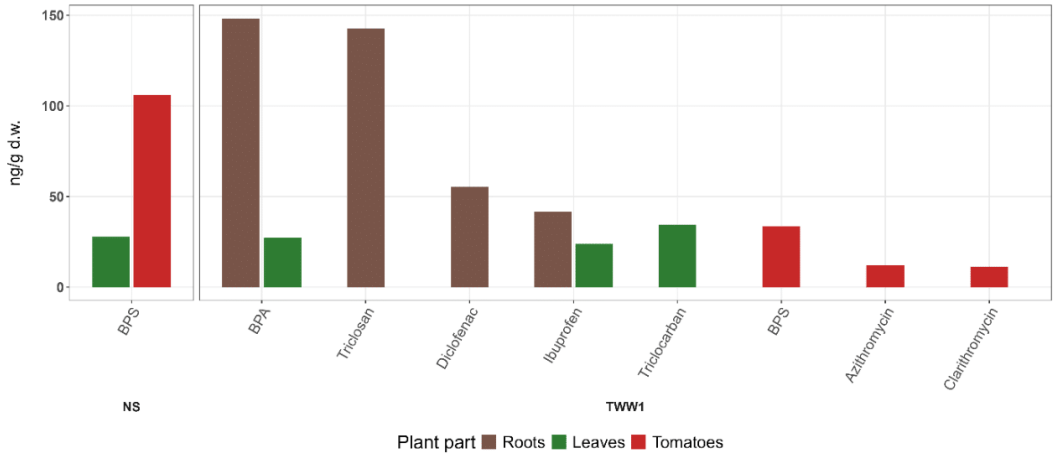


Fig. 10: Bar plots of CEC concentration (ng/g d.w.) in plant parts in NS and TWW1 treatment.

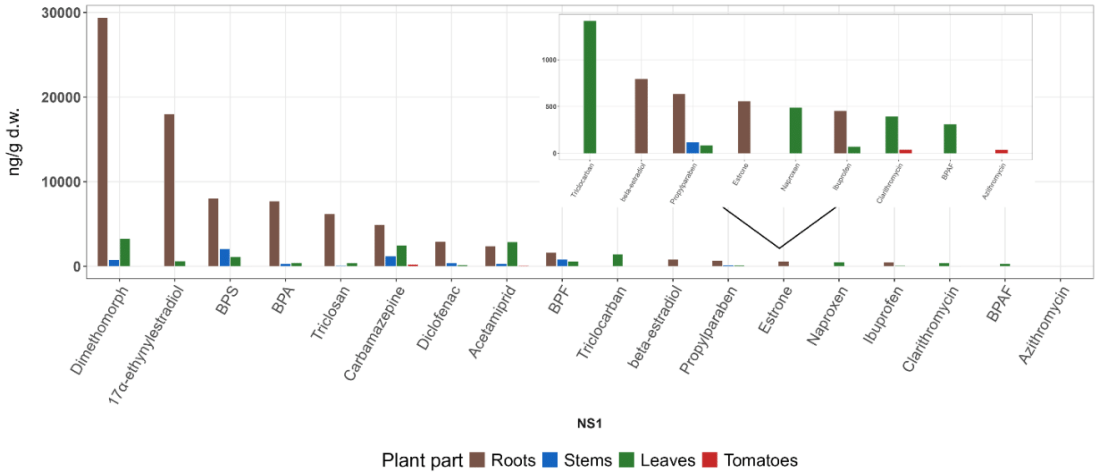
The differences in root uptake between treatments are illustrated by the RCF, which also considers the amount of CEC present in the growth media. In all treatments, except for progesterone and testosterone, RCF was>1 (Table 6), indicating that the concentration in the roots was overall higher than that in the growth medium. For most CECs, the RCF between NS2 and TWW2 was close, since they both had the same nominal concentration. The highest values in TWW2 were observed for BPS, diclofenac, ibuprofen and triclosan (Table 6). BPS has also been reported to accumulate in high concentrations in hydroponically grown cabbage; however, as a hydrophilic compound, it can also translocate to the aerial parts [62]. Based on the pH of the growth medium, diclofenac (pKa 4.15) and ibuprofen (pKa 5.3) are likely to be in their anionic forms. Consequently, they will be repelled by the negatively charged plant cell membranes, resulting in higher accumulation in the roots and reduced translocation [63].

368 Table 6: Root Concentration Factor (RCF) calculated based on irrigation water.

Roots	NS1	NS2	TWW2
17α-ethynylestradiol	214	82.8	54.9
Acetamiprid	29.6	37.5	40.6
beta-estradiol	10.3	7.0	5.4
BPA	63.1	65.1	46.2
BPF	7.2	24.5	7.9
BPS	76.4	163	382
Carbamazepine	36.2	50.9	41.6
Diclofenac	28.7	21.6	107
Dimethomorph	182	101	79.7
Estrone	7.7	4.7	5.6
Ibuprofen	8.3	61.5	341
Progesterone	-	0.4	0.3
Propylparaben	7.5	9.9	9.0
Testosterone	-	0.4	0.2
Triclosan	204	63.0	120

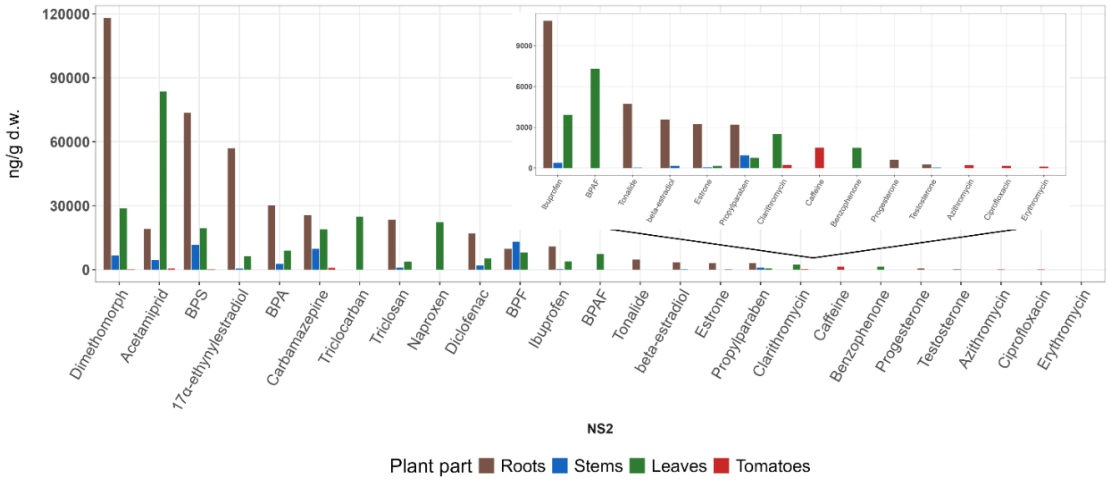
369 In stems, no compounds were quantified in the controls (NS) or in plants grown in TWW. In NS1, eight
370 compounds were >LOQ: acetamiprid (320 ng/g), BPA (290 ng/g), BPF (800 ng/g), BPS (2000 ng/g),
371 carbamazepine (1100 ng/g), diclofenac (360 ng/g), propylparaben (170 ng/g) and triclosan (125 ng/g).
372 In stems of spiked treatments with 1 mg/L of CECs, 15 compounds were quantified in NS2 and TWW1,
373 including BPA, BPF, BPS, 17α-ethynylestradiol, beta-estradiol, estrone, carbamazepine, diclofenac,
374 ibuprofen, propylparaben, triclosan, acetamiprid and dimethomorph, indicating that, although plant
375 growth was minimal, CECs translocate to the aerial parts.

376 In leaves, only BPS was found (42.4 ng/g) in the control treatment (NS), while in TWW1, BPA (50.9
377 ng/g), ibuprofen (37.5 ng/g), and triclocarban (58.6 ng/g) were quantified (Fig. 10). As in the other
378 plant tissues, translocation was more extensive in the spiked growth media. In NS1 (Fig. 11), 15
379 compounds were quantified, including BPA, BPF, BPS, BPAF, 17α-ethynylestradiol, acetamiprid,
380 carbamazepine, clarithromycin, diclofenac, dimethomorph, ibuprofen, naproxen, propylparaben,
381 triclosan and triclocarban. In NS2 (Fig. 12), 17 compounds were quantified, the same as NS1, also
382 including benzophenone and estrone. Similar to roots, most compounds were found in TWW2 (Fig.
383 13), where 19 out of 27 compounds were >LOQ, including BPA, BPF, BPS, BPAF, 17α-ethynylestradiol,
384 acetamiprid, beta-estradiol, estrone, progesterone, carbamazepine, clarithromycin, diclofenac,
385 dimethomorph, ibuprofen, naproxen, benzophenone, propylparaben, triclosan and triclocarban.



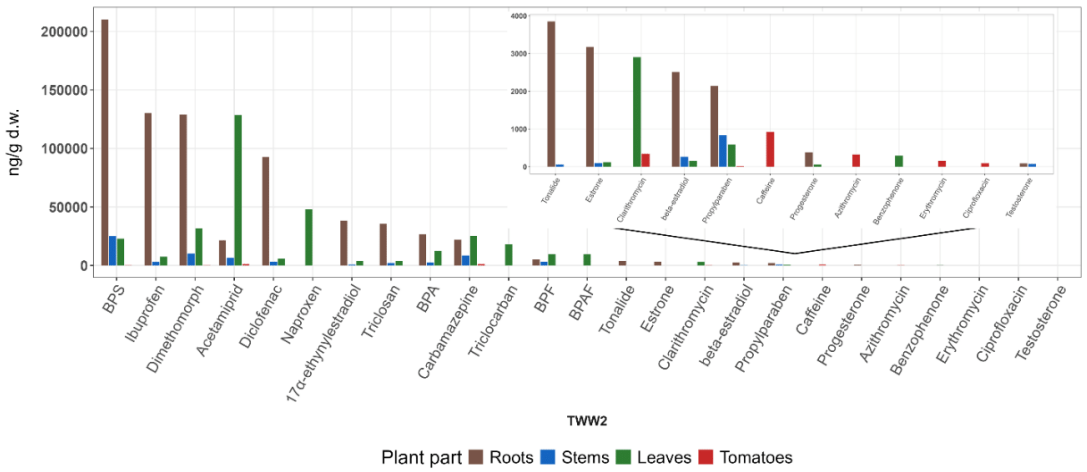
386

387 Fig. 11: Bar plots of CEC concentration (ng/g) in plant parts in NS1 treatment.



388

389 Fig. 12: Bar plots of CEC concentration (ng/g) in plant parts in NS2 treatment.



390

391 Fig. 13: Jitter plots of CEC concentration (ng/g) in plant parts in TWW2 treatment.

392 The TFs were calculated for different plant part combinations, when values were available, to evaluate
393 the internal distribution of compounds (Table 7). The TFs revealed compound-specific translocation
394 behaviours. For the majority of compounds, translocation to aerial parts was limited, with TF > 1 only
395 observed for acetamiprid (5.9), BPF (1.9), and carbamazepine (1.1) in TWW2. Most compounds were
396 translocated to leaves and did not accumulate in stems, since TFs comparing concentrations between
397 leaves and stems were >1.

398 Since plant growth was affected, it can be inferred that transpiration within the plant system was
399 limited, thereby affecting the translocation of nutrients to the aerial parts [31], [64]. Additionally,
400 hydrophobic compounds can enter the plant through its roots, but they bind strongly to the root
401 surfaces instead of dissolving in the water that moves upward through the plant [65]. Roots also
402 contain structural barriers, such as the Casparian strip, a thin, waterproof layer that surrounds the
403 inner part of the root [66]. This layer may restrict passive diffusion of CECs from entering the vascular
404 tissue (xylem) that carries water to aerial parts [67]. As a result, compounds with higher water solubility
405 can pass through cell membranes more easily and reach the xylem, where they are transported to the
406 rest of the plant [68].

407 In addition, once contaminants enter the root cells, they may be metabolised. For example, CECs can
408 be converted into less mobile or less toxic forms through processes such as conjugation with sugars
409 (glycosylation) or amino acids as part of natural detoxification [69]. These reactions increase the
410 molecule's polarity, thereby decreasing its solubility in membranes and allowing transformation
411 products to remain within root tissues [70], [71], [72].

412 Table 7: Translocation Factors (TF) among different plant parts

	Leaves/Roots				Stems/Roots			Fruits/Roots			Leaves/Stems		
	TWW	NS1	NS2	TWW2	NS1	NS2	TWW2	NS1	NS2	TWW2	NS1	NS2	TWW2
17α-ethynylestradiol	-	0.05	0.11	0.10		0.01	0.02	-	-	-	-	13	4.6
Acetamiprid	-	1.2	4.3	5.9	0.13	0.24	0.31	0.003	0.21	0.05	8.9	18	19
beta-estradiol	-	-	-	0.09	-	0.04	0.15	-	-	-	-	-	0.58
BPA	0.34	0.05	0.29	0.5	0.04	0.09	0.09	-	-	-	1.4	3.4	5.2
BPF	-	0.35	0.83	1.9	0.50	1.3	0.62	0.001	0.03	0.002	0.69	0.62	3.1
BPS	-	0.14	0.26	0.11	0.25	0.16	0.12	-	0.03	0.01	0.55	1.6	0.91
Carbamazepine	-	0.50	0.74	1.1	0.24	0.38	0.39	0.009	0.16	0.05	2.1	1.9	2.9
Diclofenac	-	0.05	0.32	0.06	0.12	0.11	0.04	-	-	-	0.40	2.7	1.7
Dimethomorph	-	0.11	0.24	0.25	0.02	0.06	0.08	-	0.004	0.002	4.4	4.4	3.1
Estrone	-	-	0.04	0.04	-	0.02	0.03	-	-	-	-	2.6	1.3
Ibuprofen	0.66	0.16	0.36	0.05	-	0.04	0.02	-	-	-	-	9.9	2.5
Progesterone	-	-	-	0.36	-	-	-	-	-	-	-	-	-
Propylparaben	-	0.13	0.23	0.27	0.26	0.29	0.39	-	-	0.007	0.49	0.81	0.71
Testosterone	-	-	-	-	-	0.11	2.1	-	-	-	-	-	-
Tonalide	-	-	-	-	-	0.01	0.02	-	-	-	-	-	-
Triclosan	-	0.06	0.16	0.11	0.02	0.04	0.06	-	-	-	2.9	4.2	1.8

413 Although translocation to fruits was smaller compared to other plant parts, different CECs were found
414 across the available treatments. In tomatoes grown in potable water, only BPS was quantified with an
415 average concentration of 106 ng/g (Table 8 and Fig. 10). In tomatoes grown in NS1, acetamiprid,
416 azithromycin, BPS, carbamazepine, clarithromycin, and dimethomorph were >LOD (29.6-221 ng/g),
417 where carbamazepine had the highest uptake. In NS2, 10 compounds were above the LOQ, namely
418 acetamiprid, azithromycin, BPS, BPF, caffeine, carbamazepine, ciprofloxacin, clarithromycin,
419 dimethomorph and erythromycin, where caffeine had the highest uptake (1,510 ng/g). In tomatoes
420 grown with treated wastewater (TWW1), only azithromycin (16.8 ng/g), BPS (45.1 ng/g), and
421 clarithromycin (17.4 ng/g) were >LOQ.

Table 8: Concentration of CEC quantified in tomato samples (ng/g dry weight)

Compounds	NS	NS1	NS2	TWW1	TWW2
BPS	106	29.6	205	45.1	365
BPF	-	-	42.4	-	52.4
Naproxen	-	-	-	-	25.4
Diclofenac	-	-	-	-	-
Propylparaben	-	-	-	-	14.6
Carbamazepine	-	221	779	-	1120
Acetamiprid	-	60.9	524	-	1030
Azithromycin	-	37.5	208	16.8	328
Erythromycin	-	-	103	-	156
Clarithromycin	-	37.7	227	17.4	345
Dimethomorph	-	37.1	113	-	269
Caffeine	-	-	1510	-	923
Ciprofloxacin	-	-	165	-	91.0

Interestingly, the concentration of BPS was lower than in control samples. The presence of BPS in control samples (leaves, fruits) may be attributed to the leaching of pipes present in the exposure media, where a small plastic aquarium pump was submerged to achieve aeriation and circulation. The migration of bisphenols from plastic pipes, made of polypropylene random copolymer (PPR), polysulfone, and polyethene (PE), as well as epoxy resins, has been previously reported [73]. However, the plant development of control plants was more extended compared to TWW1, including the size of tomato fruits and overall yield, which can explain the higher concentration in NS compared to TWW1.

In tomatoes grown in spiked treated wastewater (TWW2), acetamiprid, azithromycin, BPF, BPS, caffeine, carbamazepine, ciprofloxacin, clarithromycin, dimethomorph, erythromycin, naproxen, and propylparaben were detected in a range of 25.3-1,110 ng/g. In a soil-grown experiment, performed in parallel with the same studied compounds and similar irrigation media, and with the application of potable water and TWW, higher concentrations were found in edible plant parts irrigated with spiked potable water compared to those irrigated with spiked TWW. This difference was attributed to the reduced stability of compounds in TWW+CEC, which led to lower uptake, as the irrigation media were stored in plastic containers for up to three weeks before the addition of freshly prepared spiked media. However, this was not applicable in this experiment, where the media was prepared only once, as plants presented symptoms of poor growth in the first two weeks after planting. Thus, differences in concentrations between spiked treatments at the same level (NS2, TWW2) were not extensive.

Previous studies investigating hydroponically grown tomato plants have demonstrated that CEC uptake and translocation are strongly dependent on a compound's physicochemical properties. For instance, in hydroponic systems, carbamazepine has repeatedly shown high persistence and uptake due to its moderate hydrophobicity (log Kow: 2.45) and neutral charge, which facilitates passive diffusion through root membranes and subsequent translocation to fruits [74]. Riemenschneider et al. reported that hydroponically grown tomatoes took up 80% of the carbamazepine amount spiked within 35 days and quantified eleven transformation products. The authors noted that the parent compound was highest in fruit compared to leaves (2.5 times) [25], which was not the case in the present study.

In our previous study, BPS, BPF and naproxen were quantified in mature fruits grown under the same conditions as treatment NS1 (spiked at 100 µg/L). In that study, BPS was quantified in tomato fruit up to 2.68 ng/g, compared to 29.6 ng/g found in the current experiment [30]. The differences in plant development can also explain the differences in accumulation, as reduced growth can diminish xylem-driven translocation of nutrients to fruits [75]. These findings suggest that hydroponically grown

tomatoes can uptake a variety of CECs and translocate them into the edible fruit, although the levels and compound-specific behaviours vary. Across all treatments, contaminant distribution followed the pattern roots > leaves > stems > fruits, reflecting the retention in lower tissues and a gradual decline in concentrations along the transpiration stream.

3.3 Phytotoxic effects of TWW and CEC in tomato plant seedlings.

A preliminary experiment, which used different spiking levels to achieve the selected concentration, revealed that the ethanol concentration introduced through spiking was influencing growth. Specifically, during the first experiment, which was performed using the same set-up as the phytotoxic experiment, 1.6 ml/L was introduced to treatments S1 and S3, while 0.16 mL/L was introduced for spiking S2. The results showed that the presence of ethanol (up to 0.16% v/v) in the growth medium inhibits plant growth. The amount of ethanol also led to the formation of a hydroslime (a mixture of fungi and bacteria) around the root area in both experiments, which has an impact on the uptake of nutrients and thus the further development of plants [42], [76].

In the main phytotoxic experiment, a statistical analysis (one-way ANOVA followed by Tukey's post-hoc test) revealed a significant treatment effect on total plant mass. Plants grown in TWW (S4) had significantly lower biomass than those in all other treatments, while the highest concentration (S3) was not significantly different from the control. It is evident that the absence of added nutrients significantly influenced the growth ($p = 0.000044$, vs C) (Fig. 14-16). This finding suggests that although TWW IS known to contain nutrients, those are not sufficient to promote plant growth in hydroponics [77].

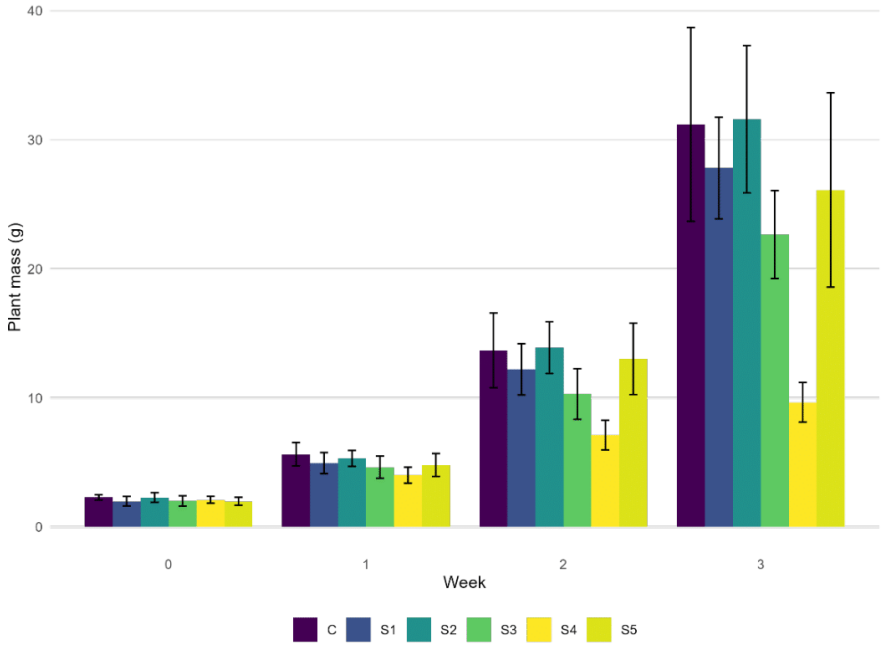


Fig. 14: Average weight (g) of tomato plants with respective standard deviation for each week for different treatments. Bars represent mean values ± standard deviation (n = 5). C: Nutrient solution; S1: 0.01 mg/L; S2: 0.1 mg/L; S3: 1 mg/L; S4: TWW; S5: TWW + nutrients.



Fig. 15: Comparison of different treatments at the end of the experiment (t=22 days) between a) C, b) S3 and c) S4.

Figure 12 shows the weekly variation in chlorophyll index (SPAD) for the six treatments. SPAD values exhibited a gradual decrease during the experiment across all treatments. This short-term reduction likely reflects physiological responses to environmental conditions and SPAD sensitivity in plants in the early growth stage [78], [79]. Despite minor fluctuations among treatments, the overall pattern was consistent, and statistical analysis (Kruskal–Wallis followed by Dunn's post-hoc test) confirmed that no significant differences occurred among treatments. These results indicate that the applied treatments had no effect on leaf chlorophyll content during the experiment.

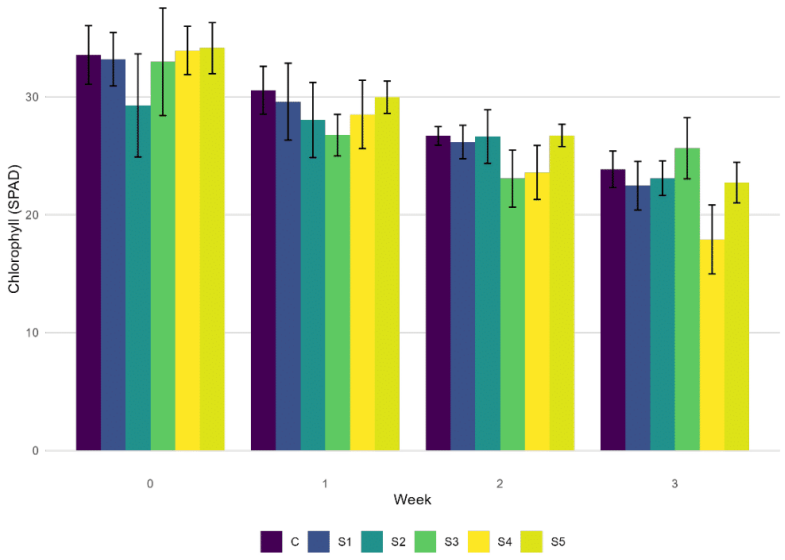


Fig. 16: Average chlorophyll index (SPAD) of tomato plants measured weekly across treatments. Bars represent mean values ± standard deviation (n = 5). C: Nutrient solution; S1: 0.01 mg/L; S2: 0.1 mg/L; S3: 1 mg/L; S4: TWW; S5: TWW + nutrients.

Overall, by comparing the phytotoxic effects on plant mass with those of the initial hydroponic experiment, we can conclude that high concentration and the mixture of CECs were not the primary factors that diminished growth in the main hydroponic experiment. Instead, a combination of additional issues could be the cause of poor growth, such as the presence of ethanol (up to 1 mL/L) and unfavourable weather conditions.

3.4 Human health risk assessment

Although the tomatoes were not ripe or at a marketable size, the risk from consumption was still calculated. The average chronic dietary exposure was estimated for different population groups (toddlers, children, adolescents, adults) using the maximum concentration measured for each CEC across treatments. Despite the measurable translocation to fruits, most quantified compounds in NS1, NS2 and TWW2, namely acetamiprid, azithromycin, caffeine, carbamazepine, ciprofloxacin, clarithromycin, dimethomorph, erythromycin, naproxen and propylparaben, had exposure estimates that were several orders of magnitude below their respective health-based reference values.

Risk was identified only for BPS, which exceeded the tolerable intake in all treatments, and BPF exceeded the tolerable intake in NS2 and TWW2. For both BPS and BPF, the suggested TDI by EFSA for BPA (0.2 ng/kg bw/day) was used as a conservative comparison, due to the lack of other health-based reference values and by considering the structural similarities. However, for both bisphenols, the respective LOQs correspond to higher values than the established EFSA TDI. Thus, when a bisphenol result is reported as <LOQ, this does not indicate concentrations below the EFSA TDI and may still imply potential risk.

While the spiked treatments (1 mg/L) included elevated concentrations, above typical ranges found in the environment, the findings of this study suggest that dietary risk from most targeted CECs was minimal, except for bisphenols. However, the fact that tomato fruits were not mature and plant growth was limited should be considered, as normal development could lead to higher uptake of compounds and additional risk above TDI values. Additionally, the study only focused on 27 CECs, while additional metabolites and transformation products of the selected compounds could also pose risks through dietary intake. Thus, future studies should evaluate in-plant metabolism, exposure risk and synergistic effects deriving from mixtures.

4. Conclusions

This study evaluated the uptake and translocation of 27 CECs in hydroponically grown tomato plants. Additionally, the stability of CECs in aqueous samples under various storage conditions is examined. Results showed that most CECs remained stable (>80%) during storage at 4 °C and −20 °C, whereas degradation was evident at room temperature and was more pronounced in TWW. Translocation to aerial parts was compound-specific and limited overall, with a distribution pattern of roots > leaves > stems > fruits. Although high exposure concentrations affected plant growth, except for bisphenols BPF and BPS, dietary exposure estimates indicated negligible risk for most CECs, although the evaluated tomatoes were immature. Regarding the phytotoxic effects of TWW and CEC on tomato plant growth, the use of ethanol at a concentration of 0.16% v/v to prepare the spiking solution resulted in reduced plant growth. In contrast, plants in TWW showed insufficient growth without the addition of nutrients. The results obtained from the phytotoxic experiment can be linked to the reduced growth of plants in the main experiment, where ethanol spiking was up to 0.1% v/v and TWW was applied without the addition of any nutrients. Future studies should focus on the transformation product formation in plants through suspect/non-target analysis and further evaluation of their risk, which may potentially be higher compared to the parent compounds.

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3.3 Uptake of CECs in Tomatoes Grown in Sludge-amended Peat and Implications for Soil Accumulation and Food Safety

3.3.1 Contaminants of emerging concern in tomatoes grown in sludge-amended peat: Uptake, translocation and risk assessment

The paper titled: "Contaminants of Emerging Concern in Tomatoes Grown in Sludge-Amended Peat: Uptake, Translocation and Risk Assessment", was authored by E. Andreasidou, A. Kovačič, L. Manzano-Sánchez, D. Heath, M. Pintar, N. Kacjan Maršič, U. Blaznik, A.R. Fernández-Alba, M.D. Hernando and E. Heath. The paper was published in Toxics in November 2025 (doi.org/10.3390/toxics13121013). I was responsible for experimental design, experimental work, sampling, method optimisation and validation. I was also responsible for data analysis, visualisation and writing of the manuscript.
This study examined the uptake of CECs by tomato plants grown in peat amended with sludge. Tomatoes were cultivated in peat substrate amended with dried sludge under various exposure scenarios, as well as in peat substrate directly mixed with anaerobically digested sludge. Dried sludge was applied at two rates corresponding to nitrogen and nitrogen/potassium requirements. Additional treatments included CEC-spiked media. Analysis was performed with LC–MS/MS.

In tomato fruits, ibuprofen (15.8 ng/g) and triclosan (17.9 ng/g) were quantified at the low amendment rate, while caffeine (381 ng/g), carbamazepine (18.1 ng/g), ciprofloxacin (306 ng/g) and ibuprofen (5.3 ng/g) were quantified at the high amendment rate. In all treatments, RCF was <1, indicating that the concentration in roots was lower than in the peat substrate. For carbamazepine, the only compound detected in each tissue, translocation from roots to leaves was the highest, followed by stems and fruit.

Although adding sludge had an impact on the plant health (leaf chlorosis and necrosis), neither sludge nor CECs had a measurable effect on tomato yield. Dietary exposure estimates were below the health-based reference values for most compounds; however, potential risk was identified for BPS when non-dried anaerobically digested sludge was applied. In terms of environmental risk, the study showed risk quotients greater > 1 for seven out of 27 CECs in treatments that included sludge, which could have implications if sludge is applied repeatedly in the field.

This publication addresses multiple aims of the thesis, as it includes a study on the uptake and translocation of CECs following sludge amendment. CECs concentrations were measured in peat substrate, tomato fruits and plant parts, based on which dietary exposure of consumers and environmental risk were calculated. Overall, the findings of this study suggest that consuming tomatoes amended with sludge poses a limited human health risk. However, the accumulation of CECs in peat substrate can have negative environmental impacts. The inclusion of the same CECs as in the experiments presented in 3.2.2 and 3.2.3 can offer valuable insights into the differences between various cultivation systems and contamination sources.

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Contaminants of Emerging Concern in Tomatoes Grown in Sludge-Amended Peat: Uptake, Translocation and Risk Assessment

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Abstract

Although sewage sludge in agriculture can promote circular economy goals, concerns remain about the transfer of contaminants of emerging concern (CECs) into crops and soils. This study evaluated the uptake and risk of 27 CECs in tomatoes cultivated in peat substrate amended with stabilised anaerobically digested (dried) sludge from a local municipal wastewater treatment plant at two rates corresponding to nitrogen and nitrogen/potassium requirements. Peat substrate served as the control. Additional treatments included CEC-spiked media and peat amended with non-dried sludge. Analysis was performed with LC–MS/MS. In tomato fruits, ibuprofen (15.8 ng/g) and triclosan (17.9 ng/g) were quantified at the low amendment rate, while caffeine (381 ng/g), carbamazepine (18.1 ng/g), ciprofloxacin (306 ng/g) and ibuprofen (5.3 ng/g) were quantified at the high amendment rate. Dietary exposure estimates were below the health-based reference values for most compounds; however, a potential risk was identified for bisphenol S when non-dried anaerobically digested sludge was applied. Soil risk quotients (RQ > 1) for several CECs at the end of the experiment indicate possible ecological concern. These findings emphasise that monitoring CECs in sludge-amended soil remains essential to ensure the safety of sludge reuse in agriculture.

Keywords: *Solanum lycopersicum* L.; bioaccumulation; pharmaceuticals; personal care products; bisphenols; pesticides; hormones; LC-MS/MS; dietary exposure; ecological risk assessment

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1. Introduction

The sustainable management of stabilised sewage sludge (also referred to as dewatered biosolids), which is a by-product of wastewater treatment, remains challenging due to contaminants, compositional variability, and regulatory constraints on its reuse [1]. Despite

this, sludge is a source of nitrogen, phosphorus, organic carbon, and micronutrients, and its application in soil can improve both chemical and physical properties, including cation exchange capacity, field capacity, and bulk density [2]. Early studies have shown that the application of sludge increased crop yields by an average of 7% [3]. In Europe, approximately 35–50% of total sludge management involves agricultural use [4]. Its use in agriculture also aligns with the EU's Circular Economy Action Plan, which promotes resource recovery and waste valorisation [5]. Compared to landfilling and incineration, it offers a cost-effective way of recycling nutrients while mitigating the environmental impact of waste sludge disposal [5].

At the European level, the agricultural use of treated sewage sludge is regulated under Council Directive 86/278/EEC, which sets limits for heavy metals to protect the health of soil, plants, animals, and humans [6]. National legislation across EU Member States often imposes different requirements or further limitations, reflecting both environmental concerns and varying levels of sludge reuse in agriculture [7,8], with most countries not supporting the use of sludge on crops intended for direct human consumption due to the risks posed by heavy metals and pathogens [9]. However, many believe that the reuse of sludge should be reconsidered, given the increasing volume of sludge produced, rising disposal costs (e.g., incineration), and the push toward sustainability.

While heavy metals and pathogens are already considered, risks from contaminants of emerging concern (CECs) remain unaddressed [10–12]. These include pharmaceuticals, personal care products, pesticides [13], and industrial chemicals [14] that are not yet regulated. Their persistence through wastewater treatment and bioactivity raises concerns about their potential accumulation in amended soils, subsequent transfer into crops, and entry into the food chain, where they can pose a risk to human health [15].

Carbamazepine, for example, has been shown to be taken up by different vegetables, including carrot, celery, lettuce, tomato, pak choi and wheat grown in biosolid-amended soils [16,17]. Similarly, trace amounts of triclocarban and triclosan have been detected in various plant parts [13]. Studies also indicate that the uptake and translocation of CEC depend not only on their physicochemical properties (e.g., log Kow, sorption affinity) but also on soil characteristics, e.g., pH, $CaCO_3$ content, organic matter and number of basic cation exchangeable sites [15,18], as well as sludge properties including pH, total organic carbon, total suspended solids and heavy metals content [19,20].

Fewer studies, however, have examined the translocation of CECs in tomatoes grown in amended soils (Table 1) [21,22]. Among them, four looked at uptake and translocation and found the presence of CECs, including carbamazepine and its metabolites in leaves and fruits [17], triclocarban present in root > shoot > fruit [22] and Per- and Polyfluoroalkyl Substances (PFASs), with higher translocation of short-chain ones, to aerial tissues [23,24]. Knowledge gaps also persist regarding the risks associated with agricultural applications. For instance, studies typically focus on a smaller group of compounds, such as triclosan, triclocarban, and carbamazepine, rather than on realistic CEC mixtures present in sludge [25,26].

The present study examines the uptake and translocation of 27 CECs, including selected pesticides (2), pharmaceuticals (9), personal care product ingredients (6), hormones (5), stimulants (1) and industrial chemicals (4), in tomato plants cultivated in pots containing peat substrate amended with dried anaerobically digested sludge, at two amendment levels covering (1) nitrogen and (2) nitrogen and potassium plant requirements. In addition, the growth media were spiked with CECs at levels higher than typically found in sewage sludge to enhance the likelihood of detecting CECs in tomato tissues. In addition, peat substrate was amended with non-dried sludge. The study aimed to evaluate the impact of sludge amendment on fruit yield and the presence of CECs in plant tissues and

growing media (soil risk quotients), as well as to assess dietary exposure from consuming tomatoes (*Solanum lycopersicum* L. cv. Rally) grown in sludge-amended media.

Table 1. Literature on uptake studies, including the growth of tomatoes in sludge/biosolids.

Compounds Studied	Plants Studied	Treatment	Compounds in Tomato Fruits	Refs
Carbamazepine, 10,11-Epoxide-carbamazepine, Dihydroxy-carbamazepine	Tomato, lettuce, wheat	Composted biosolids	Carbamazepine, Dihydroxy-carbamazepine	[17]
Synthetic musks and UV filters	Tomato	Composted biosolids (spiked up to 500 ng/g)	15 musks and UV filters, including tonalide & benzophenone	[21]
Carbamazepine, Diphenhydramine, Triclocarban	Pepper, tomato, collard, lettuce, radish	Spiked biosolids (100 ng/g)	Diphenhydramine, Triclocarban	[22]
30 PFASs	Tomato	Biosolids	PFBA; PFPeA; PFHxA, PFOA	[23]
PFASs, PBDEs and Dechloranes	Spinach, tomato	Anaerobically digested thermally dried sludge	PFASs, PBDEs and Dechloranes	[24]
12 PFASs	Lettuce, tomato, corn	Biosolids	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFBS, PFHxS	[27]
118 PPCPs or PPCP TPs, 6 parabens, and 17 hormones or hormone TPs	Barley, carrot, potato, sweet corn, tomato	Dewatered municipal biosolids	Atenolol, DEET, Minocycline, Sulfamerazine, Testosterone, Trimethoprim	[28]
Triclosan and Triclocarban	Carrot, cucumber, tomato, green bell pepper	Dewatered anaerobically digested biosolids	Triclocarban	[29]

2. Materials and Methods

2.1. Reagents and Chemicals

Analytical-grade standards (>97%) were utilised, including acetamiprid, azithromycin, benzophenone, bisphenol F (BPF), bisphenol AF (BPAF), bisphenol S (BPS), caffeine, carbamazepine, ciprofloxacin, clarithromycin, diclofenac sodium salt, dimethomorph, erythromycin, estrone (E1), 17α-ethynylestradiol (EE2), ibuprofen, methylparaben, naproxen, progesterone, propylparaben, testosterone, tonalide, triclocarban, and triclosan. These were purchased from Sigma-Aldrich (Steinheim, Germany), bisphenol A (BPA) was obtained from Merck (Darmstadt, Germany); amoxicillin trihydrate, acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA); and beta-estradiol (E2), purchased from Thermo Fisher Scientific (Waltham, MA, USA). Detailed compound information is provided in Supplementary Table S1. A selection of isotopically labelled internal standards was used, including BPA-d_{16}, $^{13}C_3$-caffeine, carbamazepine-d_{10}, clothianidin-d_3, $^{13}C_6$-diclofenac, ibuprofen-d_3, $^{13}C_6$-methylparaben, and $^{13}C_6$-propylparaben, all purchased from Sigma-Aldrich (Steinheim, Germany). Additional labelled compounds included oxybenzone-d_5 from C/D/N Isotopes Inc. (Pointe-Claire, QC, Canada), $^{13}C_{12}$-triclosan from Cambridge Isotope Laboratories (Tewksbury, MA, USA), $^{13}C_2$-beta-estradiol from Campro Scientific (Berlin, Germany), and both $^{13}C_{12}$-BPS and $^{13}C_{12}$-BPF from CanSyn Chem. Corp. (Toronto, ON, Canada).

Methanol (MeOH), acetonitrile (MeCN), and water, all of LC-MS grade, were obtained from J.T. Baker (Deventer, The Netherlands). Ultrapure water (18.0 MΩ·cm at 25 °C) was produced using a Milli-Q water purification system (Merck Millipore, Darmstadt, Germany). Formic acid (FA) and ammonium fluoride (NH_4F) were purchased from Sigma-

Aldrich (St. Louis, MI, USA), and ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA) was supplied by Kemika d.d. (Zagreb, Croatia). Individual stock solutions of each analyte were prepared in MeOH at 1 mg/mL. From stock solutions, working solutions (10, 100, and 1000 ng/mL) were prepared through serial dilutions in MeOH. The internal standard mixture (1 µg/mL) was similarly prepared in MeOH by combining isotopically labelled solutions of individual compounds (1 or 0.1 mg/mL). All prepared solutions were stored in amber glass vials at 4 °C.

2.2. Experimental Design and Treatments

Pot experiments were conducted during the 2022 growing season under glass at the University of Ljubljana (Slovenia). Tomato seedlings (*Solanum lycopersicum* L., cv. Rally) were first cultivated for a period of eight weeks, until each plant reached the 5–6 leaf developmental stage, before transplanting to the experimental pots. Each pot was filled with 1.7 kg of peat substrate (Huminsubstrat N8, Neuhaus, Germany; a high-quality white peat mixture with frozen black peat, clay and humic acids) with and without sludge, according to the specific treatment (Figure S1). The characteristics of the peat substrate are provided in Table S2.

Stabilised sludge was obtained from a local wastewater treatment plant (Domžale-Kamnik WWTP, Domžale, Slovenia). The WWTP treats approximately 25,000 m^3/day of wastewater, with 8% from industrial sources and 92% from municipal and rainwater sources. It is designed to handle an organic load equivalent to 125,000 population equivalents (PE). The dry matter content of the anaerobically digested sludge was 32%. For dried sludge application, the sludge was dried (up to 200 °C), crushed and sieved (nylon sieve, size 2 mm) (Figure S2) as a basic pretreatment, since in real case application it undergoes treatment to facilitate easier storage, transportation and application, as well as reducing biological hazards [30]. After the experiment, the peat substrate was handled and disposed of in accordance with Slovenian waste management legislation [31].

The nutrient content of the dried sludge is given in Table S3 [32–36]. Specifically, the dried sludge contained 35,300 mg/kg of N (mostly as NH_4^+), with 30% of the N potentially transformable from NH_4^+ to NO_3^-; 2238 mg/kg of P_2O_5; and 1000 mg/kg of K_2O. The experiment included sludge-based, CEC-spiked, and control treatments (Table 2). For the control, only peat substrate (PC) was used. For sludge amendments, two application rates were tested: SP1—dried sludge mixed with peat substrate (45 g per pot, 320 mg N/kg of peat substrate) to meet nitrogen requirements and SP2—to meet N and K requirements (545 g per pot, 330 mg K/kg of peat substrate) for tomato cultivation [37].

The same treatments were then spiked with CECs, resulting in concentrations of individual CEC of SP3: 0.3 mg/kg and SP4: 3.5 mg/kg. Spiking was achieved by adding an ethanol solution of CECs to the dried sludge (SP3 and SP4) or peat (P1 and P2), which were left overnight at room temperature to allow the ethanol to evaporate. The resulting mixture was then homogenised into the peat substrate in the test pots. Finally, treatment with anaerobically digested sludge without an additional drying step (SP5), consisting of a 1:1 (*w*/*w*) mixture, was applied to evaluate CEC uptake from the sludge without any additional treatment.

The pots were placed at a plant density of 3.2 plants/m^2. All plants were irrigated with potable water as needed, based on the reference evapotranspiration of 510 mm recorded at the Ljubljana Bezigrad weather station for the duration of the experiment (Figure S3) [38]. Fertiliser (ENTEC® mineral fertiliser; NPK 14:7:17; Eurochem, Mannheim, Germany) was applied only to treatments without sludge (PC, P1, P2), covering nitrogen (320 kg/ha, corresponding to 160 mg N/kg soil), phosphorus (160 kg/ha P_2O_5, corresponding to 80 mg

P/kg soil), and potassium (K_2O: 330 kg/ha, corresponding to 165 mg K/kg soil) as part of the standard growing procedure.

Table 2. Treatments included in the uptake experiment.

Abbreviation	Treatment	Addition of CEC
PC	Peat substrate (control)	-
P1	Peat substrate + CEC	0.3 mg/kg
P2	Peat substrate + CEC	3.5 mg/kg
SP1	Peat substrate + 45 g of dried sludge	-
SP2	Peat substrate + 545 g of dried sludge	-
SP3	Peat substrate + 45 g of dried sludge + CEC	0.3 mg/kg
SP4	Peat substrate + 545 g of dried sludge + CEC	3.5 mg/kg
SP5	Peat substrate + Anaerobically digested sludge (1:1, *w*/*w*)	-

For pots amended with sludge (SP1–5), deficiencies in plant growth/health were observed at 56 days after planting, including dried and yellow leaves (Figure S4). As a result, fertiliser was added to two of the five replicates to evaluate its effect, since reduced growth was attributed to nutrient deficiency. Foliar addition of Ca and B began 61 days after planting and was applied a total of three times to prevent blackening (Blossom End Rot) of the tomato fruits due to a calcium deficiency.

2.3. Sample Collection

Tomato fruits were hand-harvested at the fully ripe stage. The first harvest was performed 49 days after planting, and was followed by ten additional harvests, distributed across three defined sampling periods. The final sampling took place at 117 days after planting. During each sampling, mature fruits were placed into plastic bags, transferred to the laboratory, washed, weighed to evaluate yield and stored whole at −20 °C. Prior to sample preparation, frozen fruits were cut into pieces, weighed, freeze-dried and ground. Composite samples were prepared based on treatments, either by pooling fruits from each plant or by sampling period.

At the end of the experiment, plant tissues (roots, stems, and leaves) were collected and air-dried. Each plant part was ground using a stainless steel-bladed mill and then passed through a 2000 µm mesh sieve. In parallel, peat substrate samples were collected in triplicate from the pots at planting (day 0) and at day 117.

2.4. Sample Preparation

Sample preparation protocols for the analysis of contaminants of emerging concern (CECs) in tomato fruits, plant tissues (leaves, roots, and stems), and soil were followed using previously validated protocols [39]. Briefly, for tomato fruits, freeze-dried composite samples were spiked with isotope-labelled internal standards, extracted using solid–liquid extraction with MeOH containing 0.1% FA, followed by solid-phase extraction (SPE) using Oasis Prime HLB cartridges. Final eluates were dried under N_2, reconstituted in MeOH:Milli-Q (1:1), filtered, and stored at −20 °C prior to LC-MS/MS analysis. Plant part samples (1 g dry weight) were extracted using QuEChERS with acidified MeCN (1% FA), followed by automated µSPE (PAL® RTC autosampler). Extracts were eluted, transferred into LC vials, and stored at −20 °C until analysis. Peat samples were extracted similarly to soil (0.5 g compared to 1 g used in the original method) using solid–liquid extraction with MeOH and 0.1% FA (3 × 4 mL). The solvent from the combined extracts was removed under N_2, and the residue reconstituted in MeOH:Milli-Q (1:1). The mixture was then filtered and stored at −20 °C.

To determine CECs in dried sludge, 0.5 g of the sample was weighed into a 15 mL centrifuge tube and spiked with 25 µL of isotope-labelled internal standard mixture (1 µg/mL). Acetonitrile (1 mL) was added, and the sample was vortexed for 30 s and shaken overnight (200 rpm). Then, 4 mL MeOH with 0.1% FA was added, followed by 10 min sonication and centrifugation at 9000 rpm for 10 min. The supernatant was transferred to a 250 mL vial, and the residue was re-extracted twice. The combined extracts were diluted with 90 mL of Milli-Q water and loaded onto Oasis Prime HLB cartridges (500 mg, 6 mL). After washing with 10% MeOH in MQ (10 mL) and drying under vacuum for 30 min, analytes were eluted with 6 mL MeOH:MeCN (1:1) with 0.1% FA in three 2 mL aliquots. Eluates were dried under N_2 at 40 °C, reconstituted in 0.4 mL MeOH:Milli-Q (1:1), filtered, and stored at −20 °C before analysis.

2.5. LC-MS/MS Analysis

Samples were analysed using a liquid chromatograph (UHPLC; Shimadzu Nexera X2, Kyoto, Japan) coupled to a triple quadrupole-linear ion trap mass spectrometer (4500 QTRAP, Sciex, Framingham, MA, USA). Chromatographic separation was performed at ambient temperature using a Poroshell 120 EC-C18 column (3.0 × 100 mm, 2.7 µm; Agilent Technologies, Santa Clara, CA, USA). Electrospray ionisation (ESI) was applied in both positive and negative modes. For the positive mode, the mobile phases consisted of acetonitrile (A) and 0.1% FA in water (B). For the negative mode, 0.1% NH_4F in methanol (A) and water (B) was used. Analytical parameters are described in detail in [40].

The method was validated according to DG SANTE guidelines, assessing linearity, accuracy, recovery, matrix effects, LOD, and LOQ [41,42]. Validation results for tomato and plant parts are provided elsewhere [39]. For tomatoes, LOQs ranged between 1.11 and 287 ng/g, while for plant parts, the range was between 25 and 100 ng/g. For the peat substrate, linearity was evaluated using the calibration curves, resulting in R^2 values ranging from 0.978 to 0.999, while LODs and LOQs were between 0.04 and 11.1 ng/g and 0.13–36.4 ng/g, respectively. Recoveries ranged from 55% to 160%, with most values close to 100%, and RSDs remained below 20%. For sludge, R^2 values ranged from 0.902 to 0.999, LODs and LOQs were between 0.04 and 1.940 ng/g and 0.15–6.402 ng/g, respectively. Recoveries ranged from 41% to 157%, with most values close to 90%, while the RSD remained below 20%. Detailed results and clarifications on validation criteria are provided in the Supplementary Information (SI-2.2, Table S4).

2.6. Bioconcentration, Root Concentration and Translocation Factors

The bioconcentration factor (BCF), root concentration factor (RCF) and translocation factor (TF) were calculated to assess the uptake and internal distribution of CECs in tomato plants. Concentrations of CECs are based on dry weight (d.w.). The BCF was estimated as the ratio of the concentration of each compound in tomato fruit to the corresponding concentration in the peat substrate at the start of the experiment (day 1), as shown in Equation (1).

$$\mathrm{BCF} = \frac{Cf}{Cp} \quad (1)$$

Here, *Cf* and *Cp* are the concentration of CEC (µg/kg d.w.) in fruits and in peat substrate. The same approach was applied to estimate the RCF of tomato plants based on Equation (2):

$$\mathrm{RCF} = \frac{Cr}{Cp} \quad (2)$$

where *Cr* is the concentration of CEC in roots (µg/kg d.w.). Similarly, TF was calculated to assess compound translocation from roots to aerial plant parts, including leaves, stems,

and fruits, in various combinations such as stem/root, leaf/root, and fruit/root based on Equation (3):

$$\mathrm{TF} = \frac{Ca}{Cr} \quad (3)$$

where *Ca* is the concentration of CEC (µg/kg d.w.) in individual aerial plant parts, including stems, leaves and fruits. A TF < 1 indicates that most of the compound remains in roots, whereas a TF > 1 suggests the translocation and potential accumulation in the rest of the tissues [43].

2.7. Human Health Risk Assessment

Dietary exposure was estimated for children (toddlers), adults and adolescents by combining data from composite samples (transformed to fresh weight, considering that the evaluated tomato fruits contained 95% water) with the corresponding consumption levels from the EFSA Comprehensive European Food Consumption Database [44]. The consumption data were selected based on the Exposure hierarchy up to Level 7 (Vegetables and vegetable products up to Tomatoes) for the Slovenian Population (Table S5). Default body weight values suggested by EFSA were used for further calculations. Chronic dietary exposure from fresh tomato consumption was calculated for all CEC groups of chemicals, based on both average and high consumption levels (the latter representing the 97.5th percentile of the distribution) [45]. The estimated daily intake (EDI), expressed in mg/kg body weight (bw) per day, was calculated using Equation (4):

$$\mathrm{EDI} = \frac{Cff \times \mathrm{FC}}{\mathrm{bw}} \quad (4)$$

Here, EDI is given as mg/kg bw/day, *Cff* is the concentration of individual CEC (mg/kg fresh weight) in tomato fruit, FC is the food consumption rate (kg/day) for tomatoes in Slovenia, and bw is the corresponding body weight (kg) for the selected population group. For pesticide residues, an acute exposure was calculated using the International Estimate of Short-term Intake (IESTI) model [46]. This calculation combines the consumed portion of the food product eaten within one meal or day, reflecting the upper tail (the 97.5th percentile) of the distribution of food consumption data (Table S5), with the highest residue level in this food product. The IESTI, expressed in mg/kg body weight (bw) per day, was calculated using Equation (5):

$$\mathrm{IELSTI} = \mathrm{LPup} \times \frac{\mathrm{HRup}}{\mathrm{bw}} \quad (5)$$

where IESTI is given as mg/kg bw/day, LPup is the acute large portion for unprocessed tomatoes in Slovenia, HRup is the highest measured concentration of pesticide residue (mg/kg fresh weight) in tomato fruit, and bw is the corresponding body weight (kg) for the selected population group. The results of chronic and acute dietary exposure for each compound were compared with health-based guidance values (HBGV), including Acceptable Daily Intake (ADI), Acute Reference Dose (ARfD), Tolerable Daily Intake (TDI) or No Observed Adverse Effect Level (NOAEL) values from the literature (Table S6) [47–55], by calculating the hazard quotient (HQ). HQ was calculated from the ratio of estimated dietary intake (EDI) to health-based guidance values (HBGV), following Equation (6):

$$\mathrm{HQ} = \frac{\mathrm{EDI}}{\mathrm{HBGV}} \quad (6)$$

A hazard quotient > 1 indicates a higher daily intake than the HBGV, suggesting a potential risk of exposure [56]. It should be noted that the current assessment refers only to the risk associated with the consumption of tomatoes grown in sludge-amended peat

substrate, as a single exposure pathway. Thus, the total exposure from other potential sources is not taken into account.

2.8. Ecological Risk Assessment

To evaluate the ecological risk posed by CECs in amended peat substrate, measured environmental concentrations (MECs) from different sampling periods were compared to predicted no-effect concentrations (PNEC). This assessment followed the guidance of the European Chemicals Agency (ECHA) [57].

In the absence of terrestrial ecotoxicological data for the compounds investigated, soil PNEC values were estimated using the equilibrium partitioning method (EPM), based on aquatic toxicity data. PNEC values for freshwater organisms (µg/L) were obtained from the Norman Ecotoxicology Database, which includes experimental data or QSAR predictions suitable for preliminary screening and prioritisation [58]. Soil PNEC values (PNECsoil, µg/kg) were calculated by multiplying the aquatic PNEC values (PNECwater, µg/L) by the soil–water distribution coefficient (Koc, L/kg) of each compound, based on Equation (7) and provided in Table S7.

$$\text{PNECsoil} = (0.1176 + 0.01764 \times \text{Koc}) \times \text{PNECwater} \quad (7)$$

Equation (7) assumes equilibrium between soil and pore water and includes standard parameters for soil bulk density and porosity [59,60]. This approach is suitable for substances with log Kow values between 3 and 6 and is commonly applied as a screening process for risk when terrestrial toxicity data are unavailable. Although the EPM may either overestimate or underestimate actual risk to soil organisms, it is accepted as a conservative proxy [61].

Risk quotients (RQs) were calculated for each compound as the ratio of MEC to $\text{PNEC}_{\text{soil}}$, according to Equation (8):

$$\text{RQ} = \frac{\text{MEC}}{\text{PNECsoil}} \quad (8)$$

Interpretation of RQ values followed standard thresholds: RQ < 0.1 indicates low risk, $0.1 \leq \text{RQ} < 1$ denotes medium risk, and $\text{RQ} \geq 1$ signifies a potential high ecological risk [62].

2.9. Data Processing and Analysis

Processing of data, statistical analysis and visualisation for all measurements concerning the concentrations of CEC in different samples were carried out using R (version 4.3.3) [63]. For each matrix, concentrations < LOQ were imputed by random draws from a uniform distribution between 0 and LOQ, using matrix-specific LOQs, to preserve variability. To reduce skewness and meet the assumptions of statistical tests, concentrations were log-transformed to the base 10. Normality was tested using the Shapiro–Wilk test; the $\log_{10}$-transformed data met the assumption of normal distribution. For tomato data, pairwise comparisons of treatments were performed within each treatment and per compound using Welch's two-sample *t*-tests, with effect sizes estimated by Cohen's d. A result was considered statistically significant when $p \leq 0.05$ and $|\text{d}| > 1$.

Temporal changes in the peat substrate dataset were tested per compound and treatment using an independent two-sample Welch's *t*-test, which does not assume equal variances between groups, making it more robust for environmental data. A difference in concentration was considered significant if $p \leq 0.05$ and the effect size $|\text{d}| > 1$. For tomato yield data, which were not normally distributed, a Kruskal–Wallis test was performed across treatments, followed by pairwise Wilcoxon rank-sum tests for (i) all unique treatment pairs and (ii) fertilised vs. non-fertilised within each treatment ($p \leq 0.05$). Finally, to explore

the relationship between fruit number and total yield, a non-parametric Spearman's rank correlation analysis was conducted.

3. Results and Discussion

The levels of CECs were evaluated in peat substrate, sludge and plant parts (fruits, leaves, stems, roots) across the different treatments. For all samples, concentrations are reported as dry weight (d.w.) (Supplementary Information, SI-3).

3.1. CEC in Sludge and Amended Peat Substrate

Prior to amendment, dried sludge was analysed separately before mixing (Table S8), and samples from all pots were collected before planting (Table S9). The control pots (PC) showed very low background contamination, with only two compounds (BPS and Naproxen) being quantified with values close to their LOQs. Regarding spiked peat substrate treatments (P1, P2), although the nominal concentrations after spiking were 300 and 3500 ng/g, respectively, the average CEC concentration for P1 was 296 ± 136 ng/g and 2580 ± 1340 ng/g for P2. Methylparaben and propylparaben were not detected in P1, which could be attributed to their rapid degradability under biotic conditions and adsorption to the surfaces of the PVC pots [64,65]. In spiked sludge treatments, concentrations were also close to the nominal spiking levels (SP3: 308 ± 136 ng/g and SP4: 2030 ± 1290 ng/g) for most of the compounds. Variations in concentrations of individual compounds may result from different processes, including adsorption to Petri dishes during sludge spiking and poor homogenisation despite thorough mixing, and subsampling a small amount from the pot for analysis. For SP5 (sludge added directly in a 1:1 ratio), 12 compounds were quantified within a range of 1 to 1630 ng/g.

For each treatment, samples were collected at the end of the experiment (day 117) and CEC concentrations were compared with those at the beginning (day 0), to identify any reduction in concentration during the experimental period (Tables S10–12, Figures S5–S8). For P1, all compounds quantified at the beginning decreased significantly by the end of the experiment, except for tonalide. This musk has been shown to absorb into soil [66], resulting in slow dissipation and its presence in soil for up to one year. In the case of triclocarban, it is an antimicrobial hydrophobic compound, which may explain its recalcitrance [67,68]. For P2, although the spiked CECs were still present, all compounds quantified at the beginning showed a significant decrease by day 117. In the case of SP3 and SP4, all compounds decreased significantly, except for triclocarban.

The observed differences can be explained based on the compounds' physicochemical properties [69]. The primary parameters are the organic carbon sorption coefficient (Koc) and octanol–water partition coefficients (log Kow), which can indicate the potential for sorption; for example, compounds with log Kow > 3 (tonalide) tend to sorb to the substrate [70]. Sorption involves various mechanisms, including hydrogen bonding with organic matter, van der Waals interactions, charge transfer, cation exchange, and surface adsorption to soil minerals, which can cause the formation of non-extractable residues, limit detection, and reduce bioavailability [69,71]. The studied hormones: 17α-ethynylestradiol, beta-estradiol, estrone, progesterone, and testosterone, showed a significant reduction in concentration (Table S12) in both spiked sludge treatments. This reduction can be attributed to sorption processes, which are correlated with organic matter and moisture content [72]. Moreover, it has been shown that the presence of other hydrophobic compounds may compete with hormones for available binding sites on the substrate [70].

Other factors that can affect the behaviour of CEC in amended soils include the presence of antimicrobial compounds (e.g., triclocarban), which have been shown to alter the soil microbial community and can further influence the persistence of other compounds [71,73].

Aeration can also affect degradation, which is faster in aerobic compared to anaerobic soil [74].

3.2. Uptake and Translocation of CECs in Tomato Plant Parts (Roots, Stems and Leaves)

The concentration of CEC in plant parts (roots, stems, leaves) is presented in Table S13. The RCF and TF were calculated for those compounds where values from two different plant parts were available (Table S14). Considering that uptake in plants is concentration-dependent, most compounds were quantified across the spiked treatments (P2 and SP4). At the same time, in a non-spiked amended peat substrate, measurable concentrations were determined in roots and fruits (Figure 1). These patterns are further examined in Section 3.2.1 Roots, Section 3.2.2 Leaves, Section 3.2.3 Stems and Section 3.3 Tomato fruit, where each plant compartment is discussed separately.

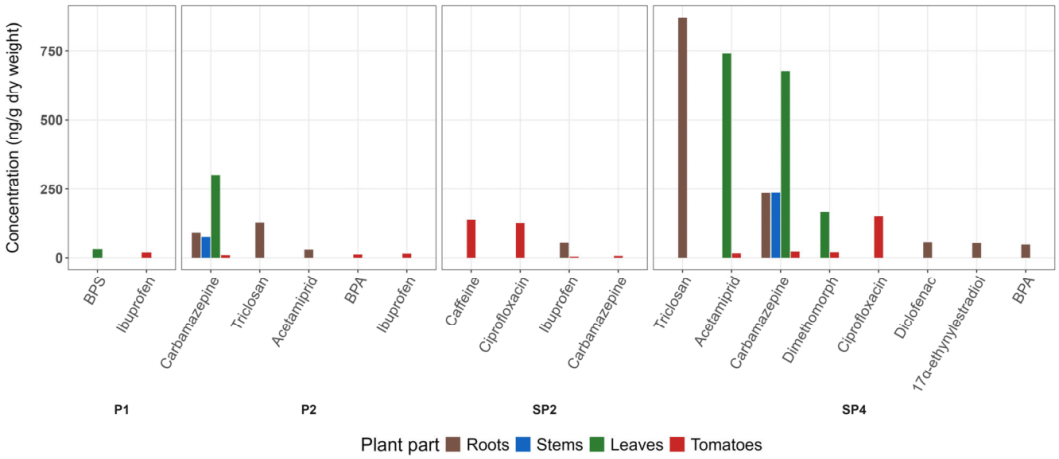


Figure 1. Bar plots of average CEC concentration (ng/g d.w.) in tomato plants grown in different treatments. P1: Peat substrate + CEC (0.3 mg/kg); P2: Peat substrate + CEC (3.5 mg/kg); SP2: Peat substrate + 545 g of dried sludge; SP4: Peat substrate + 545 g of dried sludge + CEC (3.5 mg/kg).

3.2.1. Roots

No compounds were detected in the roots of the PC. In samples grown in sludge-amended peat, no compounds were detected in SP1 (low) or SP5 (non-dried sludge), while ibuprofen was quantified (54.4 ng/g) in SP2 (high). Regarding spiked treatments, in P1, no compounds were detected above LOQ. In P2, acetamiprid (29.0 ng/g), carbamazepine (90.3 ng/g), and triclosan (129 ng/g) were >LOQ. Similarly, for SP3, no compounds were quantified, while in SP4, five CECs, 17α-ethynylestradiol (53.5 ng/g), BPA (47.3 ng/g), carbamazepine (235 ng/g), diclofenac (55.5 ng/g), and triclosan (869 ng/g) were quantifiable.

In all treatments, RCF were <1 (Table S14), indicating that the concentration in the roots was lower than that in the peat substrate. The highest RCF was 0.62 for ibuprofen in SP2 treatment. In the case of spiking with CECs at high concentration (P2 and SP4), RCFs were higher in SP4 for carbamazepine (P2: 0.056 and SP4: 0.18) and triclosan (P2: 0.035 and SP4: 0.26) indicating their higher bioavailability in spiked sludge amendment compared to spiked peat substrate, possibly due to the altered physicochemical properties of the substrate (Table S11), such as higher pH values (pH 6.4) and lower C:N ratios (20) in SP4 compared to P2 (pH 5.9 and C:N 40). Also, the addition of sludge can inhibit the degradation of both compounds [75].

Compound-specific physicochemical properties and soil characteristics influence the uptake of CECs by plant roots. Additionally, the concentration of CECs in contaminated

soil influences the extent to which CECs are absorbed by plants [76,77]. The pH of the media is also important, as it can affect their uptake and translocation, influenced by both dissociation and hydrophobicity [78].

3.2.2. Leaves

No compounds were identified across the four different non-spiked treatments: PC, SP1, SP2 and SP5. Regarding PS1, BPS was quantified in leaves in P1. In P2, only carbamazepine was quantified (301 ng/g). Regarding the spiked sludge amendments (SP3 and SP4), only acetamiprid (740 ng/g), carbamazepine (678 ng/g) and dimethomorph (168 ng/g) were quantified in SP4. The comparison between P2 and SP4, both spiked with the highest levels of CECs, showed that the total concentration in leaves was higher when CECs were derived from the addition of dried sludge (SP4) compared to peat.

3.2.3. Stems

Similar to leaves, no compounds were >LOQ in all treatments except those where a high concentration of CEC was applied (P2 and SP4). In those treatments, carbamazepine was quantified with an average concentration of 74.6 and 236 ng/g, respectively.

3.2.4. Translocation Factors

In terms of translocation from roots to stems and leaves, different compounds were determined; however, TF could be calculated only for carbamazepine in P2 and SP4, as it was the only compound detected across all the different plant parts. Specifically, in P2, the TF of leaves/roots was 3.34, while for stem/roots, 0.83 and 0.12 for fruit/roots, indicating that carbamazepine preferentially translocated from roots to leaves rather than stems or fruits. The same observation also applied to spiked sludge treatment (SP4), where the TFs from roots to leaves, stems, and fruits were 2.64, 0.73 and 0.10, respectively.

Roots can absorb neutral or cationic compounds, since they can cross cell membranes, move with the transpiration stream and accumulate in highly transpiring tissues such as leaves [79]. In peat substrate pH (5.5–6.5), compounds such as acetamiprid, carbamazepine and dimethomorph remain in their neutral form, which can in part explain the findings in leaves and stems. Molecular weight can further influence cell membrane permeability, allowing compounds with a molecular weight < 450 g/mol to transport through the xylem [80]. Nevertheless, CECs in anionic form are often repelled by negatively charged plant cell membranes, thus limiting the movement through tissues, so compounds such as 17α-ethynylestradiol, diclofenac, ibuprofen and triclosan tend to accumulate mainly in roots [81,82].

3.3. Bioaccumulation of CECs in Tomato Fruit

Tomato fruits accumulated a range of CECs, with distinct profiles depending on the treatment and whether CEC spiking was applied (Figure 2 and Table S15). The highest concentrations were found for triclosan in PC (19 ng/g), ibuprofen in SP1 (20 ng/g), ciprofloxacin in SP2 (372 ng/g) and BPS in SP5 (241 ng/g). For spiked treatments, the highest concentrations were found for ibuprofen in P1 (30.5 ng/g), P2 (30.5 ng/g), and SP3 (53.1 ng/g) and for ciprofloxacin in SP4 (408 ng/g). The BCFs ranged from 0.006 to 35, where values > 1 indicate accumulation of CEC in tomato fruits (Table S16). The highest BCF values were obtained for SP1 (ibuprofen: 6.1, triclosan: 1.3), SP2 (caffeine: 35, carbamazepine: 1.6, ibuprofen: 0.06) and SP5 (BPS: 21, carbamazepine: 12, ibuprofen: 0.6, progesterone: 0.1, triclocarban: 0.7 and triclosan: 0.2). The higher number of CECs in fruits grown in SP5 compared to SP1 and SP2 may also be due to the effect of drying, which has been shown to reduce levels of CECs [83].

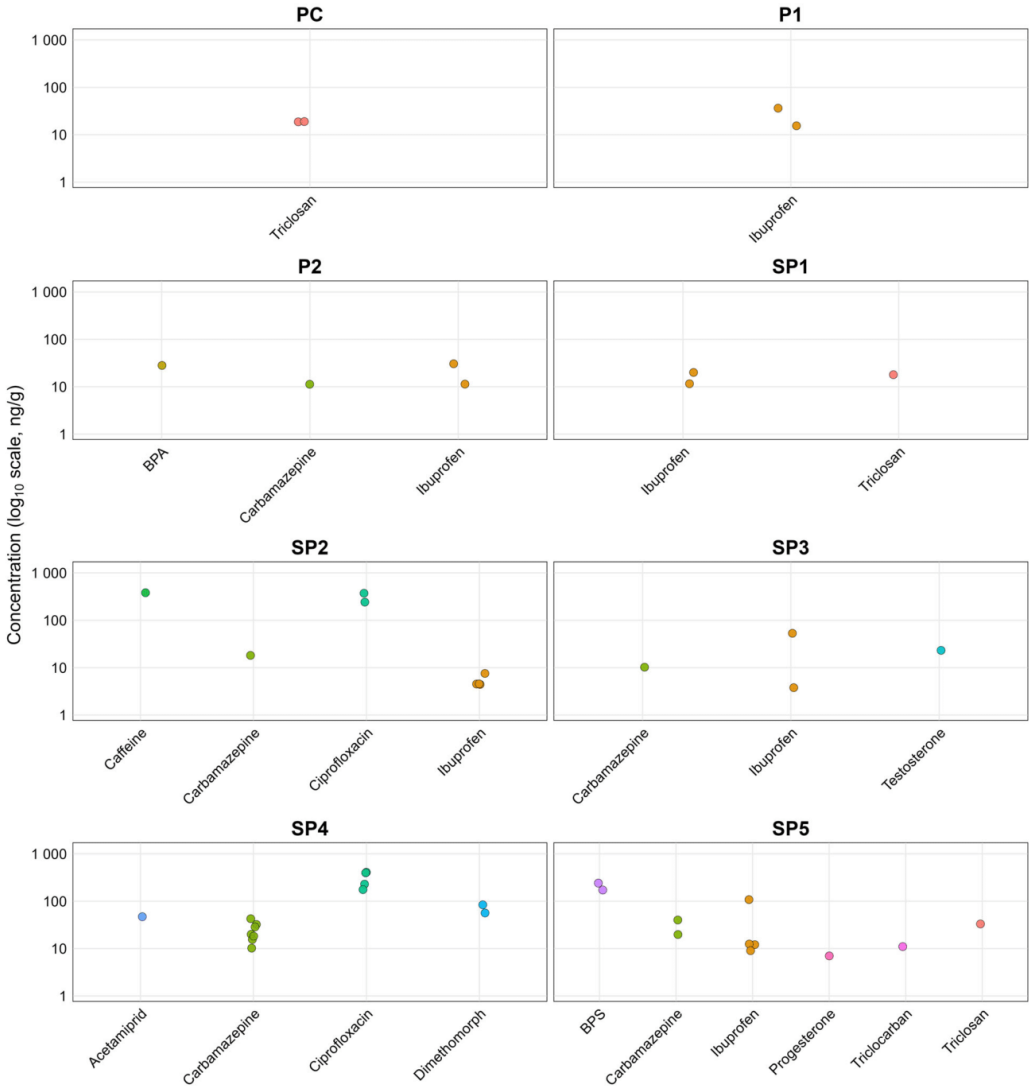


Figure 2. Jitter plots of CEC concentration (ng/g) in tomato samples across all treatments; PC: Peat substrate; P1: Peat substrate + CEC (0.3 mg/kg); P2: Peat substrate + CEC (3.5 mg/kg); SP1: Peat substrate + 45g of dried sludge; SP2: Peat substrate + 545 g of dried sludge; SP3: Peat substrate + 45 g of dried sludge + CEC (0.3 mg/kg); SP4: Peat substrate + 545 g of dried sludge + CEC (3.5 mg/kg); SP5: Peat substrate + Anaerobically digested sludge (1:1, *w*/*w*).

Within spiked treatments (P1, P2, SP3 and SP4), where different translocation factors were calculated for carbamazepine, translocation to leaves was over 100 times higher than in fruits, indicating that even in high contamination conditions, bioaccumulation in fruits was reduced compared to other plant parts. Differences between the concentrations of carbamazepine in SP4 were significantly higher compared to P2 ($p = 0.02$; effect size d = 1.9). The difference in CEC bioaccumulation observed between P2 and SP4, despite similar final CEC concentrations, may be attributed to the addition of sludge, as previously reported in the literature [15,19].

Triclosan and triclocarban have been studied previously in various crops, since they are consistently detected in sewage sludge and biosolids [84,85]. Among those studies that investigated tomatoes (Table 1), only Prosser et al. [29] were able to detect triclocarban (up to 5.2 ng/g), whereas in the present study, triclosan was quantified in SP1 (≤17.9 ng/g), and triclocarban was determined in SP5 (≤33.1 ng/g). Wu et al. [22] also studied carbamazepine and triclocarban in tomato grown in spiked biosolid-amended soil and found a higher amount of triclocarban than carbamazepine, which is opposite to the present study (SP4, carbamazepine: 42.7 ng/g; triclocarban: <LOQ). Another field study by Sabourin et al. [28] with dewatered biosolids application, detected only six out of 141 Pharmaceutical and Personal Care Products (PPCPs) in tomatoes, including testosterone, which was only quantified in tomatoes grown in spiked sludge (SP3; 23.2 ng/g), while they did not detect caffeine, carbamazepine, ciprofloxacin, ibuprofen and triclosan, which were quantified in SP1 and SP2.

Ibuprofen and diclofenac were also previously detected in tomatoes grown in spiked soil [86], with concentrations ≤ 95.2 ng/g for ibuprofen and 84.2 ng/g for diclofenac, respectively. However, diclofenac was not applicable in our study since it was not detected, whereas ibuprofen was quantified across six different treatments. Ibuprofen, a weak acid with a pKa of 4.9, is predominantly in its anionic form at the pH values of the tested media. Anions are more soluble compared to the protonated form of the molecule, which reduces sorption affinity onto organic matter and may enhance pore-water mobility [87]. Although CECs in anionic form are less prone to plant uptake, due to repellence from negative electrical potential at the cell membrane [88], other parameters and processes may also influence the uptake and translocation, including membrane permeability, ion trap, phloem transport, and sorption to proteins, as well as molecular weight, the pH around and inside the cell membranes and plant physiological characters (root lipid content and carbohydrates) [89,90].

Overall, the results demonstrate that uptake is not only associated with the concentration introduced into the substrate, but is also affected by potential interactions between the growing matrix (peat substrate and its combinations with sludge) and the compounds evaluated. These can affect their sorption, degradation, and metabolism within the plant system. Differences between this study and the available literature may be attributed to the use of a commercial peat substrate, which has different characteristics from soil.

3.4. Tomato Yield

The yield average (Table S17) between the treatments showed no significant difference ($p > 0.05$) in total weight or fruit number per replicate, likely due to limited and unbalanced replication between treatments and fertilised groups. When comparison was applied between pooled fertilised (F) and non-fertilised (NF) treatments, both yield and number of fruits had a significant difference between F and NF (higher in F, $p = 0.009$ and 0.03), while the presence or absence of CECs within treatments did not affect the yield or number of fruits ($p = 0.7$ and 0.8). Spearman's rank correlation analysis showed a statistically significant positive correlation between the number of fruits collected and the yield per replicate ($\rho = 0.96$, $p < 0.001$). This finding suggests that differences in yield weight were primarily driven by differences in fruit number rather than individual fruit size.

Plants with sludge amendment generally showed stress symptoms, including leaf chlorosis and necrosis (Figure S4, left), which may result from the potentially toxic element content in sludge and its salinity, both of which can reduce water uptake and inhibit growth [15,91]. Although the conditions for ammonium nitrification in pots with sludge were favourable (average daily air temperature during the growth period was 22.5 °C, with

sufficient substrate aeration and water content), there was enough nitrate for the growth and development of tomato plants.

Toxic symptoms in plants, which appeared in the leaves of those treated with sludge, were probably a consequence of the nitrification process in soil containing sludge, as nitrogen in sludge was mainly present as ammonium. In soil, the uptake of nitrate by plants is rapid, whereas the uptake of ammonium is slower. Ammonium binds to soil particles (clay and organic matter) and is slowly converted to nitrate over several days or weeks during nitrification by soil bacteria. During nitrification, N_2O and NO are lost to the atmosphere, potentially causing toxic symptoms in plant leaves [92,93]. Notably, after adding fertiliser, plant growth visibly improved, and there was no significant difference in yield across all treatments (Figure 3).

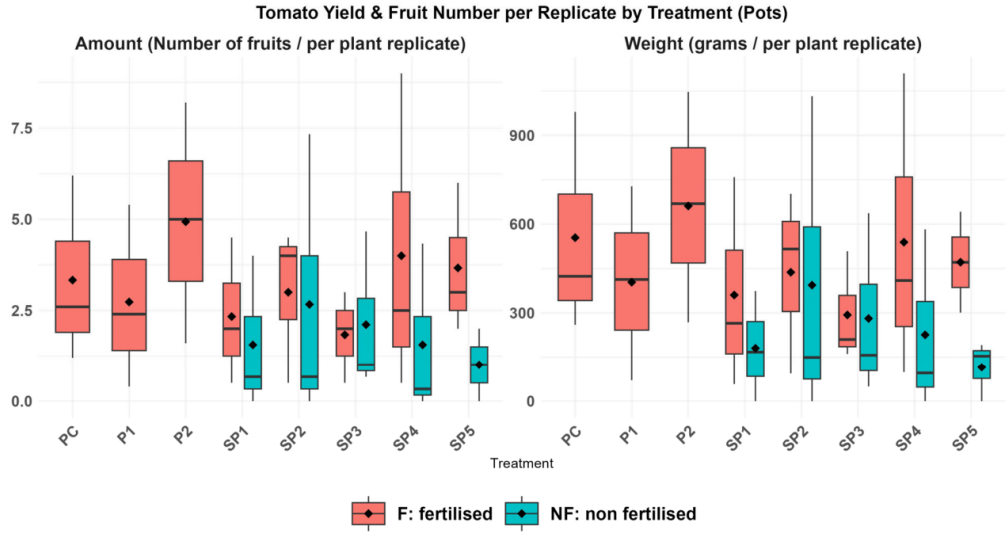


Figure 3. Boxplots of tomato yield and number of fruits per treatment, normalised per replicate.

3.5. Dietary Exposure to CECs and Health Risk Assessment for Tomato Fruits

The average chronic dietary exposure (mg kg^{-1} bw day^{-1}) was estimated for adults, adolescents, and toddlers in Slovenia under both average and high tomato consumption, using fresh-weight concentrations from each treatment (Tables S18 and S19). Risk characterisation was performed by comparing each EDI with the corresponding health-based reference values.

The data obtained show that estimated daily intakes are below the risk threshold for most compounds quantified across different treatments (HQ < 1). Risk was identified only for two bisphenols. Specifically, BPA exceeded the TDI for all age groups and consumption margins in high-spiked treatments (P2) (HQ: 1.3–18). During the mixture of sludge with peat substrate (SP5), only BPS exceeded the tolerable daily intake (HQ: 11–153). Due to the lack of health-based reference values for BPS, the suggested TDI by EFSA for BPA (0.2 ng kg^{-1} bw day^{-1}) was used as a conservative comparison and due to structural similarities. However, for both bisphenols, the respective LOQs exceed the established EFSA TDI. When a bisphenol result is reported as <LOQ, it does not mean the concentration is at or below the EFSA-TDI. Instead, it suggests that the actual value may lie anywhere from zero to a range that could still imply risk. Although this may be considered a limitation, the method was designed to quantify CECs from different groups rather than to achieve

trace-level sensitivity for bisphenols; thus, the degree of potential risk for this group cannot be precisely specified.

For the detected pesticide residues, both chronic and acute dietary exposure were calculated, and the latter was compared with the appropriate toxicological reference values. The acute exposure assessment models a high-intake event, and all estimated intakes were below the risk thresholds. This outcome was expected, as acetamiprid is an authorised pesticide in the EU for use on tomatoes, with an MRL of 0.06 mg/kg [94], whereas dimethomorph is no longer authorised and its MRL has been set at 0.05 mg/kg (LOQ) [95].

In all other treatments, no dietary exposure risk was identified for the compounds quantified in edible parts, including pharmaceuticals (carbamazepine, ciprofloxacin, ibuprofen), personal care products (triclosan, triclocarban), hormones (testosterone, progesterone), and stimulants (caffeine). However, this assessment includes only a representative target list, while metabolites and transformation products of the selected CEC could pose risks through dietary intake; further studies are needed to address risks and synergistic effects deriving from mixtures.

3.6. Ecological Risk Assessment of CECs in Peat Substrate

The potential ecological risks posed by each CEC within the growth media of different treatments, as collected at the end of the experiment, were evaluated using RQ values, which are provided in Table S20. Although the described experiment was performed in pots, where substrate was handled and disposed of in accordance with Slovenian waste management legislation after the finalisation of a growing season [31], the ecological risk assessment is considered important since the results can be extrapolated for similar applications to field-grown tomatoes, especially for scenarios with consecutive sludge applications within the years. Apart from CECs' persistence in soil following the application of sludge, another concern with the practice is the potential leaching of compounds into surface water sources and groundwater [96]. However, this aspect was not evaluated because the experimental design included pots in which leachate from individual pots was not collected. Although the method to calculate PNEC for soil may lead to overestimation, high ecological risk (RQ > 1) was found across different treatments. In control samples, two compounds exceeded the threshold: benzophenone and triclocarban.

Benzophenone had similar RQ values (1.5) when sludge was introduced at a lower dose, where triclocarban was below LOQ. In the higher sludge dose (SP2), risks were identified for triclosan, triclocarban, and tonalide, while the RQ value for benzophenone remained below 1 (0.62), indicating a medium risk. However, the evaluation of the peat substrate/dewatered sludge mix revealed the highest RQ values among the treatments where CEC was not deliberately applied. In this case, high risk was identified for BPA (18.9), benzophenone (2.5), estrone (3.7), ibuprofen (238), tonalide (296), triclocarban (770) and triclosan (131).

The limited identification of CEC and the reduced risk associated with using dried sludge amendment compared to anaerobically digested sludge are consistent with the literature. For example, torrefaction of sludge before amendment is a process that significantly reduces the CEC content in sludge and biosolids, thereby limiting their presence in the receiving substrate and their uptake by plants [97,98]. Triclosan and triclocarban are both persistent in sludge-amended soils and may pose a risk to soil health, as they can disrupt microbial communities and inhibit organic matter decomposition by suppressing microorganisms [99,100]. In spiked treatments, either spiked peat substrate or spiked sludge, RQs exceeded safety limits for most of the compounds quantified. This finding indicates that although the levels of CECs significantly decreased from the beginning to the end of the experiment, the risk remained high for most CECs (16 out of 22 CECs) in

SP4 after 117 days. Although the initially applied concentrations of CECs were higher than would be expected from sludge amendment, the results highlight the persistence of several CECs, which can affect the soil ecosystem. Their presence can impact soil processes, and the build-up of antibiotic resistance is a major concern [101,102].

3.7. Limitations and Future Work

While findings provide valuable insights into the uptake of CECs in tomato plants following sludge application, several limitations should be acknowledged. The results are based on a controlled greenhouse pot experiment and may therefore not fully reflect field conditions or commercial production. Applying sludge repeatedly over several growing seasons could also lead to the build-up of persistent CECs in the soil, thereby increasing the likelihood of their uptake and translocation to the fruits, and raising the overall risk from sludge reuse; however, this was not assessed in the current single-season study. Moreover, the uptake of CECs in plants can be influenced by variations in sludge composition and soil characteristics, limiting broader generalisation. Analytical limitations should be considered, since compounds just below the LOQ may still be present and pose potential health risks. Additionally, metabolites and transformation products of the selected CECs were not included in the analysis, which may have potentially underestimated overall exposure.

For ecological risk assessment, PNEC values derived using the equilibrium partitioning method serve as a screening tool but may lead to over- or underestimations. In the dietary exposure assessment, a deterministic (point-estimate) approach was applied, which does not account for chemical mixtures and potential synergistic effects. Although a probabilistic exposure model would provide a more realistic assessment, it was not applied due to insufficient data to characterise the distribution of individual chemicals properly.

4. Conclusions

This study looked at the uptake of CECs by tomato plants grown in sludge-amended peat. Several CECs were determined in the amended substrate, with more compounds quantified at the higher amended rate. A higher number of compounds was also quantified when non-dried anaerobically digested sludge was added. In all treatments, RCF was <1, indicating that the concentration in the roots was lower than in the peat substrate. For carbamazepine, the only compound detected in each tissue, translocation from roots to leaves was highest, followed by stems and fruit. BCFs were >1 in dried sludge for caffeine, carbamazepine, ibuprofen, and triclosan, while for non-dried sludge, BCFs were >1 for BPS and carbamazepine. Although adding sludge affected plant health, neither sludge nor CECs had a measurable effect on tomato yield. According to dietary exposure estimates, a risk was only identified for BPS when non-dried anaerobically digested sludge was applied. In terms of ecological risk, the study showed risk quotients > 1 for 7 out of 27 CECs in treatments that included sludge, which could have implications if sludge is applied repeatedly in the field. Future work should extend to monitoring non-targeted CEC residues, including their stable transformation products and address them in human and ecological risk assessment.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/toxics13121013/s1. Table S1: Names, CAS numbers, molecular formula, octanol–water partition coefficient (log Kow), pKa, organic carbon partition coefficient (Koc) and molecular weights (MW) of the investigated contaminants of emerging concern (CECs) derived from PubChem, DrugBank and Human Metabolome Database [1]; Table S2: Characteristics of peat substrate; Table S3: Analysis of nutrients in dried sludge; Figure S1 (a) Schematic showing the randomised planting scheme including different replicates (I-V), and (b) photo showing pots with 5-week-old tomato plants and the layout of the drip irrigation system; Figure S2 (a) Anaerobically

digested sludge, (b) Dried sludge and (c) Spiked sludge before solvent evaporation; Figure S3: Temperature and solar radiation measurements across the experimental period (May–September 2022, 117 days); Figure S4: Visual difference between pot amended with a high amount of sludge (SP2, left) and control (P, right); Table S4: Validation data on recovery (REC), matrix effect (ME), repeatability (REP) for high and low point, LOD, LOQ and R^2 for (a) peat substrate matrix and (b) sludge matrix. Table S5: Consumption rates for tomatoes in Slovenia, acquired from EFSA Food consumption statistics; Table S6: Health-based reference values, including Acceptable Daily Intake (ADI), Acute Reference Dose (ARfD), Tolerable Daily Intake (TDI) or No Observed Adverse Effect Level (NOAEL) for individual CEC; Table S7: Predicted environmental concentrations (PNEC) of each CEC in soil based on the PNEC of freshwater; Table S8: CEC concentration (ng/g) in dried sludge used in the experiments; Table S9: Concentration of CEC in peat substrate at the beginning of the experiment, expressed in ng/g d.w.; Table S10: Concentration of CEC in peat substrate at the end of the experiment, expressed in ng/g d.w.; Table S11: Analysis of substrate samples at the end of the experiment; Figure S5: Boxplots showing CEC concentrations at month 0 and month 3 for P1; Figure S6: Boxplots showing CEC concentrations at month 0 and month 3 for P2; Figure S7: Boxplots showing CEC concentrations at month 0 and month 3 for SP3; Figure S8: Boxplots showing CEC concentrations at month 0 and month 3 for SP4; Table S12: Month 0 vs. Month 3 comparisons per compound and treatment; Table S13: Average concentration of CEC in plant parts, expressed in ng/g d.w.; Table S14: Root Concentration Factor (RCF) calculated based on peat substrate measurement; Table S15: Concentration of CEC quantified in tomato samples, including composite samples per plant (1–5) and composite samples per harvest season (A–C); Table S16: Bioconcentration Factor (BCF) calculated based on peat substrate concentration; Table S17: Tomato yield production from different treatments and seasons; Table S18: Estimated Daily Intake (EDI) as mg/kg per body weight per day; Table S19: Hazard Quotients for CECs in tomato fruits; Table S20: Ecological risk assessment results based on Risk Quotient (RQ). [32–36,39,41–55,58].

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Abbreviations

The following abbreviations are used in this manuscript:

CECs	Contaminants of Emerging Concern
PPCPs	Pharmaceutical and Personal Care Products
PFASs	Per- and Polyfluoroalkyl Substances
PBDEs	Polybrominated Diphenyl Ethers
WWTP	Wastewater Treatment Plant
EDI	Estimated Daily Intake
ADI	Acceptable Daily Intake
TDI	Tolerable Daily Intake
NOAEL	No Observed Adverse Effect Level
MEC	Measured Environmental Concentration
PNEC	Predicted No-Effect Concentration
EPM	Equilibrium Partitioning Method
BCF	Bioconcentration Factor
RCF	Root Concentration Factor
TF	Translocation Factor

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Chapter 4

Conclusions

Treated wastewater and sludge can be valuable resources for agriculture, promoting a circular economy by recycling water and nutrients, reducing waste disposal and pressure on freshwater resources. However, these practices are not without risk, as both can introduce contaminants into agricultural systems. Overall conclusions of the work carried out within the thesis are divided into the following parts.

4.1 Development and Application of Analytical Methods for the Determination of CECs

An important part of the thesis included the development and validation of analytical methods to evaluate the presence of CECs in matrices collected from the designed experiments. An LC-MS/MS method was developed for 27 CECs, which were selected based on their occurrence and fate in wastewater and freshwater, potential adverse effects on human health, and their inclusion in regulatory frameworks.

Different sample preparation methods were optimised and validated to achieve the extraction of the selected CECs and clean-up depending on the matrix, such as tomatoes and sludge (SLE-SPE), soil and peat substrate (SLE) and plant parts (QuEChERS combined with automatic μ-SPE). For aqueous media, two different methods were used: SPE with cartridges was used for PW and TWW for a volume of 50 mL and a 96-well plate for spiked aqueous media for a volume of 1 mL.

Additionally, for wastewater samples, extraction using a newly synthesised sorbent was applied in dispersive micro-solid phase extraction (D-μ-SPE), as an alternative extraction method. The method was optimised and validated for 24 CECs, which was additionally applied to real samples from four different Slovenian WWTPs. Although the method yielded a total score of six out of 10 for the sustainability metric, compared to 2.84 for conventional SPE, SPE was applied to samples collected during the experiment to cover all 27 analytes included. However, the findings underscore the advantages of the proposed protocol, particularly the use of copolymers, since the method can successfully determine CECs in wastewater, while utilising decreased amounts of sorbent, sample volume, and organic solvent, resulting in an eco-friendly and cost-effective extraction process.

4.2 Soil-grown Tomatoes Irrigated with Treated Wastewater

The first field experiment, conducted in 2020, demonstrated that growing tomato plants in soil irrigated with treated wastewater resulted in limited uptake of CECs to the fruits. No compounds were quantified in tomatoes irrigated with PW and TWW. Only BPS (1.4

ng/g) was above its LOQ, in TWW spiked at 0.1 mg/L. In the follow-up field experiment in 2022, which included a broader set of target analytes, carbamazepine (not included in the 2020 field experiment) was the only target CEC quantified in tomatoes irrigated with TWW.

The uptake of carbamazepine in tomatoes irrigated with TWW exceeded the average concentration reported in other studies (5 ng/g), highlighting the potential influence of different parameters among the available experimental designs. These include the irrigation media (PW, TWW), irrigation system (drip, sprinkler), soil characteristics (pH, $CaCO_3$ content, organic matter) and water quality, which can differ between WWTPs (technology, size, sources). Carbamazepine also exhibited the highest concentrations across all plant parts, which can be attributed to its pK_a (13.9) and logK_{ow} (2.45) and the fact that, it is in a neutral form in all environmental compartments (PW, TWW, soil) and within various plant parts (roots, xylem, phloem, leaves, and fruit tissues), allowing it to cross the plant cell membrane and translocate through plant tissues, including fruits. The effect of CEC ionisation state was also evident based on the distribution of compounds, since anionic pharmaceuticals are repelled by negatively charged cell membranes, which restricts their movement into the xylem and limits translocation. Thus, compounds such as diclofenac and ibuprofen were mainly present in roots and did not translocate to other plant parts.

In 2022, 20 compounds were quantified across all plant parts in plants irrigated with spiked media, with the following distribution: roots > leaves > stems > fruits, in terms of the number of compounds detected and their concentrations. In fruits, six compounds were determined, including carbamazepine, which was the highest, as well as acetamiprid and dimethomorph in both spiked PW and spiked TWW. BPS, clarithromycin and azithromycin were only above the respective LOQs in spiked TWW treatment.

BPS was detected in tomato fruits irrigated with TWW+CEC in both experiments (2020 and 2022), with a higher concentration in 2022, where the spiking level was increased tenfold. To my knowledge, this was the first report of BPS uptake in tomato fruits. In 2022, BPS was detected in all plant parts, with the highest concentration in roots. As a hydrophilic compound (logK_{ow}: 1.65), BPS can translocate upwards within the plant through the transpiration stream and accumulate in different plant parts, including fruits. The potential for bioaccumulation in edible parts suggests that further studies are necessary, as the compound is a widely used alternative to BPA.

Overall, plants irrigated with TWW+CEC exhibited lower concentrations for most CECs, compared to plants irrigated with PW+CEC. This finding suggests that the reduced stability of spiked CECs in the TWW matrix, compared to PW, may influence the bioavailability and uptake of compounds. The degradation of CECs in TWW storage containers can also be linked to the presence of microorganisms, as well as the potential adsorption to suspended solids and container surface.

Although several CECs were above 2 µg/L in TWW samples collected during the experiments, none were detected in plant parts. Trace amounts may still have been present, with concentrations likely falling below the respective limits of quantification, due to analytical limitations and relatively higher limits of quantification in plant parts. The presence of soil also influences the uptake of CECs, as the major bioavailable/bioaccessible CECs for plant uptake are present in the soil pore water.

Overall, the two field experiments showed that the accumulation of CEC was limited in tomato plants irrigated with treated wastewater. Extensive uptake and translocation into fruits occurred only when compounds were present in the irrigation media at high concentrations. The overall pattern across treatments confirms that uptake is affected by concentration in TWW, compound-related parameters and interactions with soil and plant tissues.

4.3 Tomatoes Grown Hydroponically

In the 2020 experiment, out of the 14 compounds tested, BPS, BPF, and naproxen were quantified in tomatoes grown in spiked nutrient solution at 0.1 mg/L. In 2022, the experiment included 27 CECs, a higher spiking level (1.0 mg/L vs 0.1 mg/L), along with the inclusion of TWW and spiked TWW as additional treatments. During the first 14 days of the 2022 experiment, reduced plant growth was observed in spiked treatments (nutrient and TWW at 1 mg/L), accompanied by a poor root system, leaf chlorosis, and necrosis. The addition of high concentration of CECs was considered the main factor affecting the plant growth. Thus, a further irrigation treatment was introduced, with a nutrient solution spiked at 0.1 mg/L, the same as in 2020, since such issues were not encountered in the 2020 experiment. This parallel also showed minimal growth, suggesting that other factors were at play, such as the inclusion of additional CECs that could have had phytotoxic effects. Additionally, temperature differences were considered significant, with the following values recorded: June 2020, 24.8 °C (maximum: 31.7 °C); and June 2022, 29.6 °C (maximum: 36.1 °C).

In 2022, none of the collected tomatoes were ripe, and only those grown in the control (nutrient solution) were at a marketable size. A phytotoxic experiment was performed afterwards to evaluate the effect of the CEC mixture on early-stage tomato plant development. Results showed that the 0.1 mg/L treatment had no effect on plant growth compared to the control, whereas the 1 mg/L treatment partially affected plant growth. This finding further supports the idea that, in 2022, plant growth was inhibited by multiple factors, rather than just the concentration level and the selected CECs.

In tomatoes grown in a nutrient solution spiked at 0.1 mg/L in 2022, carbamazepine was the compound quantified at the highest concentration, followed by acetamiprid, clarithromycin, azithromycin, dimethomorph and BPS. Differences were identified when comparing results from 2022 with those from 2020, where BPS, BPF, and naproxen were quantified. In the 2022 experiment, BPS was found, while BPF and naproxen were only found in leaves at concentrations of 553 and 487 ng/g, respectively. The fact that neither compound was quantified in the fruits can be attributed to the extent of plant growth. In 2022, plants exhibited clear signs of stress, including small, unripe fruits and visibly reduced growth, which may have impaired xylem-driven translocation to the fruits.

Bisphenol S was also quantified in fruits and leaves in the control plants. The presence of CECs in controls could be attributed to the potential use of plastics (pipe fittings and tubs) that may contain residues of BPS. In tomatoes grown with treated wastewater, only BPS, clarithromycin, and azithromycin were detected at levels above the respective LOQs. The concentration of BPS was lower than in control samples. The differences in concentration can be attributed to the more extensive plant development in plants grown in the nutrient solution compared to those in TWW. A notable observation is that for control plants, evapotranspiration rates were higher, as the tubs had to be refilled with fresh media four times during the experiment. However, the rest of the treatments, including TWW, required limited refills (up to two times) due to visibly reduced plant growth, which likely resulted from reduced evapotranspiration rates, limiting the translocation of CECs within the plant, a known phenomenon when plants are exposed to a stressor such as organic micropollutants.

In the spiked nutrient solution treatment (1 mg/L), 10 compounds were above the LOQ in tomato fruits, namely caffeine, carbamazepine, acetamiprid, clarithromycin, azithromycin, BPS, ciprofloxacin, dimethomorph, erythromycin and BPF, from highest to lowest. In contrast, in treated wastewater spiked at the same concentration, two additional compounds were quantified in tomato fruits: naproxen and propylparaben, where

carbamazepine had the highest uptake among the 12 CECs. Across all spiked treatments, contaminant distribution followed the pattern roots > leaves > stems > fruits, reflecting that CEC can be taken up in roots and concentrations decline along the transpiration stream.

Both hydroponic experiments (2020 and 2022) revealed distinctly higher CEC uptake and translocation compared to field-grown tomatoes, likely due to the absence of soil sorption effects. Across years and treatments, the results demonstrated compound- and matrix-specific differences in uptake and translocation, similar to those observed in soil-grown tomatoes, also depending on exposure concentration.

4.4 Tomatoes Grown in Sludge-amended Peat Substrate

In pot experiments with sludge amendment, tomato fruits accumulated a range of CECs, with distinct profiles depending on the treatment and whether spiking was applied. In this case, the addition of dried sludge at two distinct levels resulted in the uptake of different compounds. When a low amount of dried sludge was applied, only triclosan and ibuprofen were quantified. In comparison, when a high amount of sludge was applied, ibuprofen, carbamazepine, ciprofloxacin, and caffeine were quantified in order from lowest to highest, while triclosan was not detected. Differences in fruit bioaccumulation were also evident when non-dried anaerobically stabilised sludge was applied, where BPS had the highest concentration, followed by carbamazepine, ibuprofen, progesterone, triclocarban, and triclosan. This finding suggests that the highest number of CECs and the highest concentration in the specific treatment can be linked to the additional pretreatment step, i.e., drying (200 °C), which could increase the sorption strength of CECs on the sludge matrix and limit its desorption capacity, making CECs less available to soil-pore water and resulting in reduced uptake.

When spiking was applied only to the peat substrate, in two concentrations, only ibuprofen was quantified in the tomato fruits at low spiking levels. In contrast, in the high-spiking treatment, ibuprofen, BPA, and carbamazepine were also quantified. Ibuprofen was in a similar concentration range in the fruits of both treatments, indicating that uptake is not only related to the applied concentration, but can also be influenced by interactions with the growing matrix (peat substrate). Sorption processes can reduce the concentration of available compounds in the pore water fraction, thereby limiting their uptake.

Differences in uptake were also evident when spiked sludge was applied. In the low spiked sludge amended peat, ibuprofen, testosterone, and carbamazepine were above their respective LOQs in fruit. Compared to spiked peat substrate, the findings reveal that sludge can alter the bioavailability of compounds, since different CECs accumulated in plants. Differences were also observed in spiked sludge at high-amendment rate, where ciprofloxacin, dimethomorph, acetamiprid and carbamazepine were quantified in fruit. Differences in uptake can also correlate with the organic carbon content in the peat substrate, combined with varying amounts of sludge, as this may lead to the sorption of CECs and reduced bioavailability.

Regarding plant parts, compounds were mainly quantified when high spiking was applied either in peat substrate or sludge. When comparing carbamazepine and triclosan, both found in high spiked treatments, root concentration factors were higher in the case of spiked sludge compared to spiked peat substrate at the same concentration. This finding was linked to the physicochemical properties of the substrate, specifically a lower pH and higher C:N ratio compared to when sludge was applied, where the C:N ratio was mainly affected by the nitrogen percentage.

Differences in growth could also explain the uptake patterns. In peat substrate, all plants had normal physiology, while plants amended with sludge showed stress symptoms (leaf chlorosis and necrosis). Plants grown in sludge showed improvement only when fertiliser was applied, which could also affect the uptake of compounds.

The only compound that was consistently quantified across all plant parts in spiked treatments was carbamazepine, for which translocation factors were calculated. Translocation to leaves was over 100 times higher than in fruits. These results demonstrate that even in scenarios with high concentration applications, bioaccumulation in fruits remains limited compared to other tissues, following a pattern of leaves > roots > stems > fruits. However, the presence of certain compounds in plant parts may still be possible, as concentrations below the LOQ cannot be determined due to analytical limitations.

Distribution patterns can also be explained in terms of plant physiology. Water and dissolved compounds absorbed by roots are primarily transported upwards through the xylem with the transpiration flow, leading to higher accumulation in leaves (main sites of transpiration). In contrast, the primary transport route of micropollutants to fruits is the phloem, which has lower water flow compared to the xylem. The mobility of CECs in phloem, due to their polarity, molecular size, and ionisation state, can affect the ability of compounds to cross membranes, thus limiting their translocation to fruits.

Overall, the results show that uptake is influenced not only by the concentration applied to the substrate but also by interactions between the growing matrix (peat or peat-sludge mixtures) and the compounds. Sorption of compounds may be subject to various mechanisms, including hydrogen bonding with organic matter and surface adsorption to soil minerals, which can lead to the formation of non-extractable residues and reduce bioavailability to plants.

4.5 Comparison of Uptake Between Different Experiments

A comparison of all five experiments: field-grown, hydroponics, and sludge-amended pots, revealed differences in the uptake and translocation of CECs.

When comparing hydroponics with field-grown tomatoes, results suggest that soil can significantly alter the bioavailability of compounds. In the 2020 experiment, hydroponically grown tomatoes had significantly higher uptake compared to field-grown tomatoes. Similarly, in 2022, concentrations in hydroponically grown tomatoes were also higher than in soil-grown tomatoes (up to two orders of magnitude).

Regarding the 10 common compounds between the two years, only BPS was present in fruits from soil-grown tomatoes in both years, although the spiking concentration increased in 2022. In hydroponics, when comparing the same concentration, BPS, BPF, and naproxen were quantified in fruits collected in 2020. In contrast, in 2022, only BPS was determined, which was attributed to the limited plant growth.

Differences were also observed when comparing soil-grown tomatoes irrigated with TWW and tomatoes grown in sludge-amended peat substrate. Although the concentrations applied are not necessarily comparable, it was evident that only a limited number of compounds were mutually present in both treatments, suggesting a trend between the bioaccumulation of CECs and the source of exposure.

The most obvious finding to emerge from the study is that carbamazepine was the most persistent compound across all experiments. It was quantified in both realistic applications and spiked treatments, including: soil-grown fruits irrigated with TWW and spiked media, fruits grown hydroponically in spiked media, fruits grown in spiked sludge-amended peat substrate and non-dried anaerobically digested sludge. Its consistent detection across all

matrices reflects its resistance to biodegradation and low sorption affinity to soil and peat at different environmental pH levels, which induces root uptake and translocation.

Both acetamiprid and dimethomorph were also present across all experiments, consistently appearing in lysimeter and hydroponic fruits under spiked conditions but not extensively in the sludge-amended peat. In the lysimeter experiments, both compounds were present in spiked conditions, indicating that they can translocate to fruits under field soil conditions. In hydroponics, the absence of a soil substrate led to higher translocation. However, in pot experiments, compounds were only determined when sludge was spiked at a high level and mixed with peat substrate. The findings can be related to the matrix applied, since both showed limited uptake in sludge-amended peat, where different interactions reduced their availability to plants.

Bisphenols (BPS and BPF) were also persistent across all experiments, found in soil-grown fruits irrigated with spiked TWW in both years. At the same time, BPA and BPF were detected in other plant parts, as well as in hydroponically grown fruits and fruits grown in peat substrate. Specifically, BPA was detected during the application of spiked peat substrate, and BPS was detected during the application of non-dried sludge. The findings indicate that even when soil is present as substrate, which induces sorption and biodegradation, bisphenols can be taken up by roots and translocate to other tissues. Their consistent presence across experiments can be attributed to a combination of factors, including their chemical stability, moderate hydrophobicity, and limited biodegradation, which enables these compounds to persist in environmental matrices and remain available for root accumulation.

In contrast, several analytes were found across only specific experiments, highlighting how different media can either suppress or enhance bioavailability. Caffeine, macrolides (azithromycin and clarithromycin) and ciprofloxacin had high concentrations in fruits grown hydroponically in spiked conditions. Caffeine and ciprofloxacin were also detected in the pot experiment in tomato fruits grown on spiked peat substrate. Ciprofloxacin was also detected when spiked sludge was applied. However, both compounds were absent in soil-grown tomatoes, either in plant parts or fruits. The observed differences in lysimeters can be attributed to the degradation of compounds in the spiked aqueous media or their strong sorption to soil minerals, which reduces their bioavailability to plants. In contrast, the sludge-amended peat substrate had different properties (pH and C:N ratio), whereas in hydroponics, the absence of sorption and continuous exposure resulted in higher concentrations of CECs.

Other analytes were unique to specific treatments. For example, ibuprofen, triclosan and triclocarban were not detected in lysimeter or hydroponic fruits but were quantified in sludge-amended fruits. Triclosan and triclocarban are both hydrophobic compounds with high sorption affinity, which affects their bioavailability/bioaccessibility. It is considered that the peat-sludge substrate enabled the desorption of compounds into pore water, resulting in their increased bioavailability and uptake by roots and subsequent translocation to aerial tissues. In contrast, during field experiments, the soil composition likely restricted desorption, resulting in reduced bioavailability/bioaccessibility and retention mainly in the roots.

Overall, the results indicate that multiple factors influence uptake and translocation in tomatoes. Hydroponic plants exhibited the highest uptake, likely due to the absence of a substrate. In contrast, uptake in soil-grown tomatoes was reduced and affected by the degradation of compounds in the aqueous medium. The peat-sludge matrix generally showed a distinct profile of determined analytes, not present in soil-grown tomatoes. These comparisons highlight that the uptake and translocation of contaminants are strongly influenced by growth conditions, application method, the nature of the growth substrate

(soil, water, or peat substrate), and the physicochemical properties of CECs (persistence, sorption-desorption behaviour, and availability in pore water).

4.6 Risk Assessment

4.6.1 Human health risk assessment

Regarding the average chronic exposure of consumers, limited risks were identified for tomatoes grown under realistic scenarios across all experiments. For carbamazepine, which was the only compound quantified in tomatoes grown in soil and irrigated with TWW, the EDI was nearly half of the respective ADI (HQ up to 0.017), even for vulnerable population groups, such as toddlers. Across all experiments, although CECs were added at high concentrations, the risk was limited for the majority of compounds in edible plant parts (18 out of the 27). Even in hydroponically grown tomatoes, where concentrations in fruits were the highest among all the experiments, exposure estimates were several orders of magnitude below the respective health-based reference values for the majority of compounds (10 out of 12 CECs). However, it should be noted that the hydroponically grown tomatoes were not ripe.

Specifically, risk was evident only for bisphenols across all experiments. For bisphenols found across the experiments, the suggested TDI by EFSA for BPA (0.2 ng/kg bw/day) was used as a conservative comparison, due to the lack of other health-based reference values and by considering the structural similarities. For BPS, risk was identified in soil-grown tomatoes (TWW+CEC), in tomatoes amended with non-dried sludge and in all tomatoes grown hydroponically. Bisphenol F was a risk in hydroponically grown tomatoes in media spiked at 1 mg/L, while BPA exceeded TDI in pots with peat substrate spiked at the higher level.

In the 2020 hydroponics experiment, although BPS and BPF were quantified in tomatoes grown in spiked nutrient solution, no risk was reported due to the selected way of risk calculation, compared to 2022. Specifically, the highest exposure estimates of BPS were compared with the available derived no-effect level of 200 µg/kg bw/day, and no health concern was reported. However, by applying the conservative limit of BPA's TDI, BPF is exceeding health-based reference values only for adults under high consumption scenarios. At the same time, BPS shows a risk for all age groups only under high consumption scenarios.

It should be noted that this assessment includes only a representative target list, indicating that even in high-contamination scenarios, dietary risks remain limited. However, this assessment follows a deterministic (point-estimate) approach, which does not account for chemical mixtures and potential synergistic effects. Additionally, metabolites and transformation products of the selected CECs may also pose a risk, potentially leading to an underestimation of overall exposure.

4.6.2 Ecological risk assessment

The potential environmental risks posed by each CEC for soil and peat substrate were evaluated. Specifically, under TWW irrigation in lysimeters, RQ values exceeded the threshold for azithromycin and triclocarban, even in samples collected 30 months after the experiment, where the soil was left fallow. The results support concerns about TWW reuse and the increase of antimicrobial resistance genes in soil, which impacts its environmental health. In soil irrigated with spiked media, ten CECs were determined after 30 months from the application and showed high RQ values. It should also be noted that the results

reflect only one growing period, whereas the repeated application of TWW may lead to the gradual accumulation of CECs from one irrigation season to the next, which can affect soil health and the uptake of CECs in plants.

In the case of peat substrate amended with sludge, risks were identified across all treatments. When dried sludge was applied, risks were identified for benzophenone and triclosan. For higher sludge doses, risks were identified for tonalide, triclosan, and triclocarban. Triclosan and triclocarban are both known to persist in sludge-amended soils and may pose a risk to soil health by disrupting soil microbial communities. Interestingly, drying sludge before amendment (a standard treatment practice to meet regulatory and hygienic requirements) resulted in a lower risk compared to the addition of non-dried sludge, where seven compounds: BPA, benzophenone, estrone, ibuprofen, tonalide, triclocarban and triclosan, had an RQ>1.

The current data highlight that both treated wastewater and sludge application may lead to the accumulation of CECs in soil. Another aspect that should be considered in reuse practices is the long-term application of TWW and sludge for consecutive years, which may result in higher concentrations in soil and facilitate the uptake of CECs, a phenomenon that may not be evident through a single growing season. Apart from CECs' persistence in soil after reuse practices, a further concern is the potential drainage of compounds in surface water sources and groundwater, which can be used as a drinking water source and may therefore pose an additional risk to humans.

4.7 Overall Conclusions

The present study has demonstrated that CECs uptake and translocation in tomato plants depend strongly on the cultivation system, the characteristics of the growing matrix, and on individual contaminant physicochemical properties. Across five tomato experiments, uptake and translocation were generally limited under realistic field conditions, with higher accumulation in fruits occurring mainly when high spiking was applied. Specific recalcitrant CECs persisted and were quantified in edible tissues, whereas others appeared only under specific exposure scenarios. Taken together, these results suggest that compounds' ionisation state and hydrophobicity strongly influence their fate in the soil-plant continuum and whether they can accumulate in edible tissues. Degradation and sorption processes in treated wastewater, soil, and sludge seem to limit the fraction that remains bioavailable/bioaccessible for plant uptake. Importantly, dietary exposure estimates showed that while many compounds remained below the risk threshold, specific groups, such as bisphenols, exceeded health-based guidance values when calculated using a conservative approach, using the TDI for BPA. Due to the limitations of deterministic dietary exposure assessment, this study did not evaluate the effect of CEC mixtures on human health. The persistence of several CECs in soils and substrates indicates that the long-term reuse of treated wastewater or sludge can contribute to environmental accumulation, highlighting the need for ongoing monitoring and management when those practices are integrated in agriculture, especially after consecutive applications.

The aforementioned findings **support** the first hypothesis, ***H1: Irrigation of tomato plants with treated municipal wastewater and/or cultivation in sludge-amended substrate results in the uptake and translocation of CECs into plant parts, including tomato fruit, at concentrations measurably higher than in plants irrigated with potable water or grown in non-amended substrate.*** Across all different experiments, multiple CECs were detected in roots, shoots, and fruits at higher concentrations under TWW irrigation or sludge amendment compared to potable water or

non-amended controls. This finding confirms that both treated wastewater irrigation and sludge amendment can lead to CEC bioaccumulation in tomatoes.

The second hypothesis, ***H2: Uptake and translocation of CECs differ between cultivation systems due to variations in exposure pathways, plant-matrix interactions, and compound bioavailability***, is also **supported**, since differences in uptake were observed across different experiments.

The third hypothesis, ***H3: Dietary exposure to individual CECs from tomatoes grown with wastewater irrigation or sludge amendment remains below established risk thresholds for the general population***, is **partially rejected**, since the bioaccumulation of BPS exceeded risk thresholds in hydroponically grown and sludge-amended tomatoes under realistic application scenarios. However, risk calculation was based on the tolerable daily intake for BPA.

The final hypothesis, ***H4: Agricultural reuse of treated wastewater and sludge leads to accumulation of CECs in soils, potentially increasing environmental risks***, is **supported**. In soil (field studies) irrigated with TWW, compounds persisted in the soil, resulting in a high risk for 30 months, while in sludge-amended peat, although CEC concentrations decreased during the experiment, a high risk was still observed. Findings indicate that repeated reuse of wastewater or sludge can contribute to CECs soil loading and long-term risks. Risks can be related to soil health, soil organisms and contamination of other environmental compartments, including surface water and groundwater.

This thesis contributes to our understanding of the uptake and translocation of CECs in tomato plants under various experimental conditions, aiming to facilitate a comparison and identify how different media affect the fate of CECs in plants. Overall, this study strengthens the idea that matrix-specific processes and interactions with CECs are correlated with plant uptake. A deeper understanding of these aspects can help ensure the safe reuse of wastewater and sludge by establishing effective measures, barriers, and monitoring systems. In addition, by providing robust experimental evidence on risks and influencing factors, this work can help increase the visibility and acceptance of wastewater reuse in agriculture in more countries. Ultimately, robust scientific data supporting both benefits and potential risks are essential for informing policymakers, guiding regulations, and building public trust, thereby facilitating broader adoption of safe and sustainable wastewater reuse practices.

Chapter 5

Future Work

The recently renewed Wastewater Treatment Directive [34] will significantly advance the safe and sustainable use of treated wastewater. One of its key priorities is the introduction of quaternary treatment processes designed to achieve >80% removal of indicator CEC from wastewaters. As a result, treated wastewater intended for reuse will contain substantially lower concentrations of pharmaceuticals, personal care products, PFAS, and other emerging contaminants. This regulation emphasises the need to evaluate the effectiveness of enhanced treatment technologies in mitigating the risk of CEC accumulation in crops and soils. Future work should involve testing the effectiveness of quaternary treatment to reduce the risk of emerging contaminants.

In contrast, the Sewage Sludge Directive [42], adopted in 1986, remains in force and establishes limits for heavy metals in sludge and soil, rules for sludge treatment, and regulates the use of sludge in agriculture. However, after nearly four decades, the Directive no longer adequately reflects today's scientific understanding or societal concerns, particularly regarding emerging contaminants and associated risks to human health and the environment. However, to establish a solid basis for revising this Directive, a comprehensive review of existing scientific and monitoring data across Europe will be essential. Such an assessment must identify not only the current state of knowledge but also the critical data gaps that hinder a complete understanding of the risks associated with CECs in sludge application.

A thorough literature review on the occurrence, persistence, bioaccumulation, and toxicity of CECs in European treated wastewater should also be performed with the aim of prioritising indicator contaminants of emerging concern [271]. Given the high number of CECs and the wide range of their physicochemical properties, such prioritisation is essential to focus on compounds most likely to pose risks under agricultural reuse scenarios. This information should be linked to available evidence on their uptake in crops, particularly in edible plant tissues. In the subsequent stage, machine learning can be incorporated into models that can predict uptake behaviour, focusing on contaminant physicochemical properties (e.g., $\log K_{ow}$, pK_a, molecular size, ionisation state, hydrogen-bond donor and acceptor counts) [272], together with relevant soil variables [273] and plant characteristics [274].

Another key aspect concerns the storage and stability of TWW before reuse. Since storage conditions (temperature, sunlight exposure, and storage time) can alter micropollutant concentrations through degradation or transformation, stability and storage tests are necessary to determine how CEC concentrations can be affected and possible formation of degradation products [142]. Additionally, repeated TWW reuse experiments should be considered to assess potential cumulative effects on soils and plants over multiple

growing seasons, including the persistence and remobilisation of CECs and their transformation products.

Future studies should focus on the role of the soil microbiome in modulating the bioavailability of CEC and the influence of TWW. Changes in microbial diversity, enzymatic activities, and functional genes can impact nutrient cycling, soil fertility, and the degradation of organic contaminants, thereby affecting their mobility and availability for plant uptake [275]. Additionally, the presence of CECs themselves may alter the structure and function of soil microbial communities, potentially affecting ecosystem services and the fate of contaminants. Investigating how different microbial communities, soil types, and reuse practices (e.g., TWW, sludge) interact within the plant rhizosphere will provide essential insights for predicting plant exposure, modelling and designing sustainable agricultural practices.

Expanded monitoring is also necessary to include a broader range of relevant micropollutants, such as micro- and nanoplastics, as well as biological contaminants, including antibiotic resistance determinants such as antibiotic-resistant bacteria and antibiotic resistance genes [269]. Moreover, establishing harmonised analytical protocols and globally accessible databases that document compound-specific uptake, distribution, and metabolism across different crop types will improve data comparability and strengthen regulatory decision-making. From a public health perspective, exposure to mixtures of substances must also be considered, including potential synergistic interactions, since co-exposure to CECs, plastic particles, and biological contaminants can result in combined or amplified toxic effects.

Furthermore, the advantages of HRMS should be fully explored in future research on CEC uptake. Non-target workflows and suspect-screening analyses can enhance the detection of substances present in wastewater, thereby expanding our understanding of the chemical landscape within agricultural systems [276]. HRMS can also be leveraged to elucidate CEC metabolism pathways in plants and to identify transformation products, including metabolites that may be more toxic than the parent compounds and therefore pose additional risks to consumers. A complementary approach, such as the use of radiolabelled CEC, could support the identification and quantification of metabolites and provide insights into uptake, translocation, and biotransformation processes [277].

Field-based studies should extend beyond commonly tested vegetables to include a wider variety of crops, such as fruit-bearing trees, cereals, and legumes, to gain a more comprehensive understanding of crop-specific accumulation patterns. Uptake studies should not evaluate only contaminant concentrations in edible tissues, but also impacts on crop quality attributes, such as colouration, soluble solids content, sugars, organic acids, fatty acids, amino acids, carotenoids, volatile organic compounds and polyphenols, which can influence the nutritional value of crops, and consecutively affect marketability and consumer acceptance [278].

In parallel, additional field-based studies should experimentally evaluate scenarios in which TWW is applied alone or in combination with biosolids, to more precisely determine the effects of the CEC source (TWW, biosolids, or their combination) on soil properties, plant uptake, and ecological risk [279]. Focus should also be placed on terrestrial organisms and the effect of TWW reuse for agriculture and irrigation of urban green spaces [20]. This practice can potentially lead to risk through alternative exposure pathways, such as the presence of CEC in nectar and pollen following wastewater irrigation practices, posing a threat towards pollinators and related ecosystems [280].

Finally, assessment of population exposure to CECs through the consumption of wastewater-irrigated crops is crucial, as only limited studies are currently available [281]. Human biomonitoring trials can determine the presence of CECs in commercial fresh products and assess their occurrence in biological samples, such as urine, following ingestion

of TWW-irrigated produce, for example, tomatoes. These evaluations should account for individual medication histories and consider all relevant exposure pathways to provide an accurate assessment of risk.

Equally important is understanding the social dimension of reuse practices, including consumer perception and acceptance of produce irrigated with treated wastewater or amended with sludge. Public acceptance of these practices can be influenced by how information is presented and communicated to consumers [282]. Science communication which highlights safety, sustainability benefits, and alignment with circular economy principles can strengthen public trust. Integrating behavioural and communication studies will be key to ensuring the successful and safe implementation of reuse practices in line with circular economy principles.

Appendix A

Contaminants of Emerging Concern

A.1 General Information on Investigated Compounds

Name	CAS number	Molecular formula	logK_{ow}	pK_a	MW (g/mol)
17α-ethynylestradiol (EE2)	57-63-6	$C_{20}H_{24}O_2$	3.67	10.3	296.4
Acetamiprid	160430-64-8	$C_{10}H_{11}ClN_4$	0.80	0.7	222.6
Amoxicillin	26787-78-0	$C_{16}H_{19}N_3O_5S$	0.87	2.6	365.4
Azithromycin	83905-01-5	$C_{38}H_{72}N_2O_{12}$	4.02	8.5	749.0
Benzophenone	119-61-9	$C_{13}H_{10}O$	3.18	-7.5*	182.2
beta-estradiol (E2)	50-28-2	$C_{18}H_{24}O_2$	4.01	10.4	272.4
Bisphenol F (4,4-BPF)	620-92-8	$C_{13}H_{12}O_2$	2.91	10.8	200.2
Bisphenol A (BPA)	80-05-7	$C_{15}H_{16}O_2$	3.43 - 3.32	9.6	228.3
Bisphenol AF (BPAF)	2467-02-9	$C_{13}H_{10}Cl_2O_2$	4.26	9.2	336.2
Bisphenol S (BPS)	80-09-1	$C_{12}H_{10}O_4S$	1.65	8.2	250.3
Caffeine	58-08-2	$C_8H_{10}N_4O_2$	-0.07	14	194.2
Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	2.45	13.9	236.3
Ciprofloxacin	85721-33-1	$C_{17}H_{18}FN_3O_3$	0.28	6.09	331.3
Clarithromycin	81103-11-9	$C_{38}H_{69}NO_{13}$	3.16	8.99	748.0
Diclofenac as sodium salt	15307-79-6	$C_{14}H_{11}Cl_2NO_2$	4.51	4.15	296.1
Dimethomorph	110488-70-5	$C_{21}H_{22}ClNO_4$	2.63 (E) 2.73 (Z)	-1.3	387.9
Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	3.06	12.4	733.9
Estrone (E1)	53-16-7	$C_{18}H_{22}O_2$	3.13	10.8	270.4
Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	3.97	4.91	206.8
Methylparaben	99-76-3	$C_8H_8O_3$	1.96	8.5	152.1
Naproxen	22204-53-1	$C_{14}H_{14}O_3$	3.18	4.15	230.3
Progesterone	57-83-0	$C_{21}H_{30}O_2$	3.87	18.9	314.5
Propylparaben	94-13-3	$C_{10}H_{12}O_3$	3.04	8.4	180.2
Testosterone	58-22-0	$C_{19}H_{28}O_2$	3.32	18.5	288.4
Tonalide	21145-77-7	$C_{18}H_{26}O$	5.70	16.3	258.4
Triclocarban	101-20-2	$C_{13}H_9Cl_3N_2O$	4.34	12.7	315.6
Triclosan	3380-34-5	$C_{12}H_7Cl_3O_2$	4.76	7.9	289.5

Name	Water Solubility (mg/L)
17α-ethynylestradiol (EE2)	4.8 at 20 °C
Acetamiprid	4.25×10^3 at 25 °C
Amoxicillin	3,433 at 25 °C
Azithromycin	514
Benzophenone	137
beta-estradiol (E2)	3.9 at 25 °C
Bisphenol F (4,4-BPF)	542.8
Bisphenol A (BPA)	120
Bisphenol AF (BPAF)	4,302
Bisphenol S (BPS)	3,518
Caffeine	21,600
Carbamazepine	152
Ciprofloxacin	36,000 at 25 °C
Clarithromycin	0.33
Diclofenac as sodium salt	2.37 at 25 °C
Dimethomorph	49.2 at 20 °C
Erythromycin	2,000
Estrone (E1)	760 at 20 ºC
Ibuprofen	21 at 25 °C
Methylparaben	2,500 at 25 °C
Naproxen	15.9 at 25 °C
Progesterone	8.81 at 25 °C
Propylparaben	500 at 25 °C
Testosterone	23.4 at 25 °C
Tonalide	1.25
Triclocarban	0.11 at 20 °C
Triclosan	10 at 20 °C

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Publications Related to the Thesis

E. Andreasidou, A. Kovačič, L. Manzano-Sánchez, D. Heath, M. Pintar, N. Kacjan Maršič, U. Blaznik, A.R. Fernández-Alba, M.D. Hernando, E. Heath, Contaminants of Emerging Concern in Tomatoes Grown in Sludge-Amended Peat: Uptake, Translocation and Risk Assessment. Toxics, vol. 13, no. 12, p. 1013, Nov. 2025, doi: 10.3390/toxics13121013.

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Submitted and in preparation papers

E. Andreasidou, A. Kovačič, D. Heath, L. Manzano-Sánchez, Š. Železnikar, M. Pintar, N. Kacjan Maršič, U. Blaznik, A.R. Fernández-Alba, M.D. Hernando, E. Heath, Hydroponically Grown Tomatoes in Treated Wastewater: Uptake of CECs and impact on plant growth. To be submitted in Environmental Research (December 2025)

Conference Paper

E. Andreasidou, A. Kovačič, L.A. Koronaiou, L. Manzano-Sánchez, D. Heath, M. Pintar, N. Kacjan Maršič, U. Blaznik, A.R. Fernández-Alba, M.D. Hernando, E. Heath, "Assessing the uptake and risk of Contaminants of Emerging Concern in tomato cultivation using treated wastewater and sludge" in 8th IMEKOFOODS, Food safety and traceability for a sustainable future, Ljubljana, Slovenia, 2025

E. Heath, E. Andreasidou, L.A. Koronaiou, L. Manzano-Sánchez, D. Heath, M. Pintar, Š. Železnikar, N. Kacjan Maršič, U. Blaznik, A.R. Fernández-Alba, M.D. Hernando,

D.A. Lambropoulou, A. Kovačič, “Treated wastewater tomato cultivation in a hydroponic system: uptake and translocation of chemicals of emerging concern” in 19th International Conference on Chemistry and the Environment - ICCE, Belgrade, Serbia, 2025

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N. Kacjan Maršič, A. Kovačič, Š. Železnikar, M. Pintar, E. Andreasidou, A. Vehar, D. Heath, N. Ogrinc, D. Kastelec, U. Blaznik, E. Heath, “The impact of contaminants of emerging concern on the safety and quality of vegetables irrigated with treated wastewater as alternative waster source” in BFestival 2024 : predstavitev raziskovalnih dosežkov Biotehniške fakultete, Ljubljana, Slovenia, 2024

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wastewater reuse: uptake of chemicals of emerging concern and risks" in 18th International Conference on Chemistry and the Environment, Venice, Italy, 2023

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E. Andreasidou, A. Kovačič, D. Heath, E. Heath, "Uptake and distribution of emerging contaminants by crops irrigated with reclaimed wastewater and sludge amendment" in 14th Jožef Stefan International Postgraduate School Students' Conference, Kamnik, Slovenia, 2022

E. Andreasidou, E. Heath, "Uptake of contaminants of emerging concern in tomato plants irrigated with treated wastewater" in Socratic Lectures, 12th International Symposium, Faculty of Health Sciences, University of Ljubljana, 2025

Biography

Education and research

- 2021 – Present: PhD student, Ecotechnologies study programme, Jožef Stefan International Postgraduate School, Ljubljana, Slovenia
- 2019 – 2021: Degree of Postgraduate Studies (M.Sc.) in "Quality Control - Chemical Analysis - Environment" with specialisation in Bioanalysis – Pharmaceutical analysis, Department of Chemistry, Aristotle University of Thessaloniki, Greece
- 2014 – 2019: Bachelor's Degree in Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Greece

Foreign experience

- 2025 (1 week): Department of Environmental Protection and Occupational Safety Engineering, The Academy of Applied Sciences Polytechnic, Belgrade, Serbia.
- 2024 (3 months): University of Almería, Department of Chemistry and Physics, European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables & Experimental Station of Arid Zones, CSIC, Almería, Spain.
- 2024 (2 months): Environmental Pollution Control Laboratory, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Fellowships, Scholarships and Grants

- 2024: Meeting Registration Grant for SETAC Europe Students: SETAC Europe 34th Annual Meeting, 2024, Seville, Spain.

Research projects

- H2020 956365: FoodTraNet - Advanced Research and Training Network in Food Quality, Safety and Security
- Project L7-4422: UPTAKE - Agricultural reuse of wastewater and sewage sludge: Uptake and distribution of contaminants of emerging concern in tomato plant as a model

Awards

- 2024: SETAC Europe 34th Annual Meeting - Science Slam, 1st place, Seville, Spain, 2024
- 2023: 1st place Award in ISO-FOOD Science Slam, 2nd ISO-FOOD Symposium, From Food Source to Health, Portorož, Slovenia, 2023
- 2023: Winner of the Public Engagement Activity Competition, FoodTraNet Summer School 2023, Almeria, Spain, 2023