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From shower to table: fate of organic micropollutants in hydroponic systems for greywater treatment and lettuce cultivation

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ABSTRACT

This study evaluated the dual functionality of hydroponic systems to grow edible crops while treating greywater (GW) containing 20 organic micropollutants (OMPs). Various conditions with differing nutrient contents were tested: raw GW, GW with struvite, and GW with commercial nutrient solution. System performance was assessed with plant growth and standard parameters and OMP removal. After 4-week exposure, all conditions produced healthy-looking plants, proving GW as a viable hydroponic growth medium. However, only the condition with commercial solution yielded plants comparable to the biotic control, indicating the necessity of nutrient supplementation. Effluent from conditions with well-developed plants met the requirements of the European water reuse legislation (EU 2020/741) for scenarios B–D (food crops not in direct contact with the reclaimed water and industrial crops), and had the highest OMP removal, showcasing the effectiveness of the system for OMP treatment. Estimated calculations of OMP detected in leaves (10/20 OMP detected, predominantly positive and small) resulted in calculated potential human health risks through lettuce intake for two compounds: atenolol and epoxycarbamazepine. These findings support a continued evaluation of the behavior of other OMPs and their transformation products in water–plant systems, and their consideration in legislation on water reuse and food safety.

Key words: edible plants, emerging contaminants, endocrine-disrupting compounds, greywater reuse, hydroponics, pharmaceuticals

HIGHLIGHTS

- Hydroponic system successful for greywater treatment and lettuce cultivation.
- Greywater is a viable medium for hydroponics but needed nutrient supplement.
- Effluent complied EU 2020/741 for reuse scenarios B, C, and D.
- Variable and moderate OMP removals, detecting 10 compounds in lettuce leaves.
- Although only atenolol and epoxycarbamazepine posed individual ingestion risks, cumulative exposure risks are expected.

1. INTRODUCTION

The escalating stress on global freshwater resources due to climate change and population growth necessitates a paradigm shift in current water management. Water reuse emerges as a crucial strategy, particularly for agricultural irrigation. Agricultural activities, in fact, are expected to surpass 70% of the total water withdrawals by 2050 (UNESCO & WSSM 2020) while food and water demand are expected to increase by more than 50% by 2050 (Karan *et al.* 2018). However, water reuse implementation remains limited, with a scarce number of countries considering this practice in their legislation. The European Union recently published the legislation (EU) 2020/741 on minimum requirements for water reuse in agriculture. Greywater (GW), defined as the fraction of domestic wastewater (WW) excluding toilet WW, constitutes 50–80% of the total domestic WW load, with daily volumes ranging from 15 to 200 L/person (Oteng-Peprah *et al.* 2018; He *et al.* 2022). GW emerges as an excellent candidate for reuse since it is considered low strength WW, with lower organic and pathogen levels than the total WW load (Oteng-Peprah *et al.* 2018), thus its treatment seems simpler and its reuse safer (Donner *et al.* 2010). Additionally, GW contains valuable nutrients for plant growth, including phosphates and nitrogen compounds (Prodanovic *et al.* 2017), making it an excellent candidate for irrigation (Misra *et al.* 2010). Indeed, studies have shown enhanced nutrient levels in plants irrigated with reclaimed GW compared to tap water (Rodda *et al.* 2011). Consequently, GW application in agriculture may reduce reliance on chemical or commercial fertilizers, the availability of which could be at risk in the near future, and also promotes more sustainable agricultural practices and resource management.

In recent years, on-site decentralized GW treatment and reuse practices have popularized, particularly for non-potable purposes like toilet flushing and irrigation, especially in water deficient areas (Noutsopoulos *et al.* 2018). Among the different options, nature-based solutions (NBSs) have gained importance due to lower operational costs, durability, and co-benefits (Vymazal 2007; Boyjoo *et al.* 2013). These systems have the capacity to achieve high removal rates of a wide spectrum of water contaminants (Ramprasad & Philip 2018) with the combined action of plants (root adsorption and plant uptake), substrate (adsorption), and microorganisms (biodegradation). Notable examples of NBS encompass green roofs, retention ponds, vegetated pavements, or constructed wetlands (CWs). NBSs have shown good performance for GW treatment, with CW among the most popular NBS. The effluent of different CWs (vertical and horizontal flow configurations, and GROW-Green Roof-top Water Recycling System) treating light domestic GW was of comparable quality to other low and high technologies, with removals exceeding 87% for BOD_5, total suspended solids (TSS), and microbial indicators (Williams *et al.* 2008). Similarly, removals over 88% were reported for standard parameters and nutrients (TN, TP) with the GROW system treating GW from a student residence (Ramprasad *et al.* 2017). The vertECO technology (hydroponic system including diverse ornamental plants) achieved removals higher than 90% for most standard parameters from hotel GW while the removal of organic micropollutants (OMPs) was reported to be variable, and greater than 95% for several compounds (Zraunig *et al.* 2019). Additionally, no detriment to the plants was reported in a study testing different CW configurations treating synthetic GW, with COD removals surpassing 87% in all cases (Comino *et al.* 2013). Incorporating edible plants into NBS enhances circularity by simultaneously addressing GW treatment (Gattringer *et al.* 2016; Xu *et al.* 2020) and food production (Barbosa *et al.* 2015; Bliedung *et al.* 2020). Among the diversity of NBS for WW treatment, hydroponic systems (soilless culture) are also widely recognized for their role in food production, by optimizing water and nutrient utilization, resulting in higher yields, less water consumption, and lower specific greenhouse gas emissions compared to conventional agricultural crops in soil (Barbosa *et al.* 2015; Martinez-Mate *et al.* 2018). The versatility of these systems offers a valuable option to promote sustainable water management, particularly in space-constrained urban environments, as they can be installed indoors, outdoors, on vertical surfaces, and other locations for simultaneously treating GW while producing edible goods. Indeed, Sangare *et al.* (2021) obtained 64% greater mass and 60% more leaves in hydroponic lettuce cultivation in raw GW compared to those grown in well water. Nutrient supplementation required for optimal lettuce growth in hydroponics can be provided, at least partially, by GW/WW (Da Silva Cuba Carvalho *et al.* 2018). In parallel, recovering P and N from WW as fertilizers holds significant promise. The application of struvite ($MgNH_4PO_4{\cdot}6H_2O$), a byproduct derived from the precipitation of magnesium, phosphate, and ammonium from WW streams, resulted in similar plant growth and nutrient uptake compared to synthetic fertilizers in hydroponic culture (Carreras-Sempere *et al.* 2021; Halbert-Howard *et al.* 2021; Arcas-Pilz *et al.* 2022). Consequently, struvite can be considered a sustainable fertilizer with the capacity to replace those synthetic in terms of N, P, and Mg. In the case of hydroponics, although nutritional requirements vary among plant species, growth stages and environmental conditions (Resh 2022), the required concentrations generally vary between 140 and 260 mg/L for N, 30 and 60 mg/L for P, and 30 and 50 mg/L for Mg (Trejo-Téllez & Gómez-Merino 2012). Hydroponic systems, hence, can offer a sustainable approach to GW treatment, compared to other conventional treatment options (Magwaza *et al.* 2020), and may benefit from both GW streams and struvite addition for nutrient supplementation, increasing the circularity. Nevertheless, such urban agriculture applications with GW/WW, promoting plant growth and sustainable agricultural practices, are still largely lacking.

Ensuring safe water reuse, especially in applications that directly impact our food supply, is of utmost importance, and therefore efficient WW treatment strategies are required. Remarkably, the ubiquitous presence in water streams of OMPs, such as pharmaceuticals (PhACs), pesticides, endocrine-disrupting compounds (EDCs), or industrial chemicals, is a point of concern for water reuse applications (Verlicchi *et al.* 2023). Also reclaimed WW/GW for irrigation may introduce contaminants in the food chain (Riemenschneider *et al.* 2016). Various studies, in fact, have highlighted the wide array of OMP present in GW (Eriksson *et al.* 2002), at different concentrations (from pg/L to mg/L). Up to 350 OMPs are identified across diverse GW sources, not completely removed during the treatment, and posing potential risks to the environment when released via irrigation (Glover *et al.* 2021). Additional risks to human health arise when plants irrigated with reclaimed water are consumed (Keerthanan *et al.* 2021). Consequently, comprehensive investigation into the occurrence and behavior of OMP in GW is needed, with the goal of integrating them in the water reuse legislations (Gulyas *et al.* 2011). The European Watch List (WL) under the Water Framework Directive is a mechanism aimed at evaluating potential water contaminants, and the risk they may pose to aquatic ecosystems in surface water. The first WL (EU 2015/495) was published in 2015 (European Union 2015) and the last one (EU 2022/1307, European Union 2022) included only 26 compounds of concern. Thus, while serving as a valuable tool for monitoring potentially hazardous compounds in various environmental matrices, it is limited due to the scarce number of considered compounds. Hence, it is necessary to study a broader array of contaminants that may pose risks to both environmental ecosystems and human health.

Some OMP enter the plants through the roots and then are gradually taken up by the shoots and fruits with the transpiration flow (Vo *et al.* 2018; Chuang *et al.* 2019), while their degradation within the plants is attributed to complex biochemical processes (Carvalho *et al.* 2014). Plant-specific characteristics such as species, lipid content or transpiration rates also affect the OMP uptake (Ravichandran & Philip 2021), with leafy crops exhibiting the highest propensity for OMP uptake, followed by root vegetables and cereals (Christou *et al.* 2019b). Additionally, the OMP behavior in the aqueous matrix and their interactions with the plant system are influenced by physico-chemical properties of the OMP, with hydrophobicity (usually expressed as log K_{ow}) considered the most important property (Carter *et al.* 2014). In addition, other properties such as charge and molecular weight (MW) of the OMP have shown to influence their behavior in water and their ability to translocate to edible parts of crops (Goldstein *et al.* 2014; Chuang *et al.* 2019). Therefore, it is of particular importance to study the OMP in hydroponics with greater plant uptake potential due to the absence of soil (Dodgen *et al.* 2013). While numerous studies have explored OMP accumulation in edible plants in hydroponic systems, they have often focused on a limited number of compounds and always using tap or deionized (DI) water with added nutrients (Wu *et al.* 2013; Chuang *et al.* 2018, 2019; Tian *et al.* 2019; Leitão *et al.* 2021b). Previous studies have reported no stress symptoms and adverse effects on plant growth due to OMP exposure (Calderón-Preciado *et al.* 2012; Chuang *et al.* 2019), while others indicated the opposite (Bartha *et al.* 2010; Carter *et al.* 2015) or attributed different effects on the plants depending on the type of OMP (Leitão *et al.* 2021a). On the other hand, the studies with edible plants in raw or treated GW (Eregno *et al.* 2017; Sangare *et al.* 2021) or WW (Da Silva Cuba Carvalho *et al.* 2018; Bliedung *et al.* 2020) have mainly focused on the removal of standard parameters, but have not included OMP. Only Kreuzig *et al.* (2021) evaluated lettuce grown in treated WW, and reported OMP removal rates ranging from 3 to 100%, along with the detection of two out of nine tested compounds in the plant leaves. No studies on GW hydroponic systems for edible plants cultivation and evaluating OMP behavior and removal are available in the literature.

Finally, it is of crucial importance to perform human health risk assessment (HHRA) studies to calculate the potential human exposure to OMP through irrigation, as well as to quantify the potential adverse effects based on the exposure concentrations (Piña *et al.* 2020). In this sense, the exposure to OMP through ingestion of various crops irrigated with raw and treated WW was reported as safe in most cases, with OMP concentrations in edible parts below the acceptable safety thresholds (Carter *et al.* 2014; Hyland *et al.* 2015; Riemenschneider *et al.* 2016; Martínez-Piernas *et al.* 2019). Similarly, studies with edible crops (lettuce, spinach, cucumber, peppers, and collards) grown in hydroponic systems using OMP-spiked DI/tap water with added nutrients have consistently demonstrated negligible risk (Shenker *et al.* 2011; Dodgen *et al.* 2013; Wu *et al.* 2013). However, other studies raised concerns about the potential risks of consuming crops irrigated with reclaimed WW due to the presence of OMP (Bartha *et al.* 2010; Keerthanan *et al.* 2021). Importantly, even though individual OMP exposure is typically considered safe, the risk derived from the exposure to numerous compounds increases because of cumulative exposure (Glover *et al.* 2021). Consequently, more studies considering the potential for plants to grow in reclaimed or raw WW, and particularly GW, as well as the risks associated with the exposure to OMP are thus necessary.

The objective of this study was to evaluate, as a proof of concept beyond the legislation constraints (i.e., cultivation of edible plants in raw GW/WW is currently forbidden), the capability of hydroponic systems to integrate

GW treatment and lettuce production by means of plant growth and contaminant removal. Furthermore, the study aimed to delineate the pathway of OMP from GW to the edible parts of plants, by evaluating their removal in the system and by assessing the associated risks to human related to OMP exposure through the consumption of plants grown in GW.

2. MATERIALS AND METHODS

2.1. Chemicals

2.1.1. Synthetic GW solution

Synthetic GW (modified from Hourlier *et al.* 2010, Supplementary material, Table S1) simulated water typically originated from baths, showers, and wash basins (light GW). All synthetic GW constituents were of reagent-grade quality, and were purchased from Scharlab (L(+)–Lactic acid, $NaHCO_3$, KNO_3, and $(NH_4)_2HPO_4$) or from Merck (glycerol, α-cellulose, $NaC_{12}H_{25}SO_4$, and Na_2SO_4). Struvite ($MgNH_4PO_4{\cdot}6H_2O$), purchased from Merck, was dissolved in approximately 100 mL of GW with around 10 mL of citric acid (1M) until reaching a pH around 3, and it was then mixed with the rest of the GW solution, causing a pH rise to values close to those of real GW (see Table 3 with the characteristics of the GWS influent). The commercial standard nutrient solution (CNS), designed for hydroponic applications, was obtained from *GroHo Hidroponía* (www.groho.es), and included five stock solutions with macronutrients (KNO_3, $Ca(NO_3)_2$, $MgSO_4$, $NH_4H_2PO_4$) and micronutrients (Fe [6%], Cu, $MnSO_4$, H_3BO_3, $(NH_4)_6Mo_7O_{24}$, and $ZnSO_4$).

2.1.2. Organic micropollutants

A selection of 20 OMP (Table 1, including the compounds acronyms) was made based on those commonly found in GW and with different physicochemical properties (i.e., pKa, log K_{ow}, MW). OMP were of analytical quality grade and purchased from LGC Group, except iopromide and venlafaxine, purchased from Merck. Individual stock solutions (1 or 10 g/L) were prepared in methanol and stored in amber glass vials at −20 °C. Caffeine stock (1 g/L) was prepared in milliQ water due to its low solubility in methanol and stored at 4 °C. The OMP mixture was prepared in methanol and spiked into the GW solution (presented in Section 2.2.1.) attaining at

Table 1 | OMPs analyzed in the experiment with their acronyms

Compound	Acronym	Use	Compound	Acronym	Use
Acetaminophen	**ACE**	Analgesics/anti-inflammatory drugs	Atenolol	**ATE**	β-Blocking agents
Diclofenac	**DCF**		Metoprolol	**MTP**	
Ibuprofen	**IBU**		Carbamazepine	**CBZ**	Psychiatric drugs
Indomethacin	**IND**		Desvenlafaxine	**DVLF[a,b]**	
Naproxen	**NPX**		Venlafaxine	**VLF[a]**	
Ofloxacin	**OFX[a]**	Antibiotics	Iopromide	**IOP[c]**	X-ray contrast agent
Sulfamethoxazole	**SFX[a]**		Caffeine	**CAF[c]**	Stimulant
Tetracycline	**TET**		1-Hydroxy-IBU	**1OH-IBU**	TP of IBU
Trimethoprim	**TRI[a]**		2-Hydroxy-IBU	**2OH-IBU**	
Gemfibrozil	**GMF**	Lipid regulators and cholesterol lowering drug	10,11-Epoxycarbamazepine 2OH-Carbamazepine	**EpCBZ** **2OH-CBZ[c]**	TP of CBZ
Ranitidine	**RAN**	Histamine H2 receptor antagonist	Metoprolol Acid	**MTPA**	TP of MTP and ATE
Bisphenol A	**BPA[c]**	Plasticizer	N-Acetyl- SFX	**N-AcSFX**	TP of SFX
Methylparaben	**mPar[c]**	Preservative	N-Desmethyl-VLF	**N-VLF**	TP of VLF

TPs were not spiked.
[a]Compounds included in the last published Watch List (EU 2022/1307).
[b]Also VLF TP and named O-Desmethyl-venlafaxine.
[c]Compounds not analyzed in plant tissues (not included in the analytical protocol).

20 μg/L of each OMP in the GW solution, except for MTP (50 μg/L, due to an error in the individual stock solution). Additionally, all possible transformation products (TPs) of the spiked OMP that are included in the available analytical protocol (Section 2.5) were also searched (Table 1). Further details of the selected OMP are indicated in Supplementary material, Table S2.

2.2. Hydroponic system

The hydroponic laboratory-scale system consisted of modular units, where each module contained four plastic (PVC) rectangular canals (5.5 cm × 8.0 cm × 100 cm), accommodating six lettuces per canal (24 lettuces per module, Figure 1). Plastic netted pots (Ø 5.5 × 4.9 cm height) with light expanded clay aggregates (LECAs) as inert substratum were introduced in the canals to accommodate the plants. Each module was connected to a reservoir on the top of the system, feeding the system by gravity. Abiotic controls were carried out in hydraulically disconnected canals and fed manually (Figure 1). A series of LED light tubes (18W, Osram, cold white and blue + red – full spectrum) were positioned 65 cm above the canals, ensuring uniform light distribution. Three sensors (Hobo® Pendant U23-001A HOBO) placed in different parts of the system recorded relative humidity and temperature at 30-min intervals.

2.3. Experimental setup

Lettuce planters *(Lactuca sativa)*, with 6–9 leaves were acquired from a local store and carefully rinsed with DI water to eliminate any soil particles adhering to their roots before the experiments. The canals were half filled with 1.8 L of water and replaced once a week. The experiment lasted 4 weeks, in line with other studies on hydroponics with edible crops (Mathews *et al.* 2014; Arcas-Pilz *et al.* 2022; Clyde-Smith & Campos 2023). The photoperiod was 14 h/day, in line with previous studies on hydroponics (Benzarti *et al.* 2008; Herklotz *et al.* 2010). The recorded average temperature was 20.1 ± 1.6 °C and the relative humidity was 52.1 ± 6.0%.

Three distinct experimental conditions were investigated, differing in nutrient composition: the baseline condition involving solely GW (GWB), GW supplemented with struvite (GWS), and GW supplemented with the CNS (GWN). Additionally, a biotic control (CTRL) was established, involving the lettuces grown in CNS dissolved in DI water. Abiotic controls (with GW and the netted pots filled with LECA but without plants) were used to assess abiotic processes such as degradation, transformation, and adsorption onto LECA. All system components and LECA were cleaned with soap, bleach, and thoroughly rinsed with DI water before the experiments.

2.4. Plant growth evaluation

Number of leaves and visual appearance of each lettuce were registered weekly, recording wilting, discoloration, number of leaves as well as number of lost leaves, and overall comparison of impression (e.g., leaf thickness, color and physical strength indicated through the leaves ability to hold itself up) between CTRL and GW conditions. Other growth parameters were only obtained at the beginning and end of the experiment (4 weeks) due to the destructive nature of the sampling. For this, two representative plants of each condition were rinsed with DI water, separated into roots and shoots (leaves), and weighted to obtain the wet mass. The leaf area (LA) was

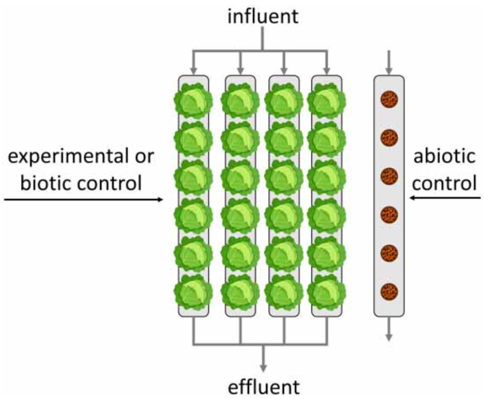

Figure 1 | Diagram of the experimental design for each of the tested GW. Each condition had a four-canal modular unit with six lettuces/canal. A separate canal with the same GW but without plants was used as the abiotic control. Lettuce vector was retrieved from vecteezy.com.

assessed using the app 'Leafscan' (version 2.1.1; updated August 30, 2020). After oven drying at 70 °C for 48 h, the samples' dry mass was obtained. The leaf dry matter content (LDMC; mg/g) and water content (%) were calculated. Additionally, the plant growth was evaluated with plant growth parameters based on the leaf area, mass production, and leaf morphology (Eregno *et al.* 2017; Fraile-Robayo *et al.* 2017; Gent 2017, 2016; Sangare *et al.* 2021):

- Relative growth rate (RGR; g/g*day), indicating the proportionate growth of the plant independent of its initial size.
- Net assimilation rate (NAR; g*cm^2/day), increase in dry matter per unit leaf area, indicating the efficiency of using the produced plant material for photosynthesis.
- Specific leaf area (SLA; cm^2/g leaf dry weight), ratio of the leaf area to the dry weight of the leaves, indicating leaf thickness/density.
- Leaf weight ratio (LWR; g/g), indicating the proportion of leaves to the whole plant and thus the dry weight involved in assimilation.

More details of the calculations and formulas are indicated in Supplementary material, Section S1.

2.5. Sampling and analyses

2.5.1. Liquid samples: analyses of standard parameters and OMP

Influent and effluent water samples from the hydroponic system were taken weekly and analyzed in duplicate, within the same week, for physicochemical parameters: (a) ions (NO_3^-, PO_4^{3-}, SO_4^{2-} Na^+, NH_4^+ and K^+; 10 mL, filtered with nylon syringe filter 0.2 μm) by ion chromatography (ICS 5000 from DIONEX); (b) COD, BOD, TSS, TOC (1 L, unfiltered) according to Standard APHA methods (American public health association *et al.* 2012). Samples for OMP analyses (2 mL, filtered with syringe filter PVDF 0.45 μm, stored at −20 °C until analysis) were analyzed by direct injection into UHPLC-MS/MS. EDCs (methylpraben and bisphenol A) and caffeine were analyzed according to Becker *et al.* (2017), while PhACs were analyzed according to Gros *et al.* (2012). Limit of detection (LOD) and limit of quantification (LOQ) are provided in Supplementary material, Table S2.

2.5.2. Plant samples: analyses of OMP

Immediately after harvesting, the lettuce leaves were rinsed with DI water and freeze-dried for 1 week. The freeze-dried samples were ground with a stainless-steel coffee grinder and stored until analysis (−20 °C). Sample preparation involved extraction by QuEChERS and dSPE PSA-C18 clean-up adapted from Montemurro *et al.* (2020) with more information provided in Supplementary material, Section S2. Matrix-match calibration curves and recovery experiments were performed with the use of laboratory-grown lettuce, free of micropollutants. The analyses of PhACs were performed by UHPLC-MS/MS according to Castaño-Trias *et al.* (2023). Recovery values in the lettuce matrix (%), as well as LOD and LOQ are provided in Supplementary material, Table S2.

2.6. Statistical analyses

The coefficient of determination (R^2), calculated with Microsoft Excel, was used to evaluate linearity between plant growth (expressed as number of leaves) in relation to pollutant/nutrient removal or water loss per week. Then, IBM-SPSS 28.0 software package was used to perform Principal Component Analysis (PCA) on the growth parameters obtained at the beginning and end of the experiments. The differences in number of leaves were assessed through univariate with a between subjects' analyses, as they were recorded weekly. The differences between the OMP removals across conditions were analyzed through a univariate Generalized Linear Model with a between subjects' factor. Further details and formulas are indicated in Supplementary material, Section S3.

2.7. Human health risk assessment

The target group for the risk assessment was the European adult population with an average body weight of 70 kg. The Hazard Quotient (HQ) was determined by comparing the estimated daily intake (EDI) of the OMP when consuming 50 g of the produced lettuce leaves (Eregno *et al.* 2017), over a reference value, that represents an exposure level at which no adverse health effect is expected. In this study, the reference values were generated from the lowest daily therapeutic dose (LDTD) for the PhACs (applying a literature safety factor (SF) or a default SF by Snyder *et al.* (2010), or the threshold of toxicological concern (TTC, Kroes *et al.* 2004) in the case of TPs. If

TTC was not available for the TP, the parent compound TTC was used for indicatory evaluation since TP potentially express higher toxicity than the parent compound. A HQ > 1 suggests that there is a potential risk to human health through the consumption of the produced lettuce leaves, thus more detailed toxicological studies would be required (e.g., De Santiago-Martín *et al.* 2020; Margenat *et al.* 2020; Tadić *et al.* 2021). Applying the state-of-the-art additivity assumption (NRMMC 2008), the combined exposure to all analyzed compounds was assessed with the Hazard Index (HI), which is the sum of the HQ of each compound. In case the concentrations exceeding the range of the available calibration curves (i.e., for DVLF, ATE, MTP, VLF, MTPA, and EpCBZ), the corresponding highest calibration curve points, different for each of the compounds, were used for calculating the risk assessment. The obtained values represent the minimum level of exposure (i.e., the lower bound of the compounds' concentration in the leaves) and used to calculate the minimum risk for each of the compounds. Thus, the presented results gave an idea of potential risks although the real accumulated concentration must be assumed to be higher. More detailed explanations, formulas and values are provided in Supplementary material, Section S4.

3. RESULTS AND DISCUSSION

3.1. Plant development

3.1.1. Plant development and nutrient uptake over the experiment

All lettuces (24 per condition) survived the 4-week experiment with a healthy appearance at harvest (see Supplementary material, Figure S1). The plants grown with conventional nutrient solution (i.e., GWN and CTRL) produced healthy looking leaves (i.e., no extraordinary discoloration/rotting, self-supporting physical strength) and thin, light-colored roots, resembling those cultivated in hydroponics using standard nutrient solutions (Lei & Engeseth 2021). In contrast, plants growing in GW or GW supplemented with struvite (GWB and GWS) produced smaller, but thicker leaves (see Chapter 3.1.2) and visibly thicker roots, a strategy known to enhance nutrient uptake (Vaillant *et al.* 2004; Eregno *et al.* 2017) as the available nutrients were probably not sufficient in GWB and GWS compared to GWN. Likewise, Da Silva Cuba Carvalho *et al.* (2018) reported the potential of WW to achieve comparable lettuce growth and nutrient absorption to tap water supplemented with fertilizers, but they also indicated that lettuces grown in WW without nutrient supplementation failed to meet market standards. Yellowish discoloration of a single leaf occurred in week 4 of some GWB and specially GWS plants, probably indicative of stress resulting from salinity and pollutant exposure (García-Valcárcel *et al.* 2016; Ramprasad & Philip 2018).

The number of leaves increased in all conditions, with 8, 6, 15, and 20 leaves produced by the end of the experiment for GWB, GWS, GWN, and CTRL, respectively. Statistical analyses (p-value < 0.01) revealed significant differences between number of leaves across all treatments, in line with Ramprasad & Philip (2018), who reported slightly more leaves in the control of *Phragmites australis* grown in hydroponics with nutrients and GW constituents (sodium dodecyl sulfate, propylene glycol, and trimethyl amine). Similarly, Rababah & Al-Shuha (2009) reported typical lettuce growth in WW effluent with nutrient supplementation in hydroponics, although control lettuces exhibited greater size, like it was observed for GWN in comparison with CTRL. Regarding the influence of the presence of OMP in water on plant development, the existing literature is not conclusive, since some studies reported negative effects (Bartha *et al.* 2010; Carter *et al.* 2015), while others did not report any effect (Calderón-Preciado *et al.* 2012; Chuang *et al.* 2019). It is hypothesized that the plants were more affected by other GW constituents (Misra *et al.* 2010), with a much higher concentration than OMP (30–200 mg/L vs. 0.02 mg/L, Supplementary material, Table S1). Furthermore, the tested OMP concentration in this study was lower than in the previously mentioned studies (20 vs. 50–100 μg/L). Although the GWN lettuces grew slightly less, their development and appearance were comparable to the control. This result places GW as a suitable medium for irrigation, particularly in decentralized systems and in water scarcity scenarios. Alternative irrigation resources are essential to create more resilient and circular systems, and the use of GW with adequate nutrient supplementation can help reduce that pressure while increasing food security. However, ideally, irrigation with GW should be able to be combined with freshwater to diminish the negative effects of its constituents on plant development.

The relationship between nutrient removal and water loss (evaporation and transpiration) exhibited linearity with the number of leaves and nutrient removal for GWN and CTRL (Figure 2), indicating enhanced nutrient and water uptake to support plant growth. In contrast, no linearity was found for GWB and GWS, as these lettuces showed poorer growth. Interestingly, while nutrients in GWB and GWS were scarce, they were not

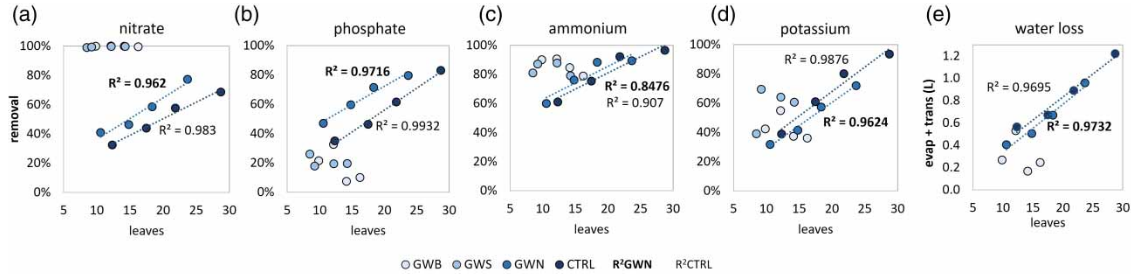

Figure 2 | Relation of the number of leaves with the nutrient removal (nitrate, phosphate, ammonium, and potassium: a, b, c, d, respectively) and the water loss (evaporation (evap) and transpiration (trans), e).

completely depleted (except for nitrogen), and although the number of leaves increased in these conditions, the relation with the nutrient and water uptake was not linear like in the other conditions, indicating inadequate growth. Additionally, the presence of sodium might had affected plant growth. Sodium concentration in this study (around 38 mg/L, Table 3) was similar in all the conditions and below the reported toxicity levels of 50 (Raval & Koradiya 2016) or 87 mg/L (Da Silva Cuba Carvalho *et al.* 2018), but early stage plants might be more sensitive. Literature indicates that the presence of sodium can stimulate the growth of roots due to salt stress, leading to lower water content and fresh biomass as well as darker color of the leaves (Bartha *et al.* 2015). Hence, the darker color of GW lettuces as well as the poor growth and thick roots of GWB and GWS can be related to both the lack of nutrients (especially N) and the stress induced by the salts present in the GW. Particularly, sodium can inhibit the plant uptake of potassium (of similar size and charge) because of competitive uptake (Vairavan *et al.* 2007). The obtained results are in line with a previous study on cucumbers grown in hydroponics with treated GW amended with nitrified urine and nutrient supplementation, where slightly lower growth was obtained compared to the control, and it was attributed mainly to the presence of sodium (Wdowikowska *et al.* 2023).

3.1.2. Plant growth assessment

A holistic interpretation of the plant growth indicators reduces possible misinterpretation of results. A consistent pattern emerged in terms of the calculated parameters (RGR, NAR, and LWR). The smallest values were observed for GWS, closely followed by GWB, with GWN exhibiting markedly higher values, while CTRL displayed slightly lower values (Table 2). Specifically, RGR (indicative of plant material increase over time) were similar for GWN and CTRL (grown with the same nutrient solution), while GWS and GWB lettuces did not receive additional nutrients and grew similarly (Table 2). The RGR of CTRL and GWN plants were in line

Table 2 | Plant growth parameters (leaf fresh and dry weights, # of leaves, and leaf area refer to the values measured at the end of the experiment), average, and standard deviation

Parameter	Unit	GWB	GWS	GWN	CTRL
Leaf fresh weight	g	2.7 ± 0.7	3.4 ± 0	25.0 ± 0.5	24.8 ± 4.7
# of leaves		18 ± 1.4	13 ± 0	23.5 ± 0.7	27 ± 0.0
Leaf area	cm^2	112.1 ± 25.9	149.7 ± 2.1	990.5 ± 22.4	1,055.9 ± 124.9
Leaf dry weight	g	0.3 ± 0.1	0.4 ± 0.0	1.4[a]	1.1 ± 0.2
Root dry weight	g	0.09 ± 0.02	0.12 ± 0.03	0.18 ± 0.0	0.16 ± 0
LDMC	mg/g	124.8 ± 3.5	102.2 ± 0.6	57.3[a]	43.5 ± 0.3
Water content in leaves	%	87.5 ± 0.4	89.8 ± 0.1	97.1[a]	95.6 ± 0.0
RGR	g/g/d	0.04 ± 0.01	0.03 ± 0.0	0.07[a]	0.06 ± 0.0
NAR	$mg/cm^2/d$	0.14 ± 0.03	0.10 ± 0.01	0.16[a]	0.10 ± 0.02
SLA	cm^2/g	344.9 ± 0.2	349.1 ± 3.1	492[a]	665.4 ± 30.7
LWR	g/g	0.78 ± 0	0.77 ± 0.02	0.84[a]	0.76 ± 0.0

[a]Standard deviation not included as there was a mistake on the measurement of the dry weight of one of the lettuces of GWN, thus only the value of one sample was used to calculate the parameters.

with the literature for medium sized lettuces grown in hydroponics under varying temperature conditions (Gent 2016). Regarding the morphology, all GW plants yielded thicker and denser leaves, as indicated by their lower SLA compared to CTRL (Table 2). The NAR evidenced that the GWN condition produced the highest dry weight, using its available dry mass more efficiently for growth and maintenance. This is further underscored by LWR, with the GWN condition displaying a higher proportion of leaves to roots most likely caused by the more abundant access to essential nutrients compared to CTRL (Hopkins 2009; Eregno *et al.* 2017). On the other hand, GWB or GWS produced fewer and smaller but thicker leaves than GWN, which is a typical morphological change characteristic of plants adapting to resource-poor conditions (Eregno *et al.* 2017). This observation is supported by the higher LDMC, lower water content (95–97% for CTRL and GWN, 87–90% for GWS and GWB), and lower SLA.

PCA applied to the initial values of the plant growth assessment (Supplementary material, Table S3) extracted three components, collectively representing 97% of the variance. Most parameters clustered on the first component (where fresh weight was the parameter with the higher factor loadings), displaying strong correlations among them, while number of leaves and LDMC fell on different components and therefore were not redundant (Supplementary material, Table S3). Therefore, the general linear model with repeated measures design was employed focusing only on these three components (total fresh weight, number of leaves, and LDMC). Tests of within-subjects effects highlighted that time was always the most significant factor for variability (Supplementary material, Table S4). The treatment (condition*time) was significant because there were different time effects (i.e., observed more growth or less).

For the first component (fresh weight), nutrient content revealed two distinct trends: plants growing with commercial nutrient solution (GWN and CTRL) exhibited similar results, while plants with lack of nutrients (GWB and GWS) yielded significantly lower estimated marginal means, displaying similarities between themselves (Supplementary material, Figure S2(a)). This emphasizes that nutrient content significantly influenced plant growth and weight. For the second component (LDMC, Supplementary material, Figure S2(b)), significant differences emerged only between GWB and CTRL, at the end of the experiments, and all conditions exhibited decreased LDMC at the end of the experiment, except for GWB. Regarding the third component (leaves, Supplementary material, Figure S2(c)), there were evident differences among all conditions. Nevertheless, the second and third components displayed greater variations among conditions, suggesting that they were less influenced by nutrient content and possibly affected by GW and/or the presence of OMP.

Limited studies have reported successful struvite application for the hydroponic growth of tomatoes (Carreras-Sempere *et al.* 2021; Halbert-Howard *et al.* 2021) and lettuce (Arcas-Pilz *et al.* 2022; Mendoza *et al.* 2023), although it was blended with other fertilizers. Struvite, in fact, is a sustainable fertilizer but rarely introduced in the literature. In this study, struvite application was not successful and the growth of the lettuces from GWS was comparable to those from GWB rather than GWN (Table 2). This could be attributed to the low water solubility of struvite, requiring pH values below 4 for near complete dissolution (Carreras-Sempere *et al.* 2021), while higher pH was applied in the current study (see GWS influent, Table 3), which exceeded 6 after 48 h, suggesting struvite reprecipitation, potentially reducing its availability to the plants. Carreras-Sempere *et al.* (2021) dissolved the struvite with HNO_3, also contributing to NO_3 input, while Arcas-Pilz *et al.* (2022) applied struvite directly in water, resulting in 50–70% undissolved struvite. In our study, struvite was dissolved with citric acid to investigate whether the Mg, P, and NH_4 supplied by struvite, along with nutrients in the GW could sustain lettuce growth. Dissolving it in HNO_3 would have led to a better nutrient balance but disguising the effects of struvite. The calculated struvite amount was based on the PO_4 content in the CNS, and successful dissolution was confirmed by PO_4 concentrations in the influent control, closely resembling those in GWS (Table 3). Notably, TSS and turbidity in the GWS effluent were higher compared to other GW conditions, possibly due to struvite precipitation, and the addition of citric acid elevated the carbon content, resulting in higher COD and TOC influent values in GWS compared to the other two GW conditions. These factors could have had a potential negative impact on plant development.

3.2. GW treatment

3.2.1. Main GW constituents and nutrients

The removal of TSS, TOC, and Na was constant with minimal weekly variability (see small standard deviation values in Table 3, in contrast to Sangare *et al.* (2021), who reported reduced removal efficiencies over time due to the accumulation of pollutants in plant tissues. Linear correlation was not found between TSS, TOC, or

Table 3 | Concentration of the standard parameters and nutrients present in the different conditions and their removal

	influent concentration, mg/L				effluent concentration, mg/L				removal, %			
	GWB	GWS	GWN	CTRL	GWB	GWS	GWN	CTRL	GWB	GWS	GWN	CTRL
EC (μS/cm)	324.3 ± 9.1	461.3 ± 8.7	2017.5 ± 15.5	1703.8 ± 47.5	223.5 ± 17.7	326.1 ± 19.9	1707.8 ± 105.8	1607.6 ± 157.3	–	–	–	–
pH	7.1 ± 0.4	4.8 ± 0.2	6.2 ± 0.2	5.6 ± 0.3	7.0 ± 0.1	7.0 ± 0.2	6.9 ± 0.4	5.4 ± 0.5	–	–	–	–
Turb	1.2 ± 0.5	5.4 ± 2.7	5.3 ± 1.4	3.7 ± 0.9	6.0 ± 3.0	15.3 ± 4.1	4.6 ± 3.7	3.5 ± 3.1	–	–	–	–
COD	594.0 ± 34.2	789.7 ± 93.1	628.9 ± 31.0		65.0 ± 9.0	116.5 ± 10.6	49.3 ± 27.3	65 ± 9.0	90.7 ± 1.1	88.1 ± 1.9	95.4 ± 2.5	
BOD	309.5 ± 31.8	346.0 ± 19.9	318.3 ± 24.4		25.4 ± 7.3	60.2 ± 10.8	22.5 ± 9.0		93.1 ± 1.6	86.0 ± 3.0	95.9 ± 0.7	
TOC	180.7 ± 7.6	237.8 ± 11.7	162.1 ± 3.7	14.4 ± 1.5	26.6 ± 2.9	34.5 ± 4.4	14.3 ± 4.8	17.6 ± 7.2	87.8 ± 1.7	88.3 ± 1.2	94.6 ± 1.1	41.9 ± 3.5
TSS	52.6 ± 12.8	68.8 ± 25.8	66.0 ± 12.8		12.3 ± 4.5	20.5 ± 7.4	15.2 ± 17.2		80.4 ± 6.6	75.4 ± 7.4	87.5 ± 10.1	
Na	38.3 ± 0.8	38.0 ± 0.2	37.2 ± 0.2		39.5 ± 1.7	40.2 ± 1.4	50.9 ± 8.2	12.9 ± 4.7	14.7 ± 8.8	14.6 ± 3.3	14.5 ± 7.3	
$N\text{-}NH_4$	21 ± 1.0	25.3 ± 0.7	43.4 ± 0.5	29.7 ± 0.4	3.5 ± 1.3	5.2 ± 1.5	13.8 ± 6.3	9.2 ± 5.8	86 ± 5.6	83.7 ± 4.2	78.4 ± 13.4	81.2 ± 15.2
K	11.9 ± 0.3	12.2 ± 0.1	191.2 ± 3.8	196.9 ± 3.5	8.2 ± 1.7	6.3 ± 2.3	144.3 ± 28.7	106.5 ± 55.8	42.5 ± 13.4	58.2 ± 14.8	50.5 ± 16.9	68.2 ± 22.1
$N\text{-}NO_3$	4.4 ± 0.1	4.8 ± 0.2	174.0 ± 5.6	168.4 ± 5.1	0.1 ± 0.0	0.1 ± 0.0	117.2 ± 28.2	158.1 ± 9.8	99.8 ± 0.2	99.4 ± 0.3	55.8 ± 15.6	50.6 ± 14.6
$P\text{-}PO_4$	18.5 ± 0.5	35.4 ± 0.4	57.1 ± 1.5	38.9 ± 1.1	18.4 ± 1.3	34.8 ± 1.7	30.9 ± 7.7	30.5 ± 6.4	17.8 ± 10.8	20.6 ± 3.7	64.4 ± 14.0	56.4 ± 19.3
$S\text{-}SO_4$	10.8 ± 0.3	17.2 ± 12.8	58.5 ± 1.9	56.0 ± 8.4	16.8 ± 0.8	16.4 ± 0.5	84.3 ± 14.5	90.9 ± 44.3	0 ± 0	31.7 ± 36.7	10.1 ± 5.5	22.1 ± 9.3

Shaded cells correspond with the parameters used to evaluate the effluent's compliance with the requirements of the different reuse scenarios of EU 2020/741.

Na removal and plant growth (number of leaves), suggesting that increased plant growth did not necessarily enhance pollutant uptake. Similar removals of TSS and organics were observed for abiotic controls (Supplementary material, Table S5) due to abiotic degradation and/or adsorption onto the LECA and, thus, the uptake by the plants is estimated to be minimal. High BOD, COD, TSS, and TOC removal were achieved in all conditions (85–96%, Table 3), in line with studies on hydroponics cultivation of honeysuckle with the same GW recipe of this study (Xu *et al.* 2020) and of lettuce with raw dishwasher GW, reporting also high nitrate and ammonium removals (Sangare *et al.* 2021). In contrast, sodium removal remained consistently low and uniform (Table 3), in line with Ramprasad & Philip (2018), reporting 20% sodium removal after a 35-day hydroponic experiment with *P. australis*, primarily accumulating in the roots through adsorption. Additionally, sodium dodecyl sufate, (typical GW constituent applied also in this study) is considered recalcitrant (Ramprasad & Philip 2018), hence high sodium removal was not expected. Comparable BOD and TSS removals were reported in various types of CWs treating GW (Williams *et al.* 2008; Ramprasad *et al.* 2017; Zraunig *et al.* 2019). Furthermore, obtained nutrient removals (i.e., N and P) from raw GW without extra supplementation (i.e., GWB condition) were in line with the results obtained in previous studies on hydroponic systems with edible plants for WW treatment, gathered in a recent review (Mai *et al.* 2023), hence confirming the efficacy of the proposed system.

3.2.2. Fate and removal of OMP

3.2.2.1. OMP removal in the system. The OMP removal over the 4-week period in the systems with plants displayed considerable variability across all conditions, leading to removals from 0 to 100% (Figure 3(a)). The average weekly removal was 62, 49, and 65% for GWB, GWS, and GWN, respectively, confirming the literature on hydroponic systems or other types of CWs for the treatment of WW (Kahl *et al.* 2017; He *et al.* 2018; Wolecki *et al.* 2019) or GW (Zraunig *et al.* 2019). The average OMP removals in the corresponding abiotic controls for GWB, GWS, and GWN were 51, 46, and 47%, respectively (Supplementary material, Table S5), indicating that OMP were removed through abiotic processes (adsorption onto LECA) or experienced abiotic degradation (e.g., hydrolysis or oxidation-reduction), as already reported (Dodgen *et al.* 2013; Bartha *et al.* 2015; Zhang *et al.* 2016). Therefore, the presence of plants improved the removal of most OMP to some extent, as generally reported in the literature (Dodgen *et al.* 2013; Chuang *et al.* 2019), but in contrast with Cardinal *et al.* (2014). GWB and GWN exhibited similar OMP removal patterns although GWN had a higher median and slightly higher average than GWB (Figure 3(a)), suggesting a removal efficiency improvement with healthier and more robust plants, consistent with previous reports (Dodgen *et al.* 2013; Chuang *et al.* 2019). Conversely, GWS presented the lowest OMP removal rates, despite having similar lettuces to GWB (Supplementary material, Figure S1, Table 2). Hence, not only plant development, but other particular characteristics of the GWS condition (higher turbidity and greater organic content, as previously mentioned, Table 3) resulted in a more complex matrix that could hinder the interactions between the OMP and the system and, consequently, decreasing their removal.

Highest removal (>90%) was recorded for RAN, OFX, TET, ACE, mPar, and BPA, while DCF, DVLF, VLF, and TRI showed the lowest removals (<30%), and the remaining compounds intermediate removal (Figure 3(b)). It should be highlighted, in fact, that very few studies are available in regarding some of the here considered OMP in hydroponics and/or GW. Comparing to literature, CBZ was removed to a greater extent than reported in

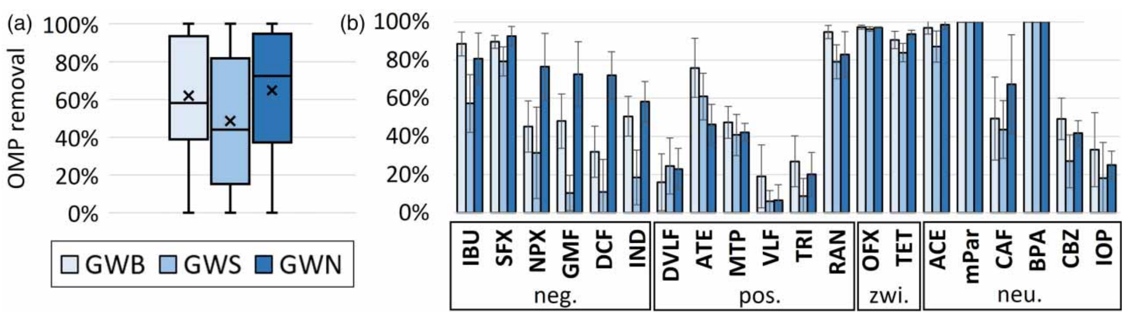

Figure 3 | OMP removal, average per condition (a) and individual removal (b). Compounds in (b) are ordered from lowest to highest MW in each of the groups. Below the compounds, the corresponding ionizable form at solution pH (7): negative (neg.), positive (pos.), zwitterionic (zwi.), and non-ionizable/neutral (neu).

studies with CW with non-edible plants (Kahl *et al*. 2017; Ravichandran & Philip 2021). Both CBZ and CAF removals (around 50%) are in line with Chuang *et al*. (2019), who evaluated their uptake by hydroponically grown lettuces. IBU removal (60–80%) happened to a lower extent than in hydroponic systems with non-edible plants (Zhang *et al*. 2016). It is worth mentioning that due to an error, MTP concentration in the synthetic GW was even higher than the average spiked (around 50 μg/L vs 20 μg/L) and thus the concentrations found in the effluent as well as the possible metabolization might be higher than with other compounds due to this higher concentration. Precisely, the intermediate removal for ATE and MTP was lower than the only available study considering these compounds (31–35% in hydroponic systems with *Iris pseudacorus*, Brunhoferova *et al*. 2021).

The removal of certain OMP remained consistent across various matrices and plant growth stages. There were not statistically significant differences on the removal of ten OMP (SFX, DVLF, MTP, VLF, TRI, ACE, mPar, CAF, IOP, and mPar) among the three experimental conditions ($p < 0.05$, Supplementary material, Table S6). This implies that their removal was not influenced by differences in the tested solutions (nutrient concentration, salinity, turbidity, carbon content, etc.) nor by plant development (no linearity between leaves and OMP removal). On the other hand, the removal of some OMP was influenced by the condition. Pairwise comparison (Supplementary material, Table S6) indicated significant differences in OMP removal for five compounds (i.e., DCF, NPX, RAN, TET, ATE) between GWB and GWN, with higher removals for DCF, NPX, and TRI in GWN, and for RAN and ATE in GWB (Figure 3(b)). GWB presented significant differences in OMP removal for more compounds with GWS (eight compounds) than with GWN (five compounds), despite having similar plants as GWB. Other issues, as mentioned before, might have influenced plant development and OMP removal in GWS.

On the relation between the removal of OMP and their properties, no direct linear correlation was observed with log K_{ow}, MW, or pKa, implying that OMP removal was not determined by a single property but by a complex interplay of factors. Neutral OMP with higher propensity of diffusion through plant cells (Chuang *et al*. 2019; Ravichandran & Philip 2021) were in general removed to a greater extent than charged compounds. Zwitterionic OFX and TET were highly removed also in the abiotic controls (Supplementary material, Table S5), indicating their degradation could be attributed to abiotic processes or adsorption onto the LECA. Compounds of smaller size and negative charge (IBU, SFX) demonstrated superior removal compared to larger ones, and the most pronounced differences between GWN and other conditions were observed for hydrophobic compounds, most likely due to the interactions with the roots.

The TPs of CBZ (i.e., EpCBZ and 2OH-CBZ) along with 1OH-IBU, N-AcSFX, and N-VLF were generally not detected in the effluent samples, and only 2OH-IBU was found at low concentrations (<4 μg/L), suggesting that substantial degradation of the parent compounds did not occur in the aqueous solution, although the formation of other not analyzed TP cannot be excluded.

3.2.2.2. OMP uptake by lettuces. Ten out of the 20 analyzed compounds in lettuces leaves were either not detected or were below LOD or LOQ limits. The remaining compounds, including DVLF, ATE, MTP, VLF, MTPA, and EpCBZ, were frequently detected above the highest point of the respective calibration curves (Table 4). It is possible that the high concentrations of MTP and MTPA found in lettuces leaves were related with the higher spiked concentration of MTP in comparison with the rest of OMP of this study. The other compounds (i.e., IBU, TRI, OFX,) ranged from 0.02 to 0.87 μg/g dry weight (dw, Table 4). Correspondingly, Wu *et al*. (2012) detected 12 out of 20 OMP in lettuces leaves grown in hydroponic solution without detecting IBU, SFX, NPX, and DCF. Similar levels were reported by Kreuzig *et al*. (2021) for lettuce grown in treated WW (0.032–0.135 μg/g for ACE, CBZ, and DCF). In contrast to this study, where no NPX was found in leaf

Table 4 | OMP concentrations in lettuce leaves, μg/g dw

	Condition	IBU	DVLF	ATE	MTP	VLF	TRI	OFX	MTPA	EpCBZ
μg/g dry weight (dw)	**GWB**	<LOQ	>0.51[a]	>1.01[a]	>0.82[a]	>1.28[a]	0.87 ± 0	<LOQ	>0.99[a]	0.84 ± 0.06
	GWS	<LOQ	>0.51[a]	>1.01[a]	>0.82[a]	>1.28[a]	0.74 ± 0.04	0.08 ± 0.01	0.58 ± 0.03	>1.06[a]
	GWN	<LOD	>0.51[a]	>1.01[a]	>0.82[a]	>1.28[a]	0.41 ± 0.01	0.17 ± 0.05	0.35 ± 0.03	1.03 ± 0.01

Not detected: NPX, GMF, DCF, IND, TET, ACE, 1OH-IBU, 2OH-IBU, N-AcSFX, N-VLF. SFX and RAN were analyzed but not recovered.
[a]Compound detected at concentrations exceeding the range of its available calibration curve.

tissue, its accumulation in lettuce tissue in hydroponic culture was reported elsewhere (Calderón-Preciado *et al.* 2012). While CBZ and SFX were not found in lettuce leaves of this study, they were commonly detected in previous studies (Herklotz *et al.* 2010; Chuang *et al.* 2019; Manasfi *et al.* 2021).

Although results of this study show very high removal for the most studied antibiotics (Figure 3(b)), they were either non-detected or detected at low concentrations within the leaves (Table 4), most likely because they tended to accumulate in the roots, where usually higher OMP concentrations are found (Chuang *et al.* 2019). In this line, hydrophobic DCF and NPX (log $K_{ow} = 4.51$ and 3.18, respectively), were removed to a significantly greater extent with well-developed plants (GWN) compared to other conditions, despite not being detected in leaf tissues, and not being removed in the abiotic control (0 and 4.6% removal for DCF and NPX, Supplementary material, Table S5). Their hydrophobic nature lead to their accumulation in the roots, with more lipid content than other plant tissues (Dodgen *et al.* 2013; Christou *et al.* 2019a). In contrast, although positively charged compounds did not exhibit overall superior removal, some of them (ATE, MTP, MTPA, VLF, and DVLF) were detected at higher concentrations in lettuce leaves, due to their greater potential to be transported with the transpiration stream (Ravichandran *et al.* 2021). Indeed, high translocation potential of some positive compounds, including MTP, was reported after being detected in all samples from lettuces grown in soil irrigated with WW (Manasfi *et al.* 2021).

As regards to TPs, they are relevant to be considered in treated water as well as in terms of plant uptake in future studies. MTPA, a metabolite of both ATE and MTP, was not spiked in the influent, but detected in both lettuce leaves (Table 4) and the effluent (average 9.5 μg/L and up to 22.9 μg/L), confirming ATE and/or MTP transformation into MTPA (Rubirola *et al.* 2014). The other TP of concern in this study is EpCBZ, detected in all lettuce samples, exceeding the calibration curve in two of the tested conditions (Table 4). As indicated previously, its parent, CBZ, is typically detected at high concentration in leaves, but it was not detected in this study. In this case, since EpCBZ was not found in any effluent sample, it is postulated that CBZ underwent metabolization within the plant, as previously reported (Kodešová *et al.* 2019). No studies were found evaluating the uptake of VLF and DVLF in edible plants, and it is important to note that DVLF is a pharmaceutical, but also a TP from VLF, and for this reason the possible metabolization cannot be ensured. Both compounds (VLF and DVLF) were spiked because they are present in the last published WL and because there are scarce studies evaluating them. Only Petrie *et al.* (2017), who spiked different concentrations of several OMP, including VLF and DVLF, found VLF in *P. australis* at concentrations up to 50 ng/g dw, while DVLF was detected at very low concentration (usually < LOQ).

To summarize, OMP can follow diverse pathways from GW to the edible parts of the plants as a function of the characteristics of OMP, plant type and growth medium. Results from this showed no linear relation between the removal of contaminants and their properties, although it must be taken into account that more contaminants have been evaluated than in other studies, and that some trends can be observed. Smaller OMP, as well as hydrophobic ones, were eliminated to a greater extent, while most of the compounds detected in high concentrations in the leaves were positively charged. This study holds significant importance as it sheds light on the concentrations in edible plant tissues of certain OMP which have seldom been investigated or remain unexplored, particularly VLF, DVL, and MTP, detected here at high concentrations.

3.2.3. Effluent quality for reuse applications

The reuse potential of the system effluent was assessed according to the European Union's reuse legislation (EU 2020/741), considering all parameters (i.e., turbidity, TSS, and BOD) except for microbiological indicators (synthetic GW without bacteria). In any case, previous research on NBS for WW treatment and reuse reported the need for an additional disinfection step to meet legislative criteria when scaling up these systems and with real GW (Winward *et al.* 2008; Arden & Ma 2018). EU 2020/471 stipulates four scenarios for the use of reclaimed water for irrigation. GWN met the turbidity limit of class A (5 NTU), however both BOD and TSS limits only met the limits suitable for classes B to D (crops not in direct contact with the reclaimed water and industrial crops). The remaining conditions also met the TSS limits for scenarios B to D (35 mg/L) and although GWB effluent approached the BOD standards for these scenarios (25 mg/L, Table 3), they did ultimately exceed the required limits for turbidity and BOD. Consequently, only the effluent from GWN had enough quality for reuse regarding the European legislation, for scenarios B to D, whose TSS and BOD requirements are the same, and the difference lies in the concentration of *E. coli*, but this parameter was not included in this study. These findings underscore that the success of the GW treatment system falls on the optimal growth of plants, which enhances removal processes and consequently results in compliance with existing legislation. Prior studies confirmed effluent from NBS for GW treatment complied with Spanish legislation (Zraunig *et al*. 2019) and with USEPA standards for reuse (Ramprasad & Philip 2018).

On the effluent quality regarding OMP, attention should be paid to those compounds with lower removals as well as those that carry greater risks for human and environmental health. Antibiotics are of special interest due to the potential for contaminated vegetables to foster antibiotic-resistant pathogens within the human organism (Keerthanan *et al*. 2021), but most of them were highly removed in this study, showing promising results. It is important to mention that the OMP concentration in this study (20 μg/L) was higher than that typically found in real GW streams for most of the tested compounds (median 0.4 μg/L for PhACs in GW, Glover *et al*. 2021), and thus their concentrations would be much lower in the effluent of a real application. Nevertheless, in several cases the spiked concentration was in the same order of magnitude and, even, concentrations of compounds such as ACE, IBU, DCF and CAF in real GW were reported to be up to one order of magnitude higher than in this study (Zraunig *et al*. 2019; Glover *et al*. 2021), thus the results obtained from these compounds can be considered comparable/similar to those systems using real GW. The outcomes of this study indicate that it is apparent that even when the effluent from a system aligns with the provided quality limits, numerous parameters remain without specific regulation in the current legislation, as it is the case of OMP.

3.3. Human health risk assessment

The only tested condition that produced lettuces of marketable size (comparable to the control) was GWN (GW supplemented with CNS). Accordingly, the risk assessment is here discussed for GWN only, as a proof of concept, although the generated data of the other conditions is also indicated in Table 5.

HHRA was only evaluated for those compounds that were detected in lettuce leaves (Table 4). OFX, MTPA, and TRI produced HQ from 0.004 to 0.04, all below the threshold of 1, indicating that risk through the individual compounds is not expected. Similarly, the HQ of MTP, VLF, and DVLF ranged from 0.09 to 0.29 (Table 5). The assessed risk using the highest quantifiable concentration (Table 4) was only indicative of whether the upper end of the quantifiable concentrations would already indicate a potential risk, but the actual concentration and therefore the potential risk must be assumed to be higher. On the other hand, most OMPs studied here are present at lower concentrations in real GW, and therefore, their concentrations in lettuce are expected to be lower in real applications than those found in this study. As regards to the compounds found in some cases at higher concentrations in real GW (ACE, IBU, and DCF), they were always not detected, or at LOQ level, in the lettuces' leaves.

The HQs of both ATE and EpCBZ were substantially above the threshold of 1 (Table 5), indicating high potential risk. Notably, these results are related to compounds classification as potentially genotoxic, warranting a

Table 5 | Risk assessed for compounds quantified in the lettuce leaves, expressed as Hazard Quotient (HQ, potential risk of individual compounds) and Hazard Index (HI, potential risk of mixture)

	Hazard characterization				Hazard quotients (HQ)[a]		
OMP	**LDTD/TTC, μg/day**	**SF**	**Ref. value μg/kg BW/day**	**Source**	**GWB**	**GWS**	**GWN**
ATE	25,000	30,000	0.012	a	7.571	6.135	3.443
EpCBZ	0.15[b]	n.a.	0.002	b	35.055	36.054	19.663
MTP	25,000	3,000	0.120	c	0.615	0.498	0.280
MTPA	90.00[b]	n.a.	1.286	b[c]	0.069	0.033	0.011
OFX	400,000	3,000	1.900	c	–	0.003	0.004
TRI	80,000	3,000	0.380	a	0.207	0.142	0.044
VLF	37,500	3,000	0.180	d	0.640	0.518	0.291
DVLF	50,000	3,000	0.237	e	0.194	0.157	0.088
Hazard Index (HI)[a]					**44.352**	**43.541**	**23.823**

LDTD was applied for parent compounds (i.e., ATE, MPT, OFX, TRI, VLF, DVLF) and TTC for transformation products (i.e., EpCBZ and MTPA). Shaded cells indicate that the compound was detected in concentrations exceeding the calibration curve used for quantification. In these cases, the EDI was calculated for the upper bound of the analytically quantifiable concentration, representing the lower bound of the compound's concentration in the leaves.

Sources: **a**: Snyder *et al*. 2010, **b**: Malchi *et al*. 2014, **c**: Semerjian *et al*. 2018, **d**: https://www.drugs.com/dosage/venlafaxine.html (SF: Snyder *et al*. 2010); **e**: https://www.drugs.com/dosage/desvenlafaxine.html (SF: Snyder *et al*. 2010).

n.a.: not applicable.

[a]HQ & HI ≤1: no risk expected; HQ & HI ≥1 possible risk must be analyzed in more detail.

[b]Transformation product: TTC applied.

[c]The TTC value of MTP (parent compound of MTPA) was applied for the risk assessment.

higher SF of 30,000 (Snyder *et al.* 2010) applied to the LDTD for ATE and the lower TTC value of 0.0021 μg/person/day (Malchi *et al.* 2014) for EpCBZ. The obtained HQ of ATE and EpCBZ were up to two orders of magnitude higher than those reported in edible crops grown in soil irrigated with WW (Prosser & Sibley 2015), as OMP are usually taken up to a lesser extent in soil due to more interactions and richer microbiological environment in soil than in hydroponics. As regards to TP, for example, EpCBZ presents a higher health risk to the consumer than its parent compound (CBZ, Malchi *et al.* 2014). Therefore, this study confirms the importance of considering these compounds in future related studies as well as in legislations.

Even if the exposure to individual OMP indicates risk for two compounds, cumulative exposure could pose additional risk for the other compounds due to the presence of multiple OMP in real GW (Glover *et al.* 2021). Hence, the HI index (Table 5) shows that the five compounds mentioned above (MTP, VLF, DVLF, ATE, and EpCBZ) were primarily contributing to HI (97%). Excluding ATE and EpCBZ (over the limit of 1 already by themselves) the HI for cumulative risk was of 0.72 with MTP and VLF contributing about 40% each, and DVLF 13%. Other quantified compounds contributed in a minor way (6 and 1% for TRI and MTPA, respectively). In contrast, in the literature negligible dietary uptakes of DCF, BPA, and NPX were reported for lettuce and collards (Dodgen *et al.* 2013), as well as negligible risk related with the consumption of lettuces exposed to 20 OMP (Wu *et al.* 2013), in both cases grown in hydroponics with 21 days exposure to the OMP, which were applied at smaller concentration than in this study (0.1–5 μg/L). Please note that DCF in this study was spiked in the water but not detected in the lettuce. In any case, attention should be paid to the uptake and translocation potential of MTP, VLF, DLVF and even more of ATE and EpCBZ.

CONCLUSIONS

The findings of this investigation offer a comprehensive exploration of GW treatment using hydroponic systems, encompassing treatment efficacy, plant growth and health, and the fate of OMP, including potential human risks arising from their presence in GW. The results underscore the potential of GW as a hydroponic growth medium for edibles, particularly lettuce. However, results emphasize the necessity for adequate nutrient supplementation when utilizing GW medium for hydroponics, as only those lettuces grown in GW fortified with commercial nutrient solution (GWN) exhibited growth comparable to control plants. Plant development was slightly affected, most likely due to GW salinity rather than OMP presence.

The system demonstrated effective removal of standard parameters from GW, surpassing 85% in all cases, except for sodium. Only GWN effluent met physicochemical quality requirements for reuse scenarios B, C, and D (food crops not in direct contact with the reclaimed water and industrial crops) set by European water reuse legislation (EU 2020/741). Furthermore, the study showcased effectiveness in OMP removal, aligning with the performance of other NBS. However, the variability in OMP removal and the low removal rates for certain compounds suggest the need for system optimization. Notably, the condition with better-developed roots (GWN) exhibited higher removal rates, particularly for hydrophobic OMP, but also displayed the lowest OMP concentrations in leaves, indicating effective plant development and OMP removal, albeit with the lowest uptake.

HHRA for the condition with robust plant growth (GWN) revealed that five out of the ten detected compounds (20 analyzed) are unlikely to pose adverse health effects under the exposure scenario of chronic ingestion of 50 g of lettuce per day. Conversely, ATE and EpCBZ demonstrated considerable potential for human health risks, whereas VLF, DVLF, and MTP may raise concerns in the context of cumulative risk of chemicals in water reuse applications.

This study provides valuable insights into OMP in the context of water reuse, edible production, and food safety. Despite effluent compliance with water reuse parameters, the low removal rates of certain OMP underscores potential issues upon effluent discharge or reuse. Consequently, it is strongly recommended to consider these compounds in future water reuse regulations, along with their TPs, which, as demonstrated in this study, may entail even greater risk than the parent compounds. Future research should prioritize optimizing these systems for enhanced removals without increasing the risks derived from plant ingestion. Finally, studies applying real GW and expanding the spectrum of evaluated OMP are required to ensure safety in reuse applications.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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