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Rayana Manasfi, Monica Brienza, Nassim Ait-Mouheb, Nicola Montemurro, Sandra Perez, et al.. Impact of long-term irrigation with municipal reclaimed wastewater on the uptake and degradation of organic contaminants in lettuce and leek. *Science of the Total Environment*, 2021, 10.1016/j.scitotenv.2020.142742 . hal-02958578

**HAL Id: hal-02958578**

**<https://hal.science/hal-02958578v1>**

Submitted on 6 Oct 2020

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## Journal Pre-proof

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PII: S0048-9697(20)36271-9

DOI: <https://doi.org/10.1016/j.scitotenv.2020.142742>

Reference: STOTEN 142742

To appear in: *Science of the Total Environment*

Received date: 11 June 2020

Revised date: 25 September 2020

Accepted date: 27 September 2020

Please cite this article as: R. Manasfi, M. Brienza, N. Ait-Mouheb, et al., Impact of long-term irrigation with municipal reclaimed wastewater on the uptake and degradation of organic contaminants in lettuce and leek, *Science of the Total Environment* (2020), <https://doi.org/10.1016/j.scitotenv.2020.142742>

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## Impact of long-term irrigation with municipal reclaimed wastewater on the uptake and degradation of organic contaminants in lettuce and leek.

Rayana Manasfi<sup>a</sup>, Monica Brienza<sup>a</sup>, Nassim Ait-Mouheb<sup>b</sup>, Nicola Montemurro<sup>c</sup>, Sandra Perez<sup>c</sup>, Serge Chiron<sup>a,\*</sup>

<sup>a</sup>UMR HydroSciences Montpellier, Montpellier University, IRD, 15 Ave Charles Flahault 34093 Montpellier cedex 5, France

<sup>b</sup>UMR G-eau, Montpellier University, INRAE, 361 rue Jean-François Breton, 34196 Montpellier cedex 5, France

<sup>c</sup>ENFOCHEM, IDAEA-CSIC, c/Jordi Girona 18-26, 08034 Barcelona, Spain

\*Corresponding author: Tel: + 33 - 411759415; Fax: + 33 - 411759414; e-mail address: serge.chiron@umontpellier.fr

### Abstract

A two years drip irrigation of lettuce and leek crops with treated municipal wastewater without and with spiking with fourteen wastewater relevant contaminants at 10 µg/L concentration level was conducted under greenhouse cultivation conditions to investigate their potential accumulation in soil and leaves and to assess human health related risks. Lettuce and leek crops were selected as a worse-case scenario since leafy green vegetable has a high potential for organic contaminants uptake. The results revealed limited accumulation of contaminants in soil and plant leaves, their concentration levels being in the range of 1 - 30 ng/g and 1 - 660 ng/g range in soil and leaves, respectively. This was likely related to abiotic and biotic transformation or simply binding processes in soil, which limited contaminants plant uptake. This assumption was underpinned by studies of the enantiomeric fractionation of chiral compounds (e.g. clambazole and metoprolol) in soil as pieces of evidence of biodegradation and by the identification of transformation products or metabolites in leaves by means of liquid chromatography - high resolution - mass spectrometry using a suspect screening workflow. The high bioconcentration factors were not limited to compounds with intermediate  $D_{ow}$  (100 to 1000) such as carbamazepine but also observed for hydrophilic compounds such as clarithromycin,

hydrochlorothiazide and the food additives acesulfame and sucralose. This result assumed that accumulation was not only driven by passive processes (e.g. lipoidal diffusion through lipid bilayer cell membranes or Casparian strip) but might be supported by carrier-mediated transporters. As a whole, this study confirmed earlier reports on the *a de minimis* human health risk related to the consumption of raw leafy green vegetable irrigated with domestic TWW containing organic contaminants residues.

*Keywords:* Reclaimed wastewater irrigation; pharmaceuticals; plant uptake; soil accumulation; risks.

#### Abbreviations

TWW: treated wastewater

PPCPs: pharmaceuticals and personal care products

RWW: raw wastewater

TPs: transformation products

DMSO: dimethyl sulfoxide

TW: tap water

WWTP: wastewater treatment plant

OM: organic matter

STWW: spiked treated wastewater

BCF: bioconcentration factor

HQ: hazard quotient

EDI: estimated daily intake

ADI: acceptable daily intake

DOM: dissolved organic matter

LODs: limits of detection

#### Introduction

In arid and semi-arid agricultural area, in both developed and developing countries, irrigation of crops with treated wastewater (TWW) is already a common practice as a result of water scarcity due to climate change as well as to continuously growing population (Carter et al., 2019; Ait-Mouheb et al., 2018). A large body of literature has demonstrated that crop plants can accumulate organic contaminants and more specifically pharmaceuticals and personal care products (PPCPs) (Fu et al.,

2019). However, studies have been carried out by using different plant-growth methods (e.g., hydroponic cultivation, crops in pots under greenhouse, field cultivation conditions) with different sets of contaminants, different types of plants and different irrigation methods resulting to some inconsistency in conclusions. Limited data is actually available under real field growing conditions of crops using appropriate irrigation regimes (Goldstein et al., 2014; Riemenschneider et al., 2016; Wu et al., 2014; Christou, 2017; Picó et al., 2019), besides most of the studies did not focus on long term introduction of contaminants in soil-plant system through irrigation, neither their fate/behavior in soil which can affect their fate and uptake in plants at the same time. Therefore, it is crucial to conduct such experiments following the risk introduced by wastewater irrigation at long-term field conditions, taking in consideration contaminants fate in the whole soil-plant system. PPCPs concentration levels, which have been found in edible parts of food crops are in the ng/g to low µg/g range, hence revealing the potential for human exposure to PPCPs as a result of irrigation with TWW (Gonzalez-Garcia et al., 2019). The European Commission has proposed in May 2018 new guidelines to facilitate water reuse in the EU for agricultural irrigation (EC European Commission, 2018) which are mainly focused on water physico-chemical constituents and pathogen indicators. However, the problem of organic contaminants has been mentioned in annexes, calling for more research in this field before including their levels as stricter requirements for water quality, if needed. Soil properties, water quality, the nature of the PPCPs and their physico-chemical properties as well as plant physiology (i.e. type of crops) have been considered major governing factors in determining plant uptake (Wu et al., 2015). Generally, plant uptake of neutrally charged PPCPs is greater than ionic compounds, because anionic PPCPs are repelled by cell membranes with negative electrical potential, and cationic species are attracted to the cell membranes thus limiting their movement into plants (Chuang et al., 2019). In addition, experimental results revealed that the potential for PPCPs uptake by crop plants decreased in the order of leafy green vegetables > root vegetables > cereals and fodder crops > fruit vegetables (Christou et al., 2019). Water flow is believed to be the primary carrier for uptake and transport of PPCPs in plants and transpiration may be a strong predictor for the accumulation of structurally diverse PPCPs in above-ground plant tissues (Nason et al., 2019). Others studies have shown the relevance of the PPCPs concentrations in soil pore water and consequently their bioavailability to

plant uptake (Li et al., 2019). In contrast, the role of the behavior/fate of PPCPs in soil under field cultivation conditions has been poorly considered because research efforts have typically focused on short-term TWW irrigation scenarios, when plants were exposed for only short-time periods to PPCPs. The role of soil as a regulator and/or attenuator of the amount of PPCPs available for plant uptake due to sorption and degradation processes has probably been underestimated. One of the research need is therefore a better understanding of the behavior and fate of PPCPs in soil following chronic or long terms irrigation of TWW in agro-food systems to investigate the relevance of PPCPs exposure time on the plant uptake processes and on the subsequent effects on PPCPs degradation. The reduction of PPCPs uptake by plants is generally related to sorption processes to soil, especially for those chemicals with strong hydrophobicity or positive charge but also to transformation processes such as photolysis at the soil-surface and biodegradation (Fu et al., 2019). Plant uptake might be therefore reduced, if attenuation processes outbalance the continuous input of chemicals. In addition, there is the potential for agricultural soil to develop accelerated biodegradation due to microorganisms adaptation processes, accounting for the non-accumulation of drugs such macrolide antibiotics over multiple crop cycles (Topp et al., 2016). Another research gap might be the role of active transport carriers in PPCPs plant uptake. Most current literature regarding PPCPs accumulation in crop plants focuses on passive processes such as diffusion across membranes, ion trapping, and sorption. However, some results suggest that in some cases plant transporter proteins may be important for accumulation of certain PPCPs, as has been assumed for metformin (Eggen and Lillo, 2016) and amitriptyline (Nason et al., 2019). Understanding the biological aspects of plant accumulation of PPCPs is important not only in environmental risk assessment but also from a remediation perspective (e.g. removal in engineered treatment wetlands). Consequently, the major aim of this work was to contribute to fill these scientific gaps by investigating the behavior of selected organic contaminants (mainly PPCPs) in the wastewater-soil-plant system for the specific conditions of greenhouse-grown of lettuce and leek irrigated with different kinds of wastewater including raw municipal wastewater (RWW), lagoon-based secondary treated wastewater (TWW) and spiked TWW with 14 pollutants under realistic agricultural scenarios over multiple crop cycles. Specific objectives were i) to investigate the impact of the fate of targeted PPCPs in soil on plant uptake ii) to understand the main driving processes of their

uptake in plant, iii) to investigate the fate of selected PPCPs in lettuce leaves to evaluate implications of irrigation with TWW containing these PPCPs for food safety and human exposure via dietary intake.

## 2. Material and methods.

### 2.1 Chemicals

Fourteen organic contaminants that were commonly detected in TWW were selected in this study including pharmaceuticals (ciprofloxacin (CIP), sulfamethoxazole (SMX), citalopram (CTP), diclofenac (DCF), valsartan (VAL), irbesartan (IRB), carbamazepine (CBZ), metoprolol (MTP), hydrochlorothiazide (HCT), clarithromycin (CLT)), food additives (acesulfame (ASF), sucralose (SUC)), the fungicide climeazole (CLB, human medicine), and the corrosion inhibitor 1H-benzotriazole (BNZ). The transformation products (TPs), valsartan acid (VAL-AC), oxcarbazepine (Ox-CBZ) and carbamazepine-10, 11-epoxide (CBZ EPX) were also investigated as they were also frequently detected in investigated TWW. Reference standards for all compounds and their TPs were obtained from Sigma-Aldrich (St Quentin-Falavier, France) and were of high purity (> 95%). Deuterated compounds including climeazole-d<sub>4</sub>, valsartan-d<sub>3</sub>, irbesartan-d<sub>6</sub>, ciprofloxacin-d<sub>8</sub>, citalopram-d<sub>6</sub>, sulfamethoxazole-d<sub>4</sub>, carbamazepine-d<sub>10</sub>, benzotriazole-d<sub>4</sub>, metoprolol-d<sub>7</sub> were used as internal standards. The above-mentioned standards were prepared individually in 100% acetonitrile, 100% HPLC water, 100% methanol or 100% DMSO according to compounds solubility and stored at -20°C. Working solutions were prepared in serial dilution in methanol by mixing all compounds and stored at -20°C. All LC-MS grade organic solvents and HPLC water were of purity higher than 99.9% and purchased from Merck (Darmstadt, Germany). Formic acid (≥ 96%, ACS reagent) and ammonium acetate were supplied by Sigma-Aldrich while ammonium fluoride was bought from Fisher Chemical (Fisher Scientific SL, Madrid, Spain). For EDTA-McIlvaine buffer preparation, di-sodium hydrogen phosphate dihydrate (Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O) was obtained from Merck, citric acid monohydrate (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>·H<sub>2</sub>O) and ethylenediaminetetraacetic acid anhydrous (EDTA) (≥ 99%) from Sigma-Aldrich.

EDTA-McIlvaine buffer preparation. 1.5 g of di-sodium hydrogen phosphate dihydrate, 1.3 g of citric acid monohydrate, and 0.372 g EDTA were dissolved in 100 mL HPLC water.

Two QuEChERS salts, BEKOLut SALT-KIT-AC (4 g  $\text{MgSO}_4$  + 1 g NaCl) known as original salt and QuEChERS Extract pouches (4 g  $\text{MgSO}_4$  + 1 g NaCl + 0.5 g di-sodium citrate sesquihydrate) known as European salts (CEN) were used for compounds extraction from soil and leave samples. The dispersive solid phase extraction salts BEKOLutPSA-Kit-04 (900 mg  $\text{MgSO}_4$  + 150 mg PSA + 150 mg C18e) were used for extracts clean-up. All the above-mentioned salts were obtained from BeKOLut (Hauptstuhl, Germany) except for the CEN salts, which were obtained from Agilent Technologies.

## 2.2 Plant-growth and exposure conditions

The experiments were conducted in a greenhouse located at the Murviel-Lès-Montpellier village (Hérault, France, 43.605034° N, 3.757292° E) where lettuces and leeks were grown in large tanks (1 m<sup>2</sup> in surface and 60 cm soil depth) and irrigated with tap water (TW), raw domestic wastewater (RWW) originating from the village (2 replicates), TWW (2 replicates) and TWW spiked with a mixture of fourteen organic contaminants at 10 µg/L concentration level, each (3 replicates), as shown in Fig. 1 Supplementing Material (SM). Those experiments were possible thanks to an administrative authorization from the Health Agency of the Occitanie region. The WWTP operated on the basis of stabilization ponds with three successive lagoons (13680, 4784 and 2700 m<sup>3</sup>, respectively) and had a nominal capacity of 1,500 Inhabitant Equivalent. The plant-growth method in greenhouse was selected to avoid water supply from rainfall but in contrast, this cultivation shaped/accelerated the evaporation rate due to hot conditions and therefore accelerated water, nutrient and contaminants plant uptake. Lettuces were cultivated during 3 successive cultivation cycles of 6 weeks in 2018 from June to December. Cultivation was then stopped during January and February 2019 and resumed for two additional cycles between March and June 2019. Leek crop was conducted during two successive cultivation campaigns of 16 weeks, one in 2018 and one in 2019. The cultivation schedule for lettuces and leeks is illustrated in Fig. 2SM. Under the greenhouse, the mean temperature and relative humidity during all cultivation cycles were  $27.2 \pm 7.2$  °C and  $54 \pm 21.7\%$ , respectively.

*Soil.* Sandy silty clay soil with 50.3% sand (19.5% very fine sand, 16.4% fine sand and 14.4% coarse sand), 25.9% silt and 24% clay (see Table 3SM for others parameters) was obtained from a local field to fill up the tanks. This texture is representative of alluvial soils near waterways where WWTPs are



very often located and where TWW are often reused in irrigation, even though this kind of soil with a rather high proportion in clay and OM (2.7%) was known to restrict the uptake of organic contaminants in comparison to sandy soils. The collected soil was characterized by a regular cation exchange capacity of 11.1 milliequivalents / 100 g soil and pH near neutrality (i.e. 7.6 in KCl). The soil was tested before starting the experiments and was found to be free of investigated organic contaminants.

*Selected crops.* Baby lettuces (*Lactuca sativa* variety Batavia) and leeks (*Allium porrum*) were obtained from a local organic market. Four lettuces and 16 leeks were placed in different tanks (see Fig. 1SM). It was known that plant species have different patterns of contaminants uptake. Lettuce growing corresponded to a worse-case scenario because leafy green vegetable constitutes the crop plants with the highest ability to uptake and accumulate PPCPs in their edible tissue in comparison to root vegetables (e.g. carrot) or fruit-bearing vegetables (e.g. tomato) (Christou et al., 2019). Leek was selected due to a long growing period of 16 weeks (6 weeks for lettuce) potentially allowing for PPCPs accumulation or conversely longer time for dissipation processes to operate. Further, lettuce and leek are consumed raw and human health risk is likely greater than for cooked vegetable. No fertilization was practiced during the experiments, neither pesticides application. Leaves were collected at mature stage after removing roots. As under field experiments, growing conditions were less controllable and reproducible some heterogeneity in results was expected. To limit this issue, three lettuces and leeks from each tank were mixed after freeze-drying and extracted separately so the analytical results were presented as a mean of two replicates.

*Irrigation regime.* Surface drip irrigation system with polyethylene irrigation pipe (16 mm i.d.) was chosen for this study, making root uptake the only relevant uptake pathway and avoiding soil runoff. In addition, drip irrigation system is the most efficient and a common irrigation practice in all arid and semi-arid regions, as it complies with the existing guidelines for a safe reuse of TWW for irrigation. RWW was selected as a worst-case scenario of irrigation water, as it can be used in some low-income countries. Non-compensate drippers working at a flow rate of 2 L/h with an inlet pressure of 1 bar were placed along the pipe. An inlet disk filter with a mesh opening size of 0.13 mm was installed to reduce the physical clogging of emitters. The irrigation was optimized to limit contaminants transport

into soil and avoiding the occurrence of any leaching events. Consequently, plants were irrigated in short intervals that were every day in summer and every week in fall and spring. Irrigation flow was 0.5 L/2d in spring and fall and 1 L/2d in summer.

*Experiments with spiked TWW (STWW):* Spiking took place manually, by routinely spiking a freshly obtained large volume of TWW with a mix solution of all studied 14 compounds. In real agricultural practices, TWW storage is actually unavoidable. This was the reason why a large volume (20 to 40 L) was spiked with selected contaminants in a tank. Such a large volume fitted to 8 - 15 d irrigation volume need according to the season. STWW was only used for lettuce irrigation.

### 2.3. Analytical procedures

Chemicals residues in soil as well as in leek and lettuce leaf samples were analyzed following the validated analytical methods described in Montemurro et al., 2020. Briefly, after harvest, leaf samples were directly transported to the analytical laboratory of Montpellier University where they were frozen, lyophilized, grounded to fine powder and stored at - 40°C until analysis. Rhizospheric soil samples and soil samples at the bottom of the tanks were collected at the end of some campaigns of the two years study. They were homogenized by a mortar and sieved at 2 mm, left under hood at room temperature for 2 or 3 d to ensure their total dryness and finally stored at - 40°C until analysis. 1 g of lyophilized lettuce and leek leaves and 10 g soil samples were extracted using QuEChERS extraction methods after spiking with a proper volume of deuterated mix. Soil, leek and lettuce leaves extracts were analyzed using the SCIEX ExionLC™ AD system coupled with the SCIEX X500R QTOF system (Sciex, Redwood City, CA, U.S.) with Turbo V™ source and Electrospray Ionization (ESI) operating in positive and negative mode. Target and non-target acquisition screening were used by employing the Multiple Reaction Monitoring (MRM<sup>HR</sup>) acquisition mode and the SWATH® acquisition mode, respectively. Shifts in mass accuracy were corrected by infusion of reserpine reference standard (C<sub>33</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>, m/z 609.28066) for positive ionization, and a cluster of trifluoroacetic acid (5(TFA-Na)<sup>+</sup> TFA<sup>-</sup>, m/z 792.85963) for negative mode. Calibrant Delivery System (CDS) was employed for mass calibration every 5 samples during the batch injection. Both acquisition modes were employed over a m/z range from 100 to 950 Da. MRM<sup>HR</sup> acquisition was employed to quantify

the target compounds, by following the precursor ion and one selected fragment ion chosen according to its highest intensity obtained on a specific collision energy (CE) and declustering potentials (DP). For MRM, MDLs and MQLs ranged from 0.01 to 0.12 ng/g and 0.04 to 0.38 ng/g, respectively. SWATH® acquisition mode was employed to identify potential TPs that might be formed in lettuce leaves during the experiments, for which analytical standards were not available. SWATH® acquisition mode allows to detect and fragment every compound in the sample in one single run, without the risk of missing a relevant analyte. Therefore, every compound in the sample has a complete MS and MS/MS spectrum, eliminating the need for re-analysis. More details on these analytical methods are made available in SM.

Chiral analysis of metoprolol and climbazole were carried out by LC-HRMS following the analytical procedures already published in Souchier et al., 2016 and Bronza and Chiron, 2017, respectively. Briefly, enantiomers of metoprolol were separated using a ASTEC vancomycin-based analytical column (Chirobiotic V) using a reverse phase isocratic mode of elution with a mobile phase consisting of water + 30 mM ammonium acetate/methanol, 10/90 (v/v). Enantiomers of CBZ were separated using a Phenomenex Lux Amylose-2 analytical column only using water/acetonitrile (35/65, v/v) as mobile phase in an isocratic mode of elution. LODs down to 10 ng/g were obtained with both analytical methods, which made them suitable for the analysis of metoprolol and climbazole in soil irrigated with STWW.

#### 2.4 Calculation of BioConcentration Factor (BCF) and Hazard Quotient (HQ)

The BioConcentration Factor (BCF) of the studied compounds in lettuce was calculated in order to estimate the ability of lettuce crop to uptake organic contaminants from soil irrigated with contaminated water (Bianco et al., 2013). Thus, BCF was calculated for each compound detected in lettuce irrigated with the several types of water used in this experiment (TWW, RWW and STWW), as the ratio of the concentration in lettuce over the concentration in soil (dry weight):

$$\text{BCF} = \text{concentration in lettuce (ng/g)} / \text{concentration in soil (ng/g)}$$

The consumption of contaminated lettuce constitutes one pathway of human exposure. The HQ was calculated as the ratio of estimated daily intake (EDI) of PPCPs and the acceptable daily intake (ADI)

which is the amount of contaminants that can be consumed daily over a person's lifespan without evocating an adverse effect. ADI values of the targeted pharmaceuticals were calculated by dividing the lowest therapeutic dose (mg/d) by a safety factor of 1000 and a body weight of 70 kg while EDI was calculated using the greatest concentration in lettuce leaves among the different lettuce crop seasons (worse case) following the equation proposed Prosser and Sidley (2015).

$$EDI = \frac{C_{lettuce} \times IR_{veg} \times \beta_{g/cup} \times \beta_{dw/ww}}{m}$$

$C_{lettuce}$  represents the concentration of contaminants in lettuce leaves (ng/d dry weight),  $IR_{veg}$  represents 2.8 cup equivalents of lettuce per day,  $\beta_{g/cup}$  represents the mass of a cup of fresh lettuce tissue, which is equal to 218.6 g of wet weight per cup (Prosser and Sidley, 2015),  $\beta_{dw/ww}$  is the wet to dry weight conversion factor for lettuce which is equal to 0.052, and  $m$  is the mass of the consumer, which is considered 70 Kg for adult (20 - 65 years of age) (USEPA, 1996).

### 3. Results and discussion

#### 3.1 Occurrence and accumulation patterns of contaminants (mainly PPCPs) in soil and leaves

Only a few studies have reported the uptake and accumulation of organic contaminants by crop plants irrigated with TWW under realistic agricultural growing and irrigation conditions. Even fewer studies have reported their concentration values both in soil and in the edible part of the crop allowing for BCF calculation. In spite of poor controllable and reproducible conditions, field studies were implemented because they enabled an appropriate risk assessment of wastewater-borne contaminants to consumers as they integrated irrigation, soil and plant processes.

The average values of physico-chemical properties of the TWW are presented in Table 1SM and complied with irrigation water quality criteria as established by FAO's guidelines (Ayers and Westcot, 1985). This lagoon-based TWW was characterized by a high DOM content (> 200 mg/L) which could prone interactions between DOM and ionizable PPCPs as well as by a high content of ammonium ions (> 50 mg/L) which could lead to rhizosphere acidification due to excess cations plant uptake over anions uptake. This acidification process could result in loss of two pH units (Nason et al., 2018). This had strong implication for compound such as climbazole with a pKa of 7.5 because at pH 5.6, it would have been 100% ionized limiting its possible plant uptake while at pH 7.6 both neutral and cationic

states of climbazole co-existed. Concentrations of investigated PPCPs were measured periodically in TWW. Highly variable levels were found and consequently, mean values are reported in Table 2SM. Irbesartan, benzotriazole and acesulfame were detected with relatively higher concentrations ( $> 450$  ng/L) than the others compounds, which were detected at concentration levels in the 5 - 200 ng/L range. Due to the low contaminants occurrence levels in small municipality effluents, it was decided to spike TWW with 14 compounds to avoid the risk of no detection in lettuce leaves. The spiking level was set to 10  $\mu\text{g/L}$  (each compound) which was a good compromise between the possibility to work at concentrations not so far from environmental concentrations and the potentiality to identify some TPs. The selection of the compounds was driven by the frequency of detection in TWW and to encompass a large range of different physico-chemical properties such as polarity ( $D_{ow}$ ), charge, water solubility, sorption ( $k_d$ ) and contrasting fate under photodegradation and aerobic/anaerobic biodegradation, expressed as half-lives values (see Table 1). For instance, X-ray contrast media, which have often been the most frequently detected compounds in domestic TWW, were excluded because their plant uptake has been found restricted under field growing conditions probably due to high molecular weight of 777-821 Da, slowing down their diffusion through cell membranes (Riemenschneider et al., 2016). No detectable chemicals residues were found in soil samples prior to the experiments and under irrigation with tap water (results not shown). This latter experiment was carried out for a rough evaluation of the phytotoxicity of RWW, TWW and spiked TWW. Dry weight of lettuces and leeks under different experimental irrigation conditions are reported in Table 4SM. Values were always higher than the dry weight of lettuces grown with tap water, excluding potential phytotoxicity of RWW, TWW and spiked TWW, which could have impacted the lettuce uptake rates across the different cultivation cycles. Following the field irrigation experiments with spiked TWW, trace residues of 13 out of 14 compounds were detected in soil samples collected near the rhizosphere at the concentration levels in the 1 - 30 ng/g (d.w.) range. One exception was sucralose, which was never detected due to its high water solubility and lack of sorption onto soil ( $k_d < 10$ ). The highest concentrations were for citalopram, climbazole, carbamazepine and clarithromycin. This result was rather well correlated with their rather high  $k_d$  values (542-1883, 123-200, 12-20 and 262-400  $\text{L kg}^{-1}$ , respectively) accounting for their retention in soil. Ciprofloxacin was detected in experiments with

TWW but not in experiments with spiked TWW. Sorption on the TWW storage tank walls or fast ciprofloxacin photodegradation (half-life of 0.5 h has been reported for direct photolysis) during the storage event might account for this difference. Compounds concentrations profiles across lettuce cultivation cycles (in weeks) in soil and in lettuce leaves are reported in Fig. 1 for spiked TWW experiments. As a general trend, analysis of soil samples revealed no carryover of contaminants over multiple crop seasons. This lack of cumulative pattern in soil was in line with previous results (Dalkmann et al., 2012; Wu et al., 2014). Only 8 out of 14 compounds were consistently detected in lettuce leaves. The frequency of detection was 100% for hydrochlorothiazide, carbamazepine, metoprolol, clarithromycin, acesulfame, sucralose, citalopram and climbazole and 25% for benzotriazole, while diclofenac, ciprofloxacin, irbesartan and valsartan were never detected. All these compounds were usually detected in higher concentrations in spring/summer than fall/winter, which confirmed a good correlation between crop transpiration and crop accumulation (Nason et al., 2018). Their distribution in the aerial organs of the plants was usually limited with concentration levels in the range of 1 - 660 ng/g (d.w.) which was consistent with those found in previous studies under field growing conditions (Fu et al., 2019). The highest concentration (i.e. 660 ng/g) was found for carbamazepine.

As far as RWW and TWW irrigation experiments were concerned, only 5 compounds were detected in soil and lettuce leaves including acesulfame, sucralose, hydrochlorothiazide, oxcarbazepine and carbamazepine as well as one human metabolite, carbamazepine-epoxide (see Fig. 2). In general, concentration levels in RWW and TWW experiments were much lower than those in spiked TWW experiments. One exception was acesulfame with concentrations in leaves exceeding those in spiked TWW experiments without any clear explanation for this experimental observation. However, this result meant that higher compound concentration in water did not necessarily mean higher concentration in leaves. In fact, many other uncontrollable processes including biotic and abiotic degradation, sorption, leaching, etc... were also taking place in soil and/or irrigation water, besides to the physico-chemical properties of CECs, soil and water, and the weather conditions, making the uptake level by crops unpredictable. Experiments were also carried out with leeks, another leafy vegetable with a longer growing period (16 weeks) than lettuce (6 weeks). Similar compounds than in lettuce

experiments were detected in leek leaves including carbamazepine, carbamazepine-epoxide, sucralose and acesulfame (see Fig. 2). One exception was sulfamethoxazole which was detected at 35.5 ng/g with TWW and 58.6 ng/g with RWW at the end of the second growing crop cycle (i.e. 64 weeks). However, concentration levels were usually lower in leek than in lettuce leaves. Longer growing period did not imply higher contaminants accumulation probably because longer time for dissipation processes to operate was also allowed. The main and common feature of all those experiments was the lack of accumulation likely related to abiotic and biotic transformation processes both in soil and leaves (see section 3.2 and section 3.3). The longer growing period could contribute to the low accumulation of CECs in leek leaves since uptaken CECs were available for metabolism for longer time before harvest. Sorption and degradation in soil likely minimized contaminants uptake by leek and lettuce.

Experimental results in spiked TWW experiments could also be interpreted according to contaminants physico-chemical properties and their persistence under photochemical and biodegradation processes. *Non-ionic compounds*: carbamazepine, hydrochlorothiazide and sucralose. Experiments confirmed that carbamazepine is highly up-taken by plants (Goldstein et al., 2018) even more than more hydrophilic compounds such as hydrochlorothiazide which was also detected in RWW and TWW experiments and previously in lettuce crop (0.18 - 0.49 ng/g) irrigated with municipal TWW at 0.18 - 0.49 ng/g concentration level (Matinez-Piermas et al., 2018). To the best of our knowledge, this was the first time that sucralose uptake by lettuce was observed. Sucralose is structurally similar to sucrose with three chlorine atoms replacing three hydroxyl groups. The presence of multiple hydroxyl groups makes this compound very soluble in water and very hydrophilic, preventing its retention in soil. In spite of its extremely high stability in the environment (Soh et al., 2011), sucralose uptake was therefore not expected due to its low ability to partition into lipophilic cell structure. The uptake process appeared to be somewhat inconsistent across the successive lettuce growing campaigns and between the different types of water. But, when this latter occurred, the uptake rate was always high. One explanation could be that sucralose was uptaken by crops via water. Aquatic plants such as *Lemna spp.* have shown the capacity to assimilate carbon from sucralose (Amy-Sagers et al., 2017). Similarly to sucrose, sucralose may be a plant-accessible sugar, possibly accounting for high uptake values in our experiments.



*Cationic compounds:* climbazole, metoprolol, citalopram and clarithromycin. As previously observed, the concentrations of cationic compounds in lettuce leaves were usually much lower than those of the non-ionic PPCPs. Citalopram and climbazole uptake was higher than metoprolol uptake because their larger accumulation in soil probably increased the available concentration for plant uptake resulting in higher accumulation in leaves. One exception was clarithromycin with a high uptake rate. Clarithromycin uptake was not expected due to its high molecular volume ( $632 \text{ cm}^3$ ) which limited its uptake to upper ground leaves, and rather low  $D_{ow}$  (3.16 at pH 7) and  $k_d$  (in the 260 - 400 L/kg range) values and was only been reported once in Bermuda grass roots grown in biosolid amended soil (John-Lepp et al., 2010). However, clarithromycin was found to be tightly bound to DOM fraction with negatively charged functional groups (Sibley and Pedersen, 2008). Those interactions were largely reversible and reached a maximum at pH close to neutrality and might make clarithromycin more available for plant uptake by limiting strong sorption on negative mineral surfaces such as clays.

*Anionic compounds:* valsartan, diclofenac, sulfamethoxazole and acesulfame. Diclofenac was never detected in leaves probably because it underwent fast degradation under photochemistry during the TWW storage event and/or at the soil surface. Valsartan was also never detected in lettuce leaves. Valsartan biodegradation in activated sludge was reported to be very fast ( $< 0.5 \text{ d}$ , Helbling et al., 2010). Even though this value was reported in activated sludge experiments, similar behavior may be expected in soil irrigated with organic rich WW because it seems to be possible to read across from half-lives determined in highly efficient biotransformation experiments such as activated sludge to soil half-lives due to similarity in enzymatic transformations of organic contaminants (Fenner et al., 2020). With a pKa of 5.6, sulfamethoxazole may partly exist as non-ionic compound in the acidic rhizosphere facilitating plant uptake. However, sulfamethoxazole has been found to be trapped as an anionic species in the cytosol (pH 7.2) and preferentially being translocated in the phloem rather than the xylem, ending in root (Chuan et al., 2019). Acesulfame was found highly up-taken in leaves similarly than in a previous field study (Riemenschneider et al., 2016), but mainly during the first growing campaign (131 ng/g, d.w.). This uptake rate steadily decreased during the following campaigns, being 26.2, 14.5 and 9.7 ng/g during the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> campaign, respectively.

*Switerionic compounds:* ciprofloxacin and irbesartan were never detected in lettuce leaf. Ciprofloxacin was probably eliminated during the



TWW storage stage due to fast photodegradation. For irbesartan, strong sorption to soil was anticipated ( $\log D_{ow}$  4.4) limiting its bioavailability.

The ability of lettuce plant to accumulate targeted contaminants from TWW irrigated soil in leaves was also assessed through the determination of BCF, which was established as the ratio of the concentration of each compound in leaves (ng/g) and in soil (ng/g). The linear regression between  $D_{ow}$  and BCF demonstrated a poor relationship, BCF being unusually high for polar compounds as it is shown by the red dots in Fig 3a, where  $D_{ow}$  lower than 500 (polar compounds) were showing BCF relatively high (Fig. 3a). This result assumed that accumulation was not only driven by passive processes (e.g. lipoidal diffusion through lipid bilayer cell membranes or Casparian strip) but might be supported by carrier-mediated transporters. In this respect, mammalian systems may provide guidance for identifying specific transporter proteins in plant systems. Human cellular uptake of hydrochlorothiazide is facilitated by diffusion through organic anion transporters (Hasannejad et al., 2004). Macrolide antibiotics and clarithromycin in particular possess several interesting features, among which are exceptionally high levels of accumulation and retention in cells and tissues. More and more data support the idea of the existence of an active transport system for macrolides in human cell (Bosnar et al., 2005). The active transport of metoprolol and citalopram have been also reported (Dobson and Kell, 2008). The contribution of transporter proteins might account for a higher BCF values for these compounds (3.5, 4.6, 12.9, 1.7 for hydrochlorothiazide, clarithromycin, metoprolol and citalopram, respectively) than that could be anticipated by their  $D_{ow}$  values (0.26, 50.1, 0.13 and 3326, respectively) and their positive charge (except for hydrochlorothiazide). This result means that potential high BCF values are not restricted to intermediate  $D_{ow}$  between 100 and 1000 such as the  $D_{ow}$  of carbamazepine. Similarly, amitriptyline, a cationic psychoactive drug, has known to be actively transported in human cell and its BCF of 0.35 in spinach was higher than that could be anticipated with its  $D_{ow} = 64\ 655$  (Nason et al., 2019). All these findings supported the existence of proteins that transport xenobiotic organic cations in plants even though they have not been specifically identified yet. These organic cation transporters have been found in all organisms and have been implicated in the uptake of the cationic antidiabetic drug metformin in plant for instance (Eggen and Lillo, 2016). The public health risk assessment associated with the intake of targeted contaminants through the

consumption of lettuce irrigated with spiked TWW, TWW and RWW was conducted through the hazard quotient (HQ) for adults (most of the targeted PPCPs are not prescribed to toddlers). Results are reported in Table 2. EDI were lower than ADI and HQ values were  $< 0.4$  for spiked TWW experiments. For TWW and RWW experiments only 4 compounds out of 14 were determined in lettuce restricting the potential toxicity additivity of mixtures of chemicals. A conservative approach for assessing the risk of a mixture of chemicals would be to sum the HQ values, which was always found  $< 0.1$ . As lettuce was selected as a worse-case scenario since leafy vegetables has a high potential for PPCPs uptake (Christou et al., 2019), the daily consumption of lettuce was considered to not pose any health problem for lettuce grown in greenhouse condition and soil irrigated with TWW during consecutive growing campaigns. This conclusion held true for 1 week.

### 3.2 Fate of contaminants in soil: chiral analysis of metoprolol and climbazole

In soil irrigated with TWW, several processes are in competition for organic contaminants removal including sorption and formation of non-extractable residues, phototransformation at the soil surface, leaching to deeper soil layers than the rhizosphere layer, plant uptake and biotransformation (Li et al., 2019). Their behavior and fate in soil is therefore very complex due to interconnected processes and it is often impossible to discriminate between abiotic and biotic processes. Enantiomeric fractionation of two chiral compounds, namely metoprolol and climbazole, was investigated during the successive lettuce growing seasons to highlight the significance of biodegradation processes over abiotic processes. Enantiomeric fractionation occurs when the biodegradation of one enantiomer of a chiral compound is favored over the other. Enantioselectivity reflects biological processes because abiotic enantioselective processes such as sorption on environmental mineral surfaces have been found negligible with respect to biodegradation-related enantioselectivity. Metoprolol and climbazole were selected because metoprolol specifically undergoes enantioselective biodegradation under aerobic conditions (Souchier et al., 2016) and climbazole under denitrifying / anaerobic conditions (Brienza and Chiron, 2017), thus covering different redox conditions in soil irrigated with TWW. The major advantage of the enantiomeric fractionation approach over isotopic fractionation has been the relative simple analysis of enantiomers ratios in a routine way using chiral LC-HRMS. The chiral analysis of metoprolol and climbazole by chiral LC-HRMS which were previously developed for assessing the

biodegradation rates of metoprolol (Souchier et al., 2016) and climbazole (Brienza and Chiron, 2017) in activated sludge were applied in this study to go deeper in the understanding PPCPs fate in soil. These methodologies were precise and reproducible with appropriate LODs of ng/g in solid matrices and were applied to a subset of field soil samples of spiked TWW experiments. As an illustrative example, Fig. 4a shows the enantiomer profile of metoprolol in a soil sample collected at the end of the last lettuce growing campaign. Enantiomers ratio showed an enantiomeric profile similar to that of metoprolol standard. This profile was constant across time and metoprolol remained racemic. This result very likely means that biodegradation did not operate because anoxic conditions already prevailed in the top layer soil (0 - 20 cm) due to the high content in LOM provided with TWW. No known TPs of metoprolol such as metoprolol acid, o-desmethyl metoprolol or  $\alpha$ -hydroxymetoprolol were detected in soil extracts (see Fig. 3SM). This constituted an additional piece of evidence for a lack of metoprolol biodegradation under anaerobic conditions. Fig. 4b shows the chiral analysis of climbazole in soil at the end of the first lettuce growing campaign. In contrast to metoprolol, climbazole enantiomeric fractionation clearly took place along the growing campaigns, supporting that biodegradation was effective under denitrifying conditions. Identification of TPs of climbazole in soil extracts using C-18 LC-HRMS revealed the occurrence of climbazole-OH which confirmed the biotransformation of climbazole but also revealed the reductive dechlorination of climbazole (see Fig. 4c) due to photochemical processes (Castro et al. 2016). Consequently, surface drip irrigation did not completely prevent from climbazole photodegradation. After irrigation, upward movement of water to the soil surface where PPCPs phototransformation could occur has to be considered and phototransformation need to be taken into account when deriving microbial degradation rates from field studies as previously suggested (Buerge et al., 2019). It is well recognized that the anoxic/anaerobic biotransformation of organic micropollutants is energetically less favorable than their aerobic counterpart, which advocates for poor efficiency of biodegradation processes in soil irrigated with domestic TWW. However, certain aerobically recalcitrant contaminants are biologically degraded under anaerobic conditions and enzymatic reactions involved in their degradation are becoming known (Ghatta et al., 2017). For instance, sulfamethoxazole (Wu et al., 2012) and irbesartan (Boix et al., 2016) are known to be quickly biotransformed under anaerobic conditions which probably

contributed to their very low occurrence levels in soil. Finally, contaminants analysis were carried out in a soil layer (50 - 60 cm) deeper than the rhizosphere layer at the end the experiments to investigate their potential leaching. The results are reported in Fig. 3b. Clarithromycin and hydrochlorothiazide were detected at high concentrations ( $481.2 \pm 67.3$  and  $169.6 \pm 30.5$  ng/g, respectively), while the others compounds were found at similar concentrations than those encountered near the rhizosphere. Many studies have reported the sorption of DOM to amorphous clay minerals (Sibley and Pedersen, 2008). Many clay surfaces in upper soil horizons irrigated with TWW were probably already coated with DOM and thus were not accessible for contaminants binding. The presence of high amount of free DOM in soil could give rise to interactions mainly through contaminant-DOM complexes. This kind of interactions was already demonstrated for clarithromycin (Sibley and Pedersen, 2008) and might also apply for hydrochlorothiazide. They could facilitate the co-transport of DOM and contaminants downward through soil horizons with the flow of water. Sorption of PPCPs to DOM has appeared to be governed by their chemical structures and difficult to be predicted at the moment (Maoz and Chefetz, 2010) probably justifying further investigations.

### 3.3 Fate of contaminants in leaves: Identification of metabolites and/or TPs

Till now, identification of metabolites in plants has been mainly carried out at high spiked concentrations (e.g. 1 mg/L) to elucidate metabolic pathways and under hydroponic conditions to discriminate between soil-generated TPs and plant-generated metabolites and because plants absorb higher levels of organic contaminants in hydroponic conditions than in soil experiments. For instance, such experiments were conducted to elucidate TPs of clarithromycin (Tian et al., 2019), carbamazepine (Martinez-Piernas et al., 2019) and ofloxacin (Tadic et al., 2020) in lettuce crop. Metabolites identification are worthy to assess the consequences of long-term exposure to PPCPs because TPs are stored in cell walls and vacuoles and because some PPCPs can be transformed into more toxic products during plant metabolism. This is the case of carbamazepine, which is transformed into carbamazepine-epoxide (Sauvêtre et al., 2018). In this study, the objective was to tentatively identify previously reported metabolites or TPs of investigated compounds in order to assess their relevance under real growing conditions by using a suspect screening workflow based on a list of

compounds and their metabolites/TPs with their respective exact  $m/z$  (see Table 6SM). In a first step, TPs with intensities lower than  $1 \times 10^4$  cps, signal to noise ratios lower than 10, isotopic ratios higher than 10%, and mass accuracy errors higher than 5 ppm were eliminated. When possible, after preliminary identification, the potential metabolites/TPs were further confirmed by including the screening of known fragments ions and the MS/MS spectrum information was compared with that reported in previous literature reports. The level of confidence for the identification of detected TPs was classified according to Schimansky et al., 2014. This approach was first applied to clarithromycin and to carbamazepine for which metabolic pathways in lettuce were previously elucidated (Martinez-Piernas et al., 2019; Tan et al., 2019). In case of carbamazepine only carbamazepine-epoxide was detected and its structure was confirmed at a level 1 of confidence through matching with an authentic standard. Fig. 5 shows results on the identification of carbamazepine epoxide at a concentration level of  $6.1 \pm 0.7$  ng/g in a lettuce leaves extract, demonstrating the reliability of the SWATH® acquisition mode for compound identification at a very a concentration level close to LODs in a complex matrix. Carbamazepine-epoxide was also detected in TWW ( $10.7 \pm 1.3$  ng/L) but not in soil. This amount found in leaves was therefore exclusively related to in-plant metabolism and not the combination of plant uptake and metabolism. This is reasonable since carbamazepine-epoxide has been known to undergo in soil hydrolysis leading to 10,11-dihydro-10-hydroxycarbamazepine and/or ring contraction leading to acridone derivatives (Li et al., 2013). Differently, valsartan acid, which was also identified with of level 1 of confidence due to the availability of an analytical standard was found in TWW ( $111.17 \pm$  ng/L) and in soil ( $1.7 \pm$  ng/L) in accordance with its high environmental persistency but not in lettuce leave excluding valsartan acid plant uptake. None of the known metabolites of clarithromycin was detected in this study, even though clarithromycin was found to be extensively and quickly metabolized in lettuce (Tian et al., 2019) with a proportion of metabolites, which was estimated to account for more than 70% of the initial clarithromycin concentration. This finding might result from very low formation rates of clarithromycin metabolites, thus escaping from analytical determination. The suspect screening approach was then extended to known bio- and photo-TPs of ciprofloxacin, citalopram, diclofenac, valsartan, irbesartan, metoprolol, hydrochlorothiazide, climbazole and acesulfame (Table 6SM). Only TPs of metoprolol and irbesartan were detected.

Metoprolol acid, a very common bio-TPs of metoprolol was never detected. In contrast, TP239 originating from the benzylic hydroxylation of metoprolol acid and TP253 resulting from further oxidation of TP239 into the corresponding carboxylic acid (see Fig. 4SM) were identified at level 2 of confidence (probable structure through matching with literature). These two TPs were previously observed in river-simulating flumes (Posselt et al., 2020) similarly to TP210 and TP226 but these two TPs were only identified at level 4 of confidence in our experiments (unequivocal molecular formula which can be unambiguously assigned with the spectral information). Finally TP213 resulting from the hydrolysis of irbesartan could be also identified at level 4 of confidence and will deserve further investigation (Fig. 5SM). Proposed structures and relative mass errors of the detected TPs and their fragments are presented in Table 3. Low mass error values ( $< 5$  ppm) were obtained comparing with their theoretical masses. Due to the lack of authentic standards, it was difficult to obtain accurate abundances of all these TPs. A semi-quantitative method was applied using the pseudo-molecular ion abundances and considering that the structure of metabolites/TPs were closely related to those of their parent compounds. Following this method their concentrations were always estimated below the concentrations of their parent compounds. All these results demonstrated that PPCPs were likely highly metabolized in lettuce, precluding the accumulation of parent compounds in leaves. The levels of metabolites/TPs concentrations in leaves were always low, limiting potential health risks.

#### 4. Conclusions

Crops irrigation with TWW represents a recognized pathway for human exposure to organic contaminants with possible health implications. However, this study confirmed that the accumulation of 14 compounds in soil and in lettuce leaves irrigated with spiked TWW at  $10 \mu\text{g/L}$  concentration level was very limited. These results relied on the implementation of real greenhouse cultivation conditions, the repeated application of spiked TWW over five successive lettuce crop cycles and a large number of compounds covering not only different physico-chemical properties but also showing contrasting behavior with respect to photo- and bio-transformation rates. This likely represented the originality of this study. Poor accumulation was also observed with non-spiked TWW and RWW and with another leafy vegetable, leek. The main reason for these low accumulation rates was an intensive degradation in soil and metabolic transformations in plant which were supported by the enantiomeric

fractionation of climbazole in soil and the identification of suspected metabolites/TPs in lettuce leaves, respectively. Metabolites/TPs concentrations were always estimated below those of the parent compounds. Unexpected pharmaceuticals such as clarithromycin and hydrochlorothiazide were yet detected in lettuce leaves. This might be linked to active transport processes similarly to what has been observed in human cells. Information on pharmacokinetics and toxicokinetics of PPCPs in humans and animals should be more mined and used to anticipate the potential plant uptake of PPCPs as even suggested by Nason et al., 2019. The food additive sucralose was also encountered at high concentration in lettuce and leek leaves for the first time because sucralose may be a plant-accessible sugar similarly to sucrose. As a whole, this study confirmed *a de nino* is human health risk related to the consumption of raw green vegetable (e.g. lettuce) irrigated with TWW. More research is today needed to fully understand the environmental risks of TWW irrigation practices and specifically the impact of such practices on long-term soil quality.

Acknowledgements: This research was financially supported by the Water and Agriculture, Food Security and Climate Change Joint Programming Initiatives (JPIs) through the research project AWARE “Assessing the fate of pesticides and waterborne contaminants in agricultural crops and their environmental risks”, the French Rhône-Méditerranée-Corse Water Agency for the experimental platform for the reuse of reclaimed wastewater in irrigation (Murviel-lès-Montpellier) project and the Spanish Ministry of Science and Innovation PCIN-2017-067. This work was also supported by the Spanish Ministry of Science and Innovation (Project CEX2018-000794-S). The authors also acknowledge SCIEX for providing the loan instrument LC/HRMS QToF X500R and Bekolut GmbH & Co. KG for the contribution with QuEChERS kits extraction. The EU is not liable for any use that may be made of the information contained therein.

## References

Ait-Mouheeb, N., Bahri, A., Thayer, B., Benyahia, B., Bourrié, G., Cherki, B., Condom, N., Declercq, R., Gunes, A., Héran, M., Kitir, N., Molle, B., Patureau, D., Pollice, A., Rapaport, A., Renault, P., Riahi, K., Romagny, B., Sari, T., Sinfort, C., Steyer, J-P., Talozzi, S., Topcuoglu, B., Turan, M., Wéry, N., Yıldırım, E., Harmand, J., 2018. The reuse of reclaimed water for irrigation

- around the Mediterranean rim: a step towards a more virtuous cycle? *Reg. Environ. Chang.* 18, 693-705.
- Amy-Sagers, C., Reinhardt, K., Larson, D., 2017. Ecological assessments show sucralose and fluoxetine affect the aquatic plant *Lemna minor*. *Aquatic Toxicol.* 185, 76-85.
- Ayers, S., Westcot, D. (Eds). *Water quality for irrigation 1985*, FAO, Rome.
- Bianco, G., Zianni, R., Anzillotta, G., Palma, A., Vitacco, V., Scrano, L., Cataldi, T.R.I. 2013. Dibenzo-p-dioxins and dibenzofurans in human breast milk collected in the area of Taranto (Southern Italy): First case study. *Anal. Bioanal. Chem.* 405, 2405-2410.
- Boix, C., Fabregat-Safont, I., Morales, E., Sancho, J., Sanchez Ramirez, J., Hernandez, F. 2016. Behavior of emerging contaminants in sewage sludge after anaerobic digestion. *Chemosphere* 163, 296-304.
- Bosnar, M., Kelneric, Z., Munic, V., Erakovic, V., Parnham, M., 2005. Cellular uptake and efflux of azithromycin, erythromycin, clarithromycin, telithromycin, and cethromycin. *Antimicrob. Agents and Chemother.* 49, 2372-2377.
- Brienza, M., Chiron, S., 2017. Enantioselective reductive transformation of climbazole: A concept towards quantitative biodegradation assessment in anaerobic biological treatment processes. *Water Res.* 116, 203-210.
- Buerge, I., Kasteel, R., Bächli, A., Poiger, T., 2019. Behavior of the chiral herbicide imazamox in soils: Enantiomer composition differentiates between biodegradation and photodegradation. *Environ. Sci. Technol.* 53, 5733-5740.
- Carter, L., Chefetz, B., Abdeen, Z., Boxall, A., 2019. Towards a framework for establishing the impacts of pharmaceuticals in wastewater irrigation systems on agro-ecosystems and human health. *Environ. Sci.: Processes Impacts* 21, 605-622.
- Castro, G., Casado, J., Rodriguez, I., Ramil, M., Ferradas, A., Cela, R., 2016. Time-of-flight mass spectrometry assessment of fluconazole and climbazole UV and UV/H<sub>2</sub>O<sub>2</sub> degradability: Kinetics study and transformation products elucidation. *Water Res.* 88, 681-690.
- Christou, A., Karaolia, P., Hapeshi, E., Michael, C., Fatta-Kassinou, 2017. Long-term wastewater irrigation of vegetables in real agricultural systems: Concentration of pharmaceuticals in soil,



- uptake and bioaccumulation in tomato fruits and human health risk assessment. *Water Res.* 109, 24-34.
- Christou, A., Papadavid, G., Dalias, P., Fotopoulos, V., Michael, C., Bayona, J-M., Fatta-Kassinos, D., 2019. Ranking of crop plants according of their potential to uptake and accumulate contaminants of emerging concern. *Environ. Res.* 170, 422-432.
- Chuang, H., Liu, C-H., Sallacha, B., Hammerschmidt, R., Zhang, W., Boyd, S., Li, H., 2019. Mechanistic study on uptake and transport of pharmaceuticals in lettuce from water. *Environ. Intern.* 131, 104976.
- Dalkmann, P., Broszat, M., Siebe, C., Willascheck, E., Sakinc, T., Huebner, J., Amelung, W., Grohmann, E., Siemen, J., 2012. Accumulation of pharmaceuticals, enterococcus, and resistance genes in soils irrigated with wastewater for zero to 100 years in Central Mexico. *PLOS One* 7(9), e45397.
- Dobson, P., Kell, DB., 2008. Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule? *Nature Reviews Drug Discovery* 7, 2005-2020.
- EC European Commission, 2018. Proposal for a Regulation of the European Parliament and of the Council on minimum requirements for water reuse. ([https://ec.europa.eu/info/law/better-regulation/initiatives/com-2018-337\\_en](https://ec.europa.eu/info/law/better-regulation/initiatives/com-2018-337_en)).
- Eggen, T., Lillo, C., 2016. Role of transporters for organic cations in plants for environmental cycling of pharmaceutical residues. In *Organic Cation Transporters*; Ciarimboli, G., Gautron, S., Schlatter, E., Eds.; Springer International Publishing: Switzerland, 243-256.
- Fenner, K., Screpanti, C., Renold, P., Rouchdi, M., Vogler, B., Rich, S., 2020. Comparison of small molecule biotransformation half-lives between activated sludge and soil: Opportunities for read-across? *Environ. Sci. Technol.* 54, 3148-3158.
- Fu, Q., Malchi, T., Carter, L., Li, H., Gan, J., Chefetz, B., 2019. Pharmaceutical and personal care products: from wastewater treatment into agro-food systems. *Environ. Sci. Technol.* 53, 14083-14090.
- Ghattas, A-K., Fischer, F., Wick, A., Ternes, T., 2017. Anaerobic biodegradation of (emerging) organic contaminants in the aquatic environment. *Water Res.* 116, 268-295.

- Goldstein, M., Shenker, M., Chefetz, B., 2014. Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. *Environ. Sci. Technol.* 48, 5593-5600.
- Gonzalez Garcia, M., Fernandez-Lopez, C., Polesel, F., Trapp, S., 2019. Predicting the uptake of emerging organic contaminants in vegetables irrigated with treated wastewater – Implications for food safety assessment. *Environ. Res.* 172, 175-181.
- Hasannejad, H., Takeda, M., Taki, K., Shin, H. J., Babu, E., Jutabha, P., et al., 2004. Interactions of human organic anion transporters with diuretics. *J. Pharmacol. Exp. Ther.* 308, 1021-1029.
- Helbling, D., Hollender, J., Kohler, H-P., Singer, H., Fenner, K., 2010. High-throughput identification of microbial transformation products of organic micropollutants. *Environ. Sci. Technol.* 44, 6621 -6627.
- Jones-Lepp, T., Sanchez, C., Moy, T., Kazemi, R., 2010. Method development and application to determine potential plant uptake of antibiotics and other drugs in irrigated crop production systems. *J. Agric. Food Chem.* 58, 11568-11573.
- Li, J., Dodgen, L., Ye, Q., Gan, J., 2013. Degradation kinetics and metabolites of carbamazepine in soil. *Environ. Sci. Technol.* 47, 3678-3684.
- Li, Y., Sallach, B., Zhang, W., Boyd, S., Li, H., 2019. Insight into the distribution of pharmaceuticals in soil-water-plant systems. *Water Res.* 152, 36-46.
- Liu, F., Ying, G-G., Yang, J-F., Zhou, L-J., Tao, R., Wang, L., Zhang, L-J and Peng, P-A., 2010. Dissipation of sulfamethoxazole, trimethoprim and tylosin in a soil under aerobic and anoxic conditions. *Environ. Chem.* 7(4), 370-376.
- Maoz, A., Chefetz, B., 2010. Sorption of the pharmaceuticals carbamazepine and naproxen to dissolved organic matter: Role of structural fractions. *Water Res.* 44, 981-989.
- Martinez-Piernas, A., Polo-Lopez, M., Fernandez-Ibanez, P., Agüera, A., 2018. Validation and application of a multiresidue method based on liquid chromatography-tandem mass spectrometry for evaluating the plant uptake of 74 microcontaminants in crops irrigated with treated municipal wastewater. *J. Chromatogr. A.* 1534, 10-21.
- Martines-Piernas, A., Nahim-Granados, S., Polo-Lopez, M., Fernandez-Ibanez, P., Murgolo, S., Mascolo, G., Agüera, A., 2019. Identification of transformation products of carbamazepine in

- lettuce crops irrigated with ultraviolet-C treated water. *Environ. Pollut.* 247, 1009-1019.
- Montemurro, N., Orfanoti, A., Manasfi, R., Thomaidis, N., Pérez, S., 2020 Comparison of high resolution MRM and Sequential Window Acquisition of All Theoretical Fragment-Ion acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce. *J. Chromatogr. A*. Available online on 19 September 2020, 461566.
- Nason, L., Miller, E., Karthikeyan, K., Pedersen, J., 2019. Effects of binary mixtures and transpiration on accumulation of pharmaceuticals by spinach. *Environ. Sci. Technol.* 53, 4850-4859.
- Nason, S., Miller, E., Karthikeyan, K., Pedersen, J., 2018. Plant-induced changes to rhizosphere pH impact leaf accumulation of lamotrigine but not carbamazepine. *Environ. Sci. Technol. Lett.* 5, 377-381.
- Picó, Y., Alvarez-Ruiz, R., Alfarhan, A., El-Sheikh, M., Alabd, S., Barcelo, D., 2019. Uptake and accumulation of emerging contaminants in soil and plant treated with wastewater under real-world environmental conditions in the Al Hayer area (Saudi Arabia). *Sci. Total Environ.* 652, 562-572.
- Posselt, M., Mechelke, J., Rutere, C., Coll, C., Jaeger, A., Raza, M., Meinikmann, K., Krause, S., Sobek, A., Lewandowski, J., Mon, M., Hollender, J., Benskin, J., 2020. Bacterial diversity controls transformation of wastewater-derived organic contaminants in river-simulating flumes. *Environ. Sci. Technol.* 54, 9, 5467-5479.
- Prosser, R., Sibley, P., 2015. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. *Environ. Intern.* 75, 223-233.
- Riemenschneider, C., Al-Raggad, M., Moeder, M., Seiwert, B., Seiwert, B., Salameh, B., Reemtsma, T., 2016. Pharmaceuticals, their metabolites, and other polar pollutants in field-grown vegetables irrigated with treated municipal wastewater. *J. Agric. Food Chem.* 64, 5784-5792.
- Sauvêtre, A., May, R., Harpaintner, R., Poschenrieder, C., Schröder P., 2018. Metabolism of carbamazepine in plant roots and endophytic rhizobacteria isolated from *Phragmites australis*. *J. Hazard. Mater.* 342, 85-95.
- Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H., Hollender, J., 2014.

- Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* 48, 2097-2098.
- Sibley, S., Pedersen, J., 2008. Interaction of the macrolide antimicrobial clarithromycin with dissolved humic acid. *Environ. Sci. Technol.* 42, 422-428.
- Soh, L., Connors, K., Brooks, B., Zimmerman, J., 2011. Fate of sucralose through environmental and water treatment processes and impact on plant indicator species. *Environ. Sci. Technol.* 45, 1363-1369.
- Souchier, M., Benali-Raclot, D., Casella, C., Ingrand, V., Chiron, S., 2016. Enantiomeric fractionation as a tool for quantitative assessment of biodegradation: The case of metoprolol. *Water Res.* 95, 19-26.
- Tadic, D., Gramblicka, M., Mistrik, R., Flores, C., Fina, B., Bayona, J., 2020. Elucidating biotransformation pathways of ofloxacin in lettuce (*Lactuca sativa* L). *Environ. Intern.* 260, 114002.
- Tian, R., Zhang, R., Uddin, M., Qiao, X., Chen, J., Gu, G., 2019. Uptake and metabolism of clarithromycin and sulfadiazine in lettuce. *Environ. Intern.* 247, 1134-1142.
- Topp, E., Renaud, J., Sumarah, M., Siburin, L., 2016. Reduced persistence of the macrolide antibiotics erythromycin, clarithromycin, azithromycin in agricultural soil following several years of exposure in the field. *Sci. Total Environ.* 562, 136-144.
- USEPA, 1996. Soil screening guidance: Technical background document. United States Environmental Protection Agency, Washington, DC, US.
- Wu, X., Conkle, J., Ernst, F., Gan, J., 2014. Treated Wastewater Irrigation: Uptake of pharmaceutical and personal care products by common vegetables under field conditions. *Environ. Sci. Technol.* 48, 11286-11293.
- Wu, X., Dodgen, L., Conkle, L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. *Sci. Total Environ.* 536, 655-666.
- Wu, Y., Williams, M., Smith, L., Chen, D., Kookana, R., 2012. Dissipation of sulfamethoxazole and trimethoprim antibiotics from manure-amended soils. *J. Environ. Sci. Health Part B* 47, 240-249.

## Figure caption

Fig. 1. Organic contaminants concentrations in rhizospheric soil and in lettuce leaves across successive crop cycles under spiked TWW irrigation at a 10 µg/L concentration level (each compound).

Fig. 2. Organic contaminants concentrations across successive crop cycles in rhizospheric soil and in lettuce and leek leaves samples irrigated with treated wastewater (TWW) and raw wastewater (RWW).

Fig. 3. a) relationship between  $D_{ow}$  of targeted contaminants and their bioconcentration factors (BCF) and b) organic contaminants concentrations in a deep soil layer (50 - 60 cm) for leaching assessment.

Fig. 4. Chiral LC-HRMS analysis of a) metoprolol (MET(S) and MET(R)) and b) climbazole (CLB(E1) and CLB(E2)) in soil irrigated with spiked treated wastewater. C) Extracted Ion chromatogram of the identified transformation products of climoazole.

Fig. 5. Identification of carbamazepine-epoxide by LC-TOFMS in lettuce leaves at a concentration level of  $6.1 \pm 0.7$  ng/g using the SWATH® acquisition mode. Upper plot: EIC m/z 253.0972; middle plot: MS spectrum; lower plot: MS/MS spectrum using m/z 253.0971 as precursor ion.

Table 1. Physico-chemical properties of investigated compounds and their reported half-lives in the literature under biodegradation and photolysis.

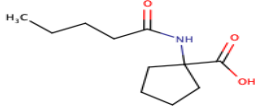
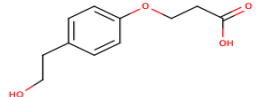
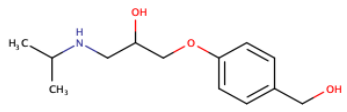

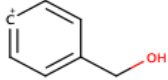
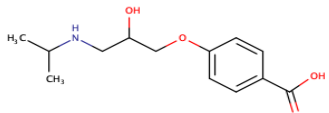
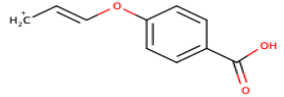
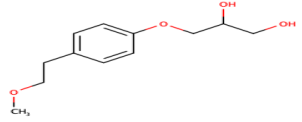
Compound	Mw (g/mol)	SW (mg/L) pH 7	pKa	Kd (soil) (L/kg)	Log D <sub>ow</sub> (pH = 7)	Molecule vol. (cm <sup>3</sup> )	Charge pH 7.5	Half-life (d) Aerobic biodeg.	Half-life (d) Anaerobic biodeg.	Half-life photolysis
Ciprofloxacin	331	< 30	6.1; 8.7	427-4844	0.28	227	Zwitterion	> 100	n.f	0.5 h
Sulfamethoxazole	253	610	1.6; 5.7	0.6-4.9	- 1.05	173	anion	4 - 10	3 - 7	3.7 h
Citalopram	324	6.1	9.78	542-1883	3.76	273	cation	n.f	n.f	14 - 65 d
Diclofenac	296	17.8	4.15	1 - 18	1.66	207	anion	0.4 - 5	> 100	0.17 h
Valsartan	435.5	16.8	3.9; 4.7	n.f	- 1.20	359	anion	0.5	n.f	stable
Irbesartan	428	0.35	4.1; 7.4	n.f	4.4	328	zwitterion	20 - 30	n.f	stable
Carbamazepine	236	153	-	12-20	2.7	187	neutral	125 - 233	n.f	34 - 42 d
Metoprolol	267	> 10000	9.7	20	- 0.58	259	cation	23	stable	26 - 41 d
Hydrochlorothiazide	298	722	7.2; 9.2	11.9	- 0.07	176	neutral	35.8	n.f	0.2 - 0.43 h
Clarithromycin	748	0.33	3.99	262 - 400	3.16	632	cation	> 100	n.f	stable
Climbazole	293	58	7.5	123 - 200	3.27	248	neutral/ cation	4.4 - 5.2	6.2	70 min
Benzotriazole	119	> 10000	8.2	0.1 - 0.8	1.17	88.3	neutral	38 - 82	n.f	42 - 54
Acesulfame	201	> 10000	2.0	0.1	- 0.55	108	anion	11 - 19	n.f	stable
Sucralose	398	> 10000	-	2.5	- 0.47	235	neutral	14 - 30	30 - 65	stable

n.f: not found

Table 2. Estimated daily intake (EDI) of targeted PPCPs based on the highest concentration determined in lettuce leaf and associated Hazard Quotient (HQ) for adult.

Compound	Lettuce irrigated with STWW				Lettuce irrigated with TWW			Lettuce irrigated with RWW		
	Greatest conc. in leave (ng/g)	EDI, (ng/kg/d)	ADI (ng/kg/d)	HQ	Greatest conc. in leave (ng/g)	EDI (ng/kg/d)	HQ	Greatest conc. in leave (ng/g)	EDI (ng/kg/day)	HQ
Ciprofloxacin	-	-	-	-	-	-	-	-	-	-
Sulfamethoxazole	-	-	-	-	-	-	-	-	-	-
Citalopram	29.1	43.3	285	0.15	-	-	-	-	-	-
Diclofenac	-	-	-	-	-	-	-	-	-	-
Valsartan	-	-	-	-	-	-	-	-	-	-
Irbesartan	-	-	-	-	-	-	-	-	-	-
Carbamazepine	660.0	982.8	2850	0.34	68.4	101.8	0.04	14.7	21.9	$7 \times 10^{-3}$
Metoprolol	7.2	10.7	360	0.03	-	-	-	-	-	-
Hydrochlorothiazide	17.6	26.2	360	0.07	9.6	14.3	0.04	6.1	9.1	0.03
Clarithromycine	128.8	191.3	7140	0.03	-	-	-	-	-	-
Climbazole	10.7	15.9	-	-	-	-	-	-	-	-
Benzotriazole	11.8	17.6	-	-	-	-	-	-	-	-
Acesulfame	131.5	195.8	$5 \times 10^6$	$3 \times 10^{-5}$	148.7	221.4	$4 \times 10^{-5}$	131.5	195.8	$4 \times 10^{-5}$
Sucralose	632.0	941.2	$15 \times 10^6$	$6 \times 10^{-5}$	146.4	218.0	$1 \times 10^{-5}$	116.8	173.9	$1 \times 10^{-5}$

Table 3. Proposed structures and mass error of the detected TPs of metoprolol and irbesartan in lettuce leaves.

TPs	Molecular formula	RT (min)	Observed m/z	Theoretical m/z	Polarity	Mass error (ppm)*	Proposed structure
Irbesartan sTP213	C <sub>11</sub> H <sub>19</sub> NO <sub>3</sub>	4.5	214.1438	214.1443	POS	-2.3349	
Metoprolol sTP210	C <sub>11</sub> H <sub>14</sub> O <sub>4</sub>	2.7	211.0965	211.09644	POS	0.2842	
Metoprolol sTP239	C <sub>13</sub> H <sub>21</sub> NO <sub>3</sub>	2.1	240.1593	240.15944	POS	-0.5829	
Metoprolol sTP239-fragment 91	C <sub>7</sub> H <sub>7</sub>	–	91.0543	91.05453	POS	-2.5260	
Metoprolol sTP239-fragment 107	C <sub>7</sub> H <sub>7</sub> O	–	107.0493	107.04926	POS	0.3737	
Metoprolol sTP253	C <sub>13</sub> H <sub>19</sub> NO <sub>4</sub>	1.5	254.1386	254.1386	POS	0.0000	
Metoprolol sTP253-fragment 177	C <sub>10</sub> H <sub>9</sub> O <sub>3</sub>	–	177.0549	177.0545	POS	2.2592	
Metoprolol sTP226	C <sub>12</sub> H <sub>18</sub> O <sub>4</sub>	2.2	225.1129	225.1127	NEG	0.8884	

\* Mass error (ppm) = ((observed mass - theoretical mass) / (theoretical mass)) x 1000000



**Declaration of competing interests**

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof

Credit Author Statement

Rayana Manasfi: Analysis – writing – original draft, review & editing

Monica Brienza: Experimental data interpretation

Nassim Ait-Mouheb: Implementation of field experiments

Nicola Montemurro: Analytical methods for organic contaminants

Sandra Perez: Analytical methods for organic contaminants

Serge Chiron: Conceptualization – writing, review & editing

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Graphical abstract

Highlights

- ▶ Low accumulation of PPCPs in soil and in lettuce leave over successive crop cycles
- ▶ Clarithromycin was found a compound of concern for leaching and plant uptake in spiked water study
- ▶ Enantiomer fractionation of climbazole was a good indicator of biodegradation in soil
- ▶ SWATH® acquisition mode was reliable for TPs identification at low concentrations
- ▶ *A de minimis* human health risks related to consumption of raw leafy green vegetable

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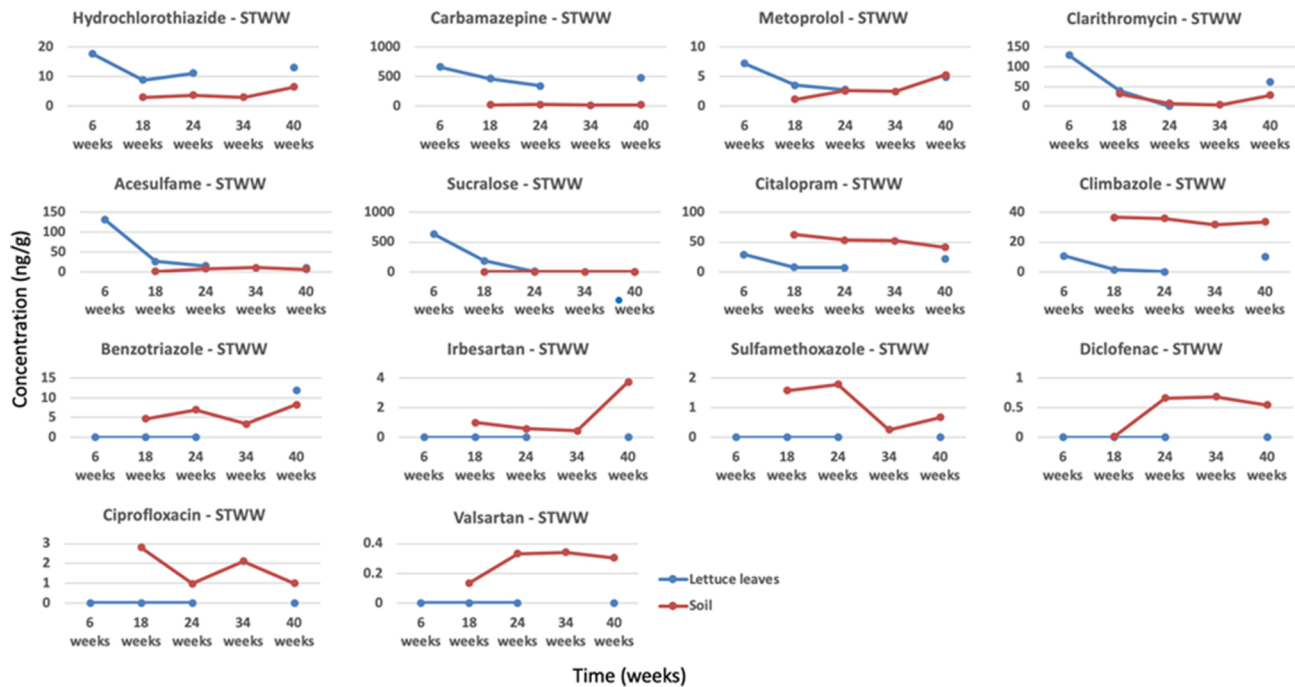


Figure 1

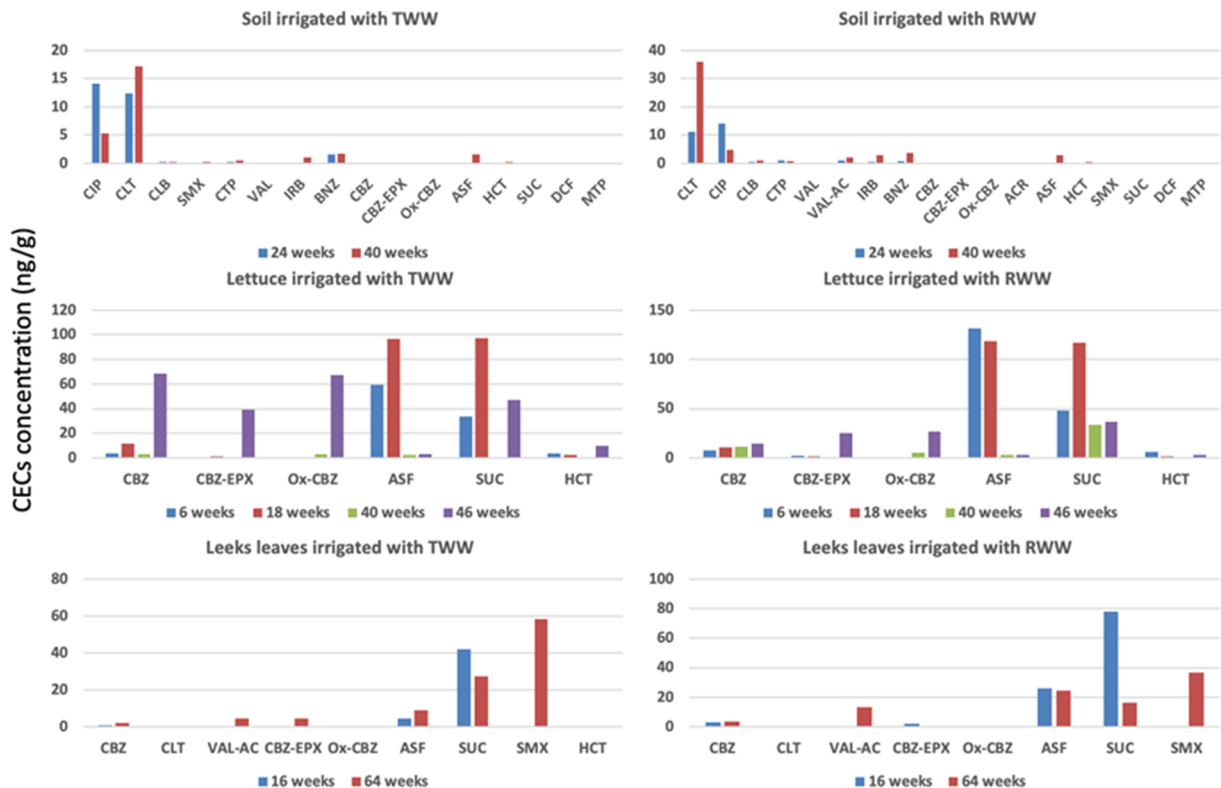


Figure 2

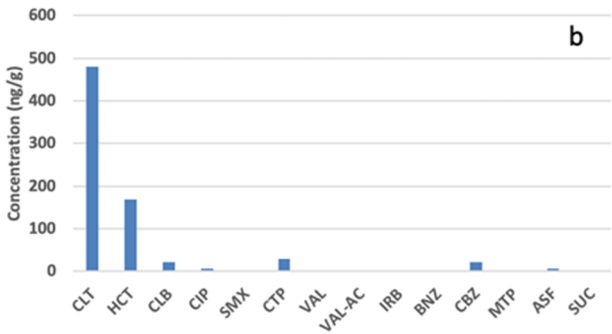
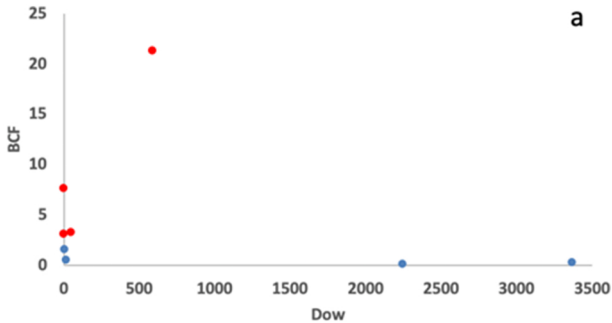


Figure 3

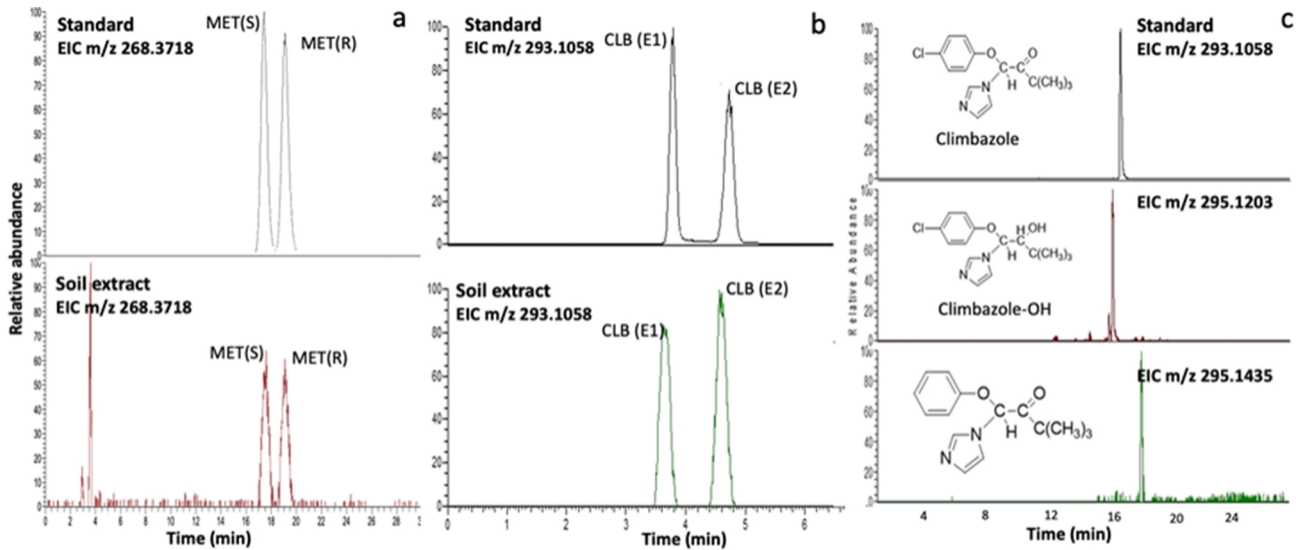


Figure 4

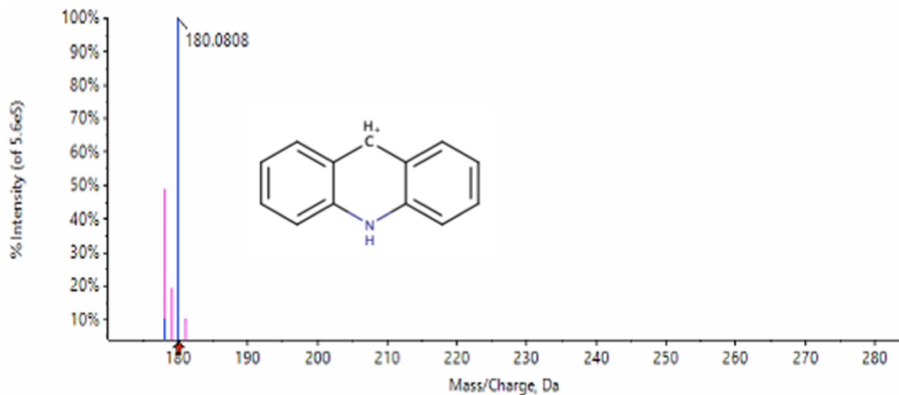
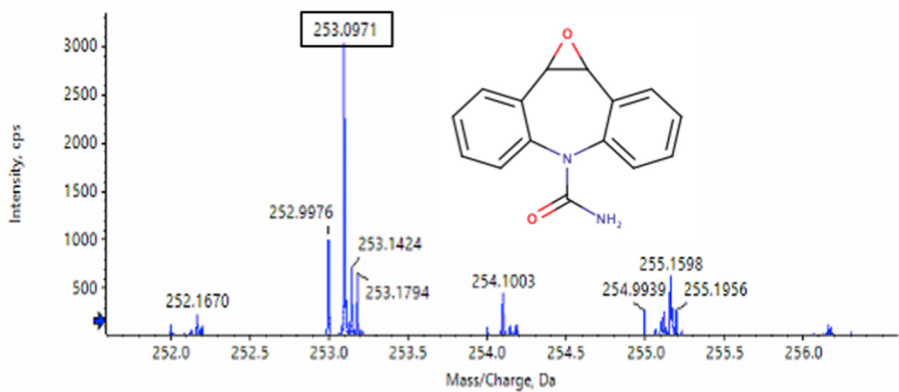
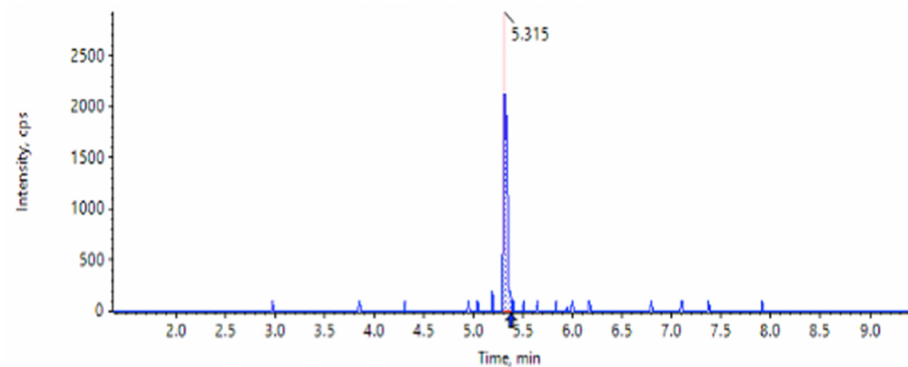


Figure 5