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Predicting the uptake of emerging organic contaminants in vegetables irrigated with treated wastewater – Implications for food safety assessment

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Abstract

Emerging organic contaminants (EOCs) undergoing incomplete removal during wastewater treatment may be found in treated wastewater (TWW) used for irrigation of agricultural products. Following uptake into edible plant parts, EOCs may eventually enter in the food chain, with associated human exposure. In the present study, we used a newly developed steady-state plant uptake model with added phloem transport to predict the uptake of four EOCs (carbamazepine, ibuprofen, ketoprofen and naproxen) into three varieties of lettuce. Input data were derived from an experimental study with vegetables grown in greenhouse and irrigated with TWW spiked with CBZ at 0, 30, 60, 120 and 210 µg/L in each variety of lettuce. Predicted carbamazepine concentrations in leaves were on average 82% higher than in roots, with good agreement between measured and calculated data. We subsequently predicted the uptake of anti-inflammatory compounds ibuprofen, ketoprofen and naproxen, for which the chemical analysis could not provide concentrations above detection limit. These three substances are weak acids and predicted concentrations in roots were higher than in the edible leaves, mainly due to phloem transport downwards. The daily dietary intake of all four EOCs was estimated for consumption of leafy vegetables, being far below usual therapeutic doses.

Keywords. Pharmaceuticals, treated wastewater reuse, lettuce, greenhouse, human risk, modelling

Introduction

Emerging organic contaminants (EOCs) are defined as organic chemicals of widespread human use (e.g., pharmaceuticals) that can have detrimental effects to living organisms in the environment [1, 2]. Although generally found in the environment at comparably low concentrations (ng L^{-1} to $\mu\text{g L}^{-1}$), their short- and long-term effects are not completely understood [3]. Nowadays, some EOCs are already involved in monitoring plans and prioritization initiatives [4]. Following human use, EOCs can reach treated wastewater (TWW) treatment facilities, where they are typically not efficiently removed via conventional treatment processes [5]. Land application of sludge and irrigation with reclaimed water in agricultural production are the major routes for EOC dissemination in agroecosystems. Furthermore, EOCs have been detected in surface water used for irrigation in agriculture [6, 7]. A number of EOCs have been found to accumulate in food crops, hence revealing the potential for human exposure to EOCs as a result of irrigation [8, 9, 10, 11, 12].

Water scarcity is a growing concern globally and, due to continuously growing population, the pressure on water resources will grow, particularly in arid and semiarid regions [13]. Agriculture is the sector that consumes the highest water amounts at global level, accounting today for approximately 70% of total freshwater consumption. Nonetheless, FAO suggests increasing crop irrigation but with improved water management [14]. The EU Water Framework Directive [15] identified water reuse as an important measure and maximization of water reuse is a specific action within the EU's 'A Blueprint to Safeguard Europe's Water Resources' [16]. Accordingly, a water reuse policy should focus on enhancing TWW reuse in agricultural irrigation. The European Commission has proposed in May 2018 new guidelines to facilitate water reuse in the EU for agricultural irrigation [17]. These guidelines describe the requirements for safe reclaimed water reuse for irrigation. Additional or stricter requirements for water quality may be set at a later stage, for example for EOCs.

Few studies have been conducted on the occurrence of pharmaceuticals in TWW throughout the Murcia Region [18] and on their uptake in crops irrigated with TWW [19]. The Region of Murcia (located in southeastern Spain) has a high and growing production of vegetables and fruits for the Spanish [20] and European market. The climate of this region is semi-arid Mediterranean, with an average annual temperature of 18 °C and scarce annual rainfall, around 300 mm [21]. Irrigated agriculture in the region (which has millennial tradition [22]) has outspread and restructured in recent decades [23], with the irrigated area (188,534 ha)

currently representing 17% of the total regional area. Despite scarce natural water resources, there is high and growing water demand for urban (including tourism along the coast), industrial and irrigation purposes [24]. In the last case, this challenge has been addressed (among other solutions) through the use of TWW. [25]. Currently, 93 treatment facilities in the region treat ca. $115 \text{ Hm}^3 \text{ year}^{-1}$ of generated TWW (equivalent to 10% of the natural water resources [24, 26]), which thus represents an alternative water resource for agriculture.

In recent years, analytical methods have been developed for the determination of pharmaceutical compounds in order to improve sensitivity and accuracy to be able to quantify low concentrations in environmental samples [27, 28]. For data interpretation, priority setting and forward prediction, a number of empirical and process-based models have been developed for the prediction of uptake and accumulation of EOCs in vegetables and fruits [29, 30]. Process-based models are typically based on multi-compartment approaches, combining the description of chemical transport with that of plant physiology. Although providing for more detailed predictions, process-based models have been used less frequently due to the larger data requirement and to the necessity of adapting them to the specific conditions of the study area [31]. Furthermore, their use has been limited to sewage sludge application [32], hence lacking application to TWW-irrigated systems. Many emerging contaminants in TWW are ionizable. The transport of ionic compounds requires the consideration of additional processes, such as ion trap and electrical interaction [33]. Weak acids may also undergo ion trapping in the alkaline phloem sap [33] with subsequent downwards transport, while phloem transport is of marginal relevance for neutral compounds.

This study aimed to (i) adapt and parameterize a simple steady-state model for the specific situation of greenhouse-grown, TWW-irrigated lettuce in Murcia, (ii) test this simulation tool against experimental data of an EOC (carbamazepine, CBZ), (iii) predict the uptake of other EOCs (ibuprofen, IBP; ketoprofen, KTP; naproxen, NPX) for this specific situation, and (iv) to evaluate implications of irrigation with TWW containing these EOCs for food safety and human exposure via dietary intake.

Materials and methods

Experimental section

Empirical data used in this study were taken from the experiment described in [19]. Briefly, the experiments were conducted in a greenhouse where three varieties of lettuce (*Lactuca sativa*) were grown in a coconut fiber substrate and irrigated with the WWTP effluent. The lettuces were irrigated with TWW mixed with CBZ at 0, 30, 60, 120 and 210 µg/L in each variety of lettuce (Iceberg lettuce var. Gitana, Mini Romane var. Jabera and Oak leaf var. Kiprien) for a period of two weeks in order to study CBZ uptake in the leaves and roots of the lettuces. Drip irrigation was used, making root uptake the only relevant uptake pathway of CBZ.

At the end of the experiment, roots and leaves of lettuces were separated and analyzed with an UPLC Acquity I-Class System and HR-QTOF-MS maXis Series (Daltonik GmbH, German, Bruker). The materials of lettuce (roots and leaves) were cut into small fragments, freeze dried and stored at -20°C until analysis. The dried plant tissue samples were extracted using a method developed by Wu et al., 2012b [28] and Wu et al., 2013 [34] and analysis procedure was performed according to Martínez-Alcalá et al., 2017 [35].

Briefly, a 0.2 g aliquot of freeze-dried plant material was extracted in an ultrasonic water bath (50/60 Hz, Fisher) for 20 min using 20 mL of methyl tert-butyl ether (MTBE) as the solvent. Then, it was centrifuged at 3,000 rpm for 20 min and the supernatant was decanted into a 40 mL glass vial and the residue was extracted using one more time 20 mL of acetonitrile. The combined extracts were dried under nitrogen at 30 °C and re-dissolved in 1 mL methanol, followed by the addition of 20 mL DI water. The aqueous solution was loaded onto a hydrophilic-lipophilic balance cartridge (Oasis HLB; 60 mg, 3 mL; extraction cartridges from Waters Corporation, Dublin, Ireland), which was preconditioned with 5 mL of MTBE, 5 mL of methanol and 5 mL of DI water. The sample was loaded onto the cartridges at a rate of 15 mL/min. After the cartridge was dried with nitrogen, the analytes were then eluted using 5 mL of methanol followed by 5 mL of 10/90 (v/v) methanol/MTBE. The resulting extract was dried and brought to a final volume of 1 mL using methanol. Samples were filtered using nylon filters (25 mm, 0.45 µm pore size) before instrumental analysis. Each sample was prepared in duplicate. Limits of detection (LOD) and quantification (LOQ) for CBZ in

WWTP effluent, were 1 ng L⁻¹ and 4 ng L⁻¹ respectively, for roots were 0.05 and 8.02 ng·g⁻¹ dw, respectively, and for leaves 0.10 and 9.01 ng·g⁻¹ dw, respectively.

Chemicals

The physicochemical properties of the investigated EOCs (CBZ, IBP, KTP and NPX) are listed in Table 1. The listed properties were used as input for the plant uptake model. Notably, CBZ is a neutral compound (pK_a is well beyond the range of typical pH conditions), while IBP, KTP and NPX are weak acids.

Table 1. Physicochemical properties of the EOCs.

Property	CBZ	IBP	KTP	NPX
CAS number	298-46-4	15687-27-1	22071-15-4	22204-53-1
Molar mass (g/mol) ^a	236.27	206.28	254.28	230.26
pK _a [charge] ^a	13.9[0]	4.3[0/-]	4.1[0/-]	4.4[0/-]
log K _{OW} ^a	2.30	3.50	3.10	3.20
log K _{OW ion} ^a	0	-0.47	0.65	-0.84
log K _{AW} ^a	-8.35	-5.21	-9.06	-7.85
log K _{HSA} ^a	3.74	4.42	5.21	5.16

^a [36] ACD/i-Lab (<https://weblab.acdlabs.com/iLab2/index.php>)

Model section

A dynamic plant uptake model was adapted to study the accumulation of pharmaceuticals in roots and leaves. Most existing plant uptake models were developed for neutral compounds [31, 32, 37, 38, 39, 40], while most pharmaceuticals are ionisable [41]. The recently used dynamic model [31] was modified to consider additional processes relevant for ionic compounds, such as (i) adsorption to proteins, (ii) speciation and ion trapping [33] and (iii) phloem transport of weak acids.

(i) *Adsorption to proteins.* The partitioning between plant cells and water is calculated for the neutral and for the ionic species. The partition coefficient is the endpoint of diffusion between two different phases. Neutral organic compounds distribute mainly into the water W and the lipid phase L (the latter described by the K_{OW}) while ionic compounds may predominantly adsorb to proteins. For the partitioning between plant cells and water K_{PW} (L/L), the following equation was used:

$$K_{PW} = W + L a K_{OW}^b + \text{Protein } K_P \quad (1)$$

where W is the water content (L/L), L is the lipid content (g/g), $a = 1.22$ L/kg is 1/density of octanol (unit conversion), b is an empirical exponent (0.85, average derived from [42]). The Partition Coefficient n-Octanol to Water (K_{OW}) is either entered for the neutral compound or for the ion. Protein is the protein content (0.18 L/L in the cytoplasm, else 0) and K_P is the adsorption coefficient to proteins. Estimated or measured values are usually available for human serum albumin (K_{HSA}) and were also used here (ACD/i-Lab, Table 1).

(ii) *Speciation, electrical attraction and ion trapping.* The fraction of neutral and ionic molecules, and their distribution between the four plant compartments (cytoplasm, vacuoles, phloem and xylem) was calculated using the cell model [43, 44], similarly to earlier studies [45]. The cell model solves the Nernst-Planck equation for diffusion of ions across charged cell membranes and thus intrinsically considers the effects of electrical repulsion and attraction of anions and cations, respectively, by the negatively charged (-120 mV) plasmalemma membranes [42].

(iii) *Phloem transport.* Sugars photosynthesized in the leaves are transported to all growing plant parts and to roots via the phloem. During the day, the phloem flow is only a few percent of the transpiration stream in the xylem. However, during night, downward phloem flow of water can match the upward flow of water (own observation, unpublished). Phloem sap is weakly alkaline (pH = 8), and therefore, the transport is mostly relevant for weak acids which are trapped in phloem. In the present study, the phloem flow was assumed to be 10% of the xylem flow.

Considering the irrigation practice used experimentally, root uptake was considered as the dominant pathway of EOC uptake from irrigation water, while direct uptake from leaves was assumed negligible. Transport of chemical is via xylem from soil to roots to leaves, and via phloem from leaves to roots. In the analytical steady-state solution, the concentration of C_1 in soil is constant, C_2 is the concentration in roots, C_3 is the concentration in leaves. The underlying system of differential equations, describing the chemical mass balances in the plant, is

$$\frac{dC_2}{dt} = k_{12}C_1 - k_2C_2 + k_{32}C_3 \quad (2)$$

$$\frac{dC_3}{dt} = k_{23}C_2 - k_3C_3 \quad (3)$$

with the steady-state solution:

$$C_2 = \frac{k_{12}k_3C_1}{k_2k_3 - k_{23}k_{32}} \quad (4)$$

$$C_3 = \frac{k_{23}C_2}{k_3} \quad (5)$$

where k_{12} is transfer rate from 1 (soil) to 2 (roots); k_2 is loss rate from 2 (roots) and k_{32} is the transfer rate within the phloem from (3) leaves to roots (2). Similarly, k_{23} is the transfer rate via xylem from 2 (roots) to 3 (leaves), and k_3 is total loss from leaves. Units are d^{-1} (rates k) and mg/kg (concentrations C). Transfer rates were calculated based on experimental measurements (e.g., water transpiration by plant, plant mass), on physico-chemical properties (pKa, KOW, K_hSA, allowing for the prediction of partitioning to soil and to plant tissues) and text-book knowledge (e.g., phloem flows, intracellular pH and charge). Transfer rates were derived as described in [30] and [40], except for the transport rate via phloem transport from leaves to roots, rate k_{32} . The change of mass of chemical in roots due to transport of chemical from leaves via phloem is phloem flow Q_P (L/d) times concentration of chemical in the phloem sap C_P (mg/L). The phloem is loaded in the leaves; K_{PL} (L/kg) is the ratio of the chemical concentration in phloem (mg/L) and leaves (mg/kg) and is calculated using the cell model under consideration of partitioning to lipids and proteins, electrical attraction or repulsion, and ion trap effects. For the mass transport in phloem dm_P/dt (mg/d) follows then that $dm_P/dt = Q_P K_{PL} C_L$. Eq. 2 (change of concentration in roots) requires conversion to concentration, which is achieved by dividing with root mass. The resulting transfer rate from leaf to roots, k_{32} (d^{-1}), is then

$$k_{32} = K_{PL} \frac{Q_P}{M_{roots}} \quad (6)$$

where Q_P is phloem flow (L/d, set to 10% of xylem flow) and M_{roots} is the mass of roots (kg, Table 2). The rate k_{32} was also added to k_3 as a loss for the leaf (dividing by M_{leaves} instead of M_{roots}). The lettuces were grown under hydroponic conditions and were planted in bags of coconut fiber substrate. The dense root growth in the coconut fiber substrate did not allow to extract water and analyse the liquid phase concentration of the chemicals in the pot. The concentration in coconut fibers was measured at the end of the experiment, and the

equilibrium concentration in the aqueous phase within the C_{solution} (mg/L) pot was calculated from

$$C_{\text{solution}} = \frac{V_w C_w}{C_w + K_d M_{\text{coco}}} \quad (7)$$

where V_w is the volume of water (0.5 L), C_w is the (nominal) concentration in the irrigation water (mg/L), K_d (L/kg), is the distribution coefficient between coconut fiber and water (measured concentration in coconut fibers divided by C_w) and M_{coco} is the mass of coconut fibers in the pot (2 kg). The calculated C_{solution} was used to determine the concentration in soil (C_1) based on soil-water partitioning coefficient. The model and the code with description is available under gnu3-license at homepage.env.dtu.dk/stt/2017Release_Plant_Model/index.htm [46].

Plant data

Plant properties can significantly affect the outcome of the model simulations, and site- and plant-specific input data [30, 33] are preferable for conducting simulations of plant uptake. The investigated variants of lettuce were: (i) Iceberg lettuce var. Gitana, (ii) Mini Romane var. Jaber and (iii) Oak leaf var. Kiprien. Their growth was measured in a parallel experiment in August 2016. Data derived from the middle of the growth period (day 13) are shown in Table 2. Growth rates were derived by a fit of the logistic growth curve to the measured weights. Dry weight data, protein content and lipid content of lettuce were taken from a nutrition table [47] (Table 2).

A preliminary sensitivity analysis revealed that the simulation results changed about a factor of 2, compared to calculations with default data when the growth parameters (plant mass and growth rates) were adapted to the measured conditions (concentrations of CBZ in roots decreased and in leaves increased, while for the anti-inflammatory compounds both concentrations increased). Protein content was found to have a greater influence on the model output than the lipid content, in particular for the three weak acids, for which adsorption to proteins leads to retention in roots. The consideration of phloem transport leads to a strong decline of calculated concentrations in leaves of the weak acids (IBP, KTP, NPX). The water content only affected the results in dry weight, because the re-calculation from fresh to dry

weight requires to divide by the dry matter content, while the fresh weight concentration was almost unaffected.

Table 2. Input data for the simulation of plant uptake measured data for three lettuce variants.

Plant	Leaf mass	Growth rate (leaves)	% Dry weight	Transpiration	Protein	Lipid
	g	1/d	(%)	L/d	% wt	% wt.
Iceberg	460	0.27	5.3	2.79	1.3	0.2
Romane	1000	0.17	5.3	2.79	1.3	0.2
Oak leaf	625	0.21	5.3	2.79	1.3	0.2
Default	1000	0.035	20	1.0	none	2

Human exposure of EOCs predicted in lettuce

The daily dietary intake (DDI) of each chemical per kilogram bodyweight (ng/kg d) was calculated [48] by multiplying the consumption of leafy vegetables (g/d lettuce, endive and similar, Spain average 9.83 g/d) [49] with the prediction of concentration (C (ng g⁻¹)), and dividing by the bodyweight (bw) (kg, Spain average 70 kg):

$$DDI = \frac{C \times consumption}{bw} \quad (8)$$

Results

Prediction the uptake of CBZ in lettuce

Uptake of CBZ in leaves and roots by lettuce plants was evaluated for the three varieties of lettuce (Iceberg lettuce var. Gitana, Mini Romane var. Jabera and Oak leaf var. Kiprien). The predicted concentrations of CBZ for all three varieties were higher in leaves than in roots. CBZ is not strongly adsorbing, it is also not volatile or rapidly degraded, and therefore it is translocated to leaves and accumulates there. In Figures 1 and 2 the measured and predicted results are shown. The simulation results for the three varieties of lettuce differ not only due to varying growth, but also due to different (measured) adsorption to coconut fibers. The higher concentrations of CBZ detected in leaves are relevant because the leaves of lettuce are consumed.

The predicted concentrations of CBZ showed a strong relationship to the measured results, with coefficients of determination (R^2) above 90% both for leaves and roots. This strong linear relationship holds for all three varieties of lettuce studied. However, there is a systematic underestimation of uptake into roots with subsequent overestimation of translocation to leaves. This indicates that adsorption of CBZ to roots is somewhat higher than calculated. According to the model calculations, adsorption is mainly due to the protein fraction (and not to lipids) of the plant tissue.

Figure 1. Comparative graphics in Iceberg lettuce (IL), Mini Romane lettuce (MRL) and Oak leaf lettuce (OLL) of the results of CBZ in the experiment and the prediction of the model in leaves

Figure 2. Comparative graphics in Iceberg lettuce (IL), Mini Romane lettuce (MRL) and Oak leaf lettuce (OLL) of the results of CBZ in the experiment and the prediction of the model in roots

Prediction the uptake of IBP, KTP and NPX in lettuce

The plant uptake model was used to evaluate and predict the uptake of IBP, KTP and NPX into the leafy vegetables grown in Murcia. The uptake predictions were obtained for the three varieties of lettuce (Iceberg lettuce var. Gitana, Mini Romane var. Jabera and Oak leaf var. Kiprien), and results are shown in Figures 3 and 4. Plant accumulation followed the order of $KTP > NPX > IBP$ in leaves and $NPX > IBP > KTP$ in roots. Interestingly, predicted concentrations in roots are far above those in leaves, in agreement with other studies [50, 51]. The uptake and translocation of weak acids ($pK_a > 3$) is known to be strongly influenced by ion trapping in phloem ($pH=8$), where these substances are present almost exclusively as ionized species with limited permeability through plant cells. Higher accumulation in roots may have therefore been determined by combined ion trapping and phloem transport from leaves to roots.

The plant uptake model used by Hurtado et al. (2016) [40] had not been able (and was not applied) to reproduce the measured concentrations of IBP, nor the observed trend (i.e. root concentrations $\gg$ leaf concentrations) and the main difference being the inclusion of the two processes of adsorption to proteins and transport leaf-to-roots with phloem. Both processes are highly relevant for weak acids (e.g., as a result of ion trapping in the phloem), while for

neutral lipophilic compounds they do not play a relevant role. Most likely, the predicted concentrations overestimate empirical ones, as predicted concentrations in roots are at a level that is clearly above LOD. However, ibuprofen and probably also ketoprofen and naproxen can be readily metabolised in the root zone and inside the plant, a process that has not been considered in the calculations due to a lack of existing degradation rate values.

As can be seen in Figures 3 and 4 at a EOCs dose of $210 \mu\text{g L}^{-1}$ in the irrigation water (mixture of IBP, KTP and NPX), resulted in a prediction concentration in Iceberg lettuce leaves of $25.20 \text{ ng g}^{-1} \text{ dw}$ for IBP, $72.60 \text{ ng g}^{-1} \text{ dw}$ for KTP and $48.60 \text{ ng g}^{-1} \text{ dw}$ for NPX with the respective values in roots being $612.30 \text{ ng g}^{-1} \text{ dw}$ for IBP, $426.90 \text{ ng g}^{-1} \text{ dw}$ for KTP and $1444.30 \text{ ng g}^{-1} \text{ dw}$ for NPX. The results in the prediction concentration in Mini Romane lettuce leaves were $30.60 \text{ ng g}^{-1} \text{ dw}$ for IBP, $83.80 \text{ ng g}^{-1} \text{ dw}$ for KTP and $64.50 \text{ ng g}^{-1} \text{ dw}$ for NPX with the respective values in roots of $764.00 \text{ ng g}^{-1} \text{ dw}$ for IBP, $584.40 \text{ ng g}^{-1} \text{ dw}$ for KTP and $2121.60 \text{ ng g}^{-1} \text{ dw}$ for NPX. In Oak leaf lettuce leaves, resulted in a prediction concentration of $28.40 \text{ ng g}^{-1} \text{ dw}$ for IBP, $83.50 \text{ ng g}^{-1} \text{ dw}$ for KTP and $59.00 \text{ ng g}^{-1} \text{ dw}$ for NPX with the respective values in roots of $692.80 \text{ ng g}^{-1} \text{ dw}$ for IBP, $505.00 \text{ ng g}^{-1} \text{ dw}$ for KTP and $1780.60 \text{ ng g}^{-1} \text{ dw}$ for NPX.

Figure 3. Comparative graphics of predictions of ibuprofen (IBP), ketoprofen (KTP) and naproxen (NPX) in leaves in Iceberg lettuce (IL), Mini Romane lettuce (MRL) and Oak leaf lettuce (OLL)

Figure 4. Comparative graphics of predictions of ibuprofen (IBP), ketoprofen (KTP) and naproxen (NPX) in roots in Iceberg lettuce (IL), Mini Romane lettuce (MRL) and Oak leaf lettuce (OLL)

Human exposure of IBP, KTP and NPX predicted in lettuce

The assessment of human exposure via dietary intake was done only for the edible parts of lettuce, i.e. the leaves. The concentrations of IBP, KTP and NPX predicted for roots are thus of no relevance for human exposure.

The highest concentration predicted in the leaves of the three varieties of lettuce were 28 ng g⁻¹ dw of IBP, 80 ng g⁻¹ dw of KTP and 57 ng g⁻¹ dw of NPX, leading to a daily uptake of 575 ng/d (IBP), 786 ng/d (KTP) and 560 ng/d (NPX). For the risk assessment of the DDI, we compared these numbers to the lowest therapeutic dose (LTD) with adverse effects [52, 53], which ranges from 1.4 to 17 mg kg bw⁻¹ d⁻¹ (Table 3). Of course, a therapeutic intake of these pharmaceuticals is not intended to last life-long, while the ingestion data are annual average. There should thus be a sufficient distance (> factor 10 to factor 1000, [54]) between DDI and LTD to consider the consumption as safe. This is ensured for all three chemicals. The estimated quantities of IBP, KTP and NPX ingested per day according to the prediction results, are clearly far below the acceptable limits.

Maximum measured concentrations of IBP, KTP and NPX in effluent water from ten wastewater treatment plants in the region of Murcia [18] were 1.39 $\mu\text{g/L}$ for KTP, 3.7 $\mu\text{g/L}$ for NPX, and 0.36 $\mu\text{g/L}$ (IBP), i.e. 57 to 583 times lower the highest spiked dosage in the experiments, which was used for the risk assessment, giving an additional safety margin.

Table 3. Predicted daily dietary intake DDI of EOC by consumption of irrigated lettuce leaves compared to the lowest therapeutic dose LTD with adverse effects.

EOC	DDI of EOC consumed ($\text{ng}\cdot\text{kg bw}^{-1}\cdot\text{day}^{-1}$)	LTD ($\text{ng}\cdot\text{kg bw}^{-1}\cdot\text{day}^{-1}$)
IBP	3.9	$17 \cdot 10^6$ ^b
KTP	11.2	$1.4 \cdot 10^6$ ^b
NPX	8.0	$4 \cdot 10^6$ ^a

^a [52] Bruce et al., 2010

^b [53] Webb et al., 2003

Discussion

Comparison to other findings

A number of studies have evaluated the uptake of CBZ, IBP, NPX and KTP in vegetables (including lettuce) irrigated with TWW. Overall, CBZ has exhibited the most prominent uptake, while anti-inflammatory compounds could be rarely quantified. In a field study with 19 different EOCs at <1–225 ng/L concentrations in irrigation water, Wu et al. (2014) [10] observed comparably high uptake of CBZ in lettuce and two other leafy vegetables (cabbage and spinach), while no quantifiable levels could be determined for IBP and NPX in lettuce. Following irrigation with TWW, CBZ concentrations of 100–500 ng g^{-1} dw were observed in leaves of cucumber and tomato plants, while IBP and KTP could not be quantified [55], thus reflecting observations made in the current study at the lowest spiking level. Furthermore, CBZ uptake and its subsequent transformation to 10,11-epoxycarbamazepine in carrot and potato has been reported [56]. Conversely, IBP and NPX could be quantified in lettuce irrigated with reclaimed water, while no detectable levels of CBZ could be determined [57]. IBP uptake into plants has been measured in a pot experiment with perlite and lettuce [40]. The concentration in irrigation water was 4, 10, 20 and 40 $\mu\text{g/L}$ and measured concentrations of IBP at the highest nominal concentration at the end of the experiment was in roots 223 $\mu\text{g/kg}$ and in edible leaves 23.5 $\mu\text{g/kg}$. Thus, like in our calculation, concentration in roots was clearly higher than in leaves.

Data from that study were used with the goal to identify the biodegradation of emerging organic contaminants in the soil-plant system [58]. However, IBP was not included in the calculations because the plant uptake model was only capable to calculate uptake of neutral compounds. This gap has been closed with the model expansion presented in this study. The two model approaches are, apart from the described adaptations, basically identical.

Limitations and shortcomings of model simulations

The plant uptake model for ionisable compounds (such as pesticides and pharmaceuticals) contains parameters that are rarely known in detail and variable in time. The model therefore cannot be expected to predict exact concentrations. The main purpose is to understand the underlying processes of uptake and transport in plant and the analyses of the most relevant parameters and processes for a given set of conditions. The steady-state 2x2-matrix solution used here cannot consider changes of growth or input conditions. To test the model, it has been compared to earlier versions for neutral compounds (e.g., with the model described in Trapp 2015 [31]) which have been extensively validated. With identical input data, both model types deliver the same results as long as ionisation can be neglected. Conversely, the results for mostly ionised substances have to be considered carefully, as $\log K_{ow}$ (or $\log D$) is not a reliable predictor of adsorption and membrane permeability of ions [59].

Risk stemming from metabolites

In the current study, the uptake and accumulation of pharmaceuticals was simulated by considering negligible dissipation in plant tissues, due to the absence of data on in-plant degradation kinetics. Nevertheless, it has been reported that many pharmaceuticals and other organic substances are metabolized in plants, and their intermediate or end products still contain bioactive functional moieties [55, 60, 61]. Among these transformation products (TPs), 10,11-epoxycarbamazepine was found to be the major metabolite of CBZ in sweet potato and carrot leaves [56] and demonstrated a toxic potency even higher than that of the parent compound CBZ [62]. For IBP, the enantiomeric fractionation of racemic ibuprofen was observed [40]. Therefore, neglecting the formation of TPs of pharmaceuticals taken up by plants may underestimate their risk to ecosystem and human health. Based on the present dataset, we can justify predictions of the uptake of parent pharmaceuticals in lettuce. On the other hand, in-plant degradation can significantly lower uptake and accumulation [40, 63],

and has not been considered in the calculations above. To date, human ingestion of parent pharmaceuticals and respective TPs from TWW-irrigated vegetables (including lettuce) has been investigated and demonstrated only for CBZ [64], and further work is required to estimate the risk associated to chronic exposure.

Conclusions

This study presents the first test of a newly adapted steady-state model (with phloem transport to roots) for plant uptake of EOCs. For the only quantifiable chemical (carbamazepine), model simulations showed comparably good agreement to empirical results obtained in a greenhouse experiment with TWW irrigated lettuce, confirming higher uptake in leaves than in roots. For three anti-inflammatory compounds (ibuprofen, ketoprofen, naproxen), simulation results were used to predict accumulation in edible lettuce parts and concurrently estimate human exposure via food ingestion. For the irrigation of leafy vegetables with TWW, we can with some certainty exclude the occurrence of relevant human exposure and associated health risks.

Overall, the presented approach could be used to identify yet uninvestigated EOCs for their potential plant uptake. The outcome can be used for an initial risk assessment, and for priority setting among the numerous pharmaceuticals and personal care products present in urban TWW, particularly when TWW is reclaimed for irrigation of agricultural products. Further studies should nevertheless confirm the validity of the presented modelling approach for a larger set of EOCs and testing conditions.

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References

- [1] Kümmerer, K. Emerging contaminants versus micro-pollutants. *Clean. - Soil, Air, Water* **2011**, 39 (10), 889–890.
- [2] Petrie, B., Barden, R., Kasprzyk-Hordern, B. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* **2015**, 72, 3–27.
- [3] Mandaric, L., Celic, M., Marcé, R., Petrovic, M. Introduction on Emerging Contaminants in Rivers and Their Environmental Risk. In *Emerging Contaminants in River Ecosystems: Occurrence and Effects under Multiple Stress Conditions*, vol. 46. *Springer International Publishing*, **2015**, 3–25.
- [4] NORMAN. Norman network. www.norman-network.net. **2017** (Accessed 24May 2018).
- [5] Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E. Occurrence, fate and effects of pharmaceutical substances in the environment- a review. *Chemosphere*, **1998**, 36, 357–393.
- [6] Calderón-Preciado, D. Matamoros, V. Bayona, J.M. Occurrence and potential crop uptake of emerging contaminants and related compounds in an agricultural irrigation network. *Sci. Total Environ.* **2011**, 412–413, 14–19.
- [7] Kümmerer, K. Antibiotics in the aquatic environment—a review—part I. *Chemosphere*, **2009**, 75 417–434.
- [8] Miller, E. L.; Nason, S. L.; Karthikeyan, K. G.; Pedersen, J. A. Root uptake of pharmaceuticals and personal care product ingredients. *Environ. Sci. Technol.* **2016**, 50, 525–541.
- [9] Wu, X. Q.; Dodgen, L. K.; Conkle, J. L.; Gan, J. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. *Sci. Total Environ.* **2015**, 536, 655–666.
- [10] Wu, X.; Conkle, J. L.; Ernst, F.; Gan, J. Treated wastewater irrigation: uptake of pharmaceutical and personal care products by common vegetables under field conditions. *Environ. Sci. Technol.* **2014**, 48, 11286–11293.
- [11] Carter, L. J.; Harris, E.; Williams, M.; Ryan, J. J.; Kookana, R. S.; Boxall, A. B. Fate and uptake of pharmaceuticals in soil-plant systems. *J. Agric. Food Chem.* **2014**, 62, 816–825.
- [12] Christou, A., Agüera, A., Bayona, J.M., Cytryn, E., Fotopoulos, V., Lambropoulou, D. The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: the knowns and unknowns of the fate of antibiotics and

- antibiotic resistant bacteria and resistance genes—a review. *Water Res.*, **2017**, 123, 448–467.
- [13] Gerland, P. Raftery, A.E. Sevcíková, H. Li, N. Gu, D. Spoorenberg, T. Alkema, L. Fosdick, B.K. Chunn, J. Lalic, N. Bay, G. Buettner, T. Heilig, G.K. Wilmoth, J. World population stabilization unlikely this century, *Science*, **2014**, 346, 234–237.
- [14] K. Frenken, K., Gillet, V. Irrigation Water Requirement and Water With drawal by Country, Food and Agriculture Organization of the United Nations (FAO), *AQUASTAT (Ed.)*, **2012**, p. 264.
- [15] European Commission (EC). Directive 2000/60/EC (Water Framework Directive). Official Journal of the European communities. **2000**. December 2000.
- [16] EC European Commission. **2012**. A Blueprint to Safeguard Europe's Water Resources. <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52012DC0673&from=EN>
- [17] 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
- [18] Fernández-López, C., Guillén-Navarro, J. M., Padilla, J. J., Parsons, J. R. Comparison of the removal efficiencies of selected pharmaceuticals in wastewater treatment plants in the region of Murcia, Spain. *Ecol Eng*, **2016**, 95, 811–816.
- [19] González García, M., Fernández-López, C., Pedrero-Salcedo, F., Alarcón, J.J. Absorption of carbamazepine and diclofenac in hydroponically cultivated lettuces and human health risk assessment. *Agric Water Manag.* **2018**, 206, 42–47.
- [20] INE, **2017**. Instituto Nacional de Estadística. Censo Agrario 2014. Available in. <http://www.ine.es>. (Accessed 29 May 2018).
- [21] Aparicio, J., Candela, L., Alfranca, O., García-Aróstegui, J.L. Economic evaluation of small desalination plants from brackish aquifers. Application to Campo de Cartagena (SE Spain). *Desalination*, **2017**, 411, 38–44.
- [22] Gil-Olcina, A. Clima e hipótesis de cambio climático en la región geográfica del sureste ibérico. *Invest. Geográficas*, **2009**, 49.
- [23] Martínez-Paz, J.M., Perni, A., Ruiz-Campuzano, P., Pellicer-Martínez, F. Economic valuation of irrigation systems supply failures in the Segura basin (Valoración económica de los fallos de suministro en los regadíos de la cuenca del Segura). *Revista Española de Estudios Agrosociales y Pesqueros*, **2016**, 244, 35–67.
- [24] CHS, **2016**. Plan Hidrológico de la Cuenca del Segura. Confederación Hidrográfica del Segura. Memoria. Ministerio de Medio Ambiente, Madrid.

- [25] Qadir, M., Sharma, B.R., Bruggeman, A., Choukr-Allah, R., Karajeh, F. Non-conventional water resources and opportunities for water augmentation to achieve food security in water scarce countries. *Agric Water Manag*, **2007**, 87 (1), 2-22.
- [26] ESAMUR, **2017**. Entidad de Saneamiento y Depuración de Aguas Residuales de la Región de Murcia. <http://www.esamur.com>. (Accessed 29 January 2018).
- [27] Camacho-Muñoz, D., Martín, J., Santos, J.L., Aparicio, I., Alonso, E. An affordable method for the simultaneous determination of the most studied pharmaceutical compounds as wastewater and surface water pollutants. *J. Sep.Sci.* **2009**, 32, 3064–3073.
- [28] Wu X, Conkle JL, Gan J. Multi-residue determination of pharmaceutical and personal care products in vegetables. *J Chromatogr. A.* **2012b**, 1254: 78–86.
- [29] Trapp, S., T. Eggen, Simulation of the plant uptake of organophosphates and other emerging pollutants for greenhouse experiments and field conditions. *Environ. Sci. Pollut. Res.* **2013**, 20, 4018–4029.
- [30] Terzaghi, E., Zacchello, G., Scacchi, M., Raspa, G., Jones, K.C., Cerabolini, K.C., DiGuardo, A. Towards more ecologically realistic scenarios of plant uptake modelling for chemicals: PAHs in a small forest. *Sci. Total Environ.* **2015**, 505, 329–337.
- [31] Trapp, S. Calibration of a plant uptake model with plant- and site-specific data for uptake of chlorinated organic compounds into radish, *Environ. Sci.Technol.* **2015**, 49, 395–402.
- [32] Prosser, R.S., Trapp, S., Sibley, P.K. Modeling uptake of selected pharmaceuticals and personal care products into food crops from biosolids-amended soil. *Environ. Sci. Technol.* **2014**, 48, 11397–11404.
- [33] Polesel, F., Plósz, B.G., Trapp, S. From consumption to harvest: Environmental fate prediction of excreted ionizable trace organic chemicals. *Water Res.*, **2015**, 84, 85–98.
- [34] Wu, X., Ernst, F., Conkle, J. L., & Gan, J. Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables. *Environ. International.* **2013**, 60, 15-22.
- [35] Martínez-Alcalá, I, Guillén-Navarro, J.M., Fernández-López, C. Pharmaceutical biological degradation, sorption and mass balance determination in a conventional activated-sludge wastewater treatment plant from Murcia, Spain. *Chem. Eng. J.* **2017**, 95, 811–816.
- [36] ACD/i-Lab (<https://weblab.acdlabs.com/iLab2/index.php>).

- [37] Trapp, S.; Matthies, M.; Schunert, I.; Topp, E. M. *Environ. Sci. Technol.* **1990**, 24, 1246-1252.
- [38] Trapp S. Fruit tree model for uptake of organic compounds from soil and air. SAR - QSAR *Environ. Res.*, **2007**, vol 18, 3-4, 367-387.
- [39] Fantke, P., Juraske, R., Anton, A., Friedrich, R., Jolliet, O. Dynamic multicrop model to characterize impacts of pesticides in food. *Environ. Sci. Technol.*, **2011**, 45, 8842-8849.
- [40] Hurtado, C., Domínguez, C., Pérez-Babace, L., Cañameras, N., Comas, J., Bayona, J.M., (2016). Estimate of uptake and translocation of emerging organic contaminants from irrigation water concentration in lettuce grown under controlled conditions. *J. Hazard. Mater.*, **2016**, 305, 139-148.
- [41] Manallack, D. T. The pKa distribution of drugs: Application to drug discovery. *Perspect. Med. Chem.*, **2007**, 1, 25-38.
- [42] Briggs, G.G., Bromilow, R.H., Evans, A.A. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pest. Sci.*, **1982**, 13, 495-504.
- [43] Trapp S. Plant uptake and transport models for neutral and ionic chemicals. *Environ. Sci. Pollut. Res.*, **2004**, 11, 33-39.
- [44] Trapp, S.; Horobin, R. W. *Eur. Biophys. J.* **2005**, 34, 959.
- [45] Buchholz, A., Trapp, S. How active ingredient localisation in plant tissues determines the targeted pest spectrum of different chemistries. *Pest Manage. Sci.*, **2016**, 72, 929-939.
- [46] DTU Plant model. homepage.env.dtu.dk/stt/2017Release_Plant_Model/index.htm
- [47] Elmadfa, I., Aign, W., Muskat, D., Fritsche, D., Cremer, H. D. Die grosse GU Nährwert Tabelle. *Gräfe und Unzer*, **1991**, Giessen, Germany.
- [48] Legind, C. N., Trapp, S. Modeling the exposure of children and adults via diet to chemicals in the environment with crop-specific models. *Environ. Pollut.*, **2009**, 157, 778-785.
- [49] MAPA, 2017. <https://www.mapama.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/panel-de-consumo-alimentario/ultimos-datos/default.aspx>
- [50] Dodgen, L.K., Li, J., Parker, D., Gan, J.J. Uptake and accumulation of four PPCP/EDCs in two leafy vegetables. *Environ. Pollut.*, **2013**, 182, 150-156.

- [51] Tanoue, R., Sato, Y., Motoyama, M., Nakagawa, S., Shinohara, R., Nomiyama, K. Plant uptake of pharmaceutical chemicals detected in recycled organic manure and reclaimed wastewater. *J. Agr. Food Chem.*, **2012**, 60, 10203–10211.
- [52] Bruce, G. M., Pleus, R. C., Snyder, S. A. Toxicological Relevance of Pharmaceuticals in Drinking Water. *Environ. Sci. Technol.*, **2010**, 44, 5619–5626.
- [53] Webb, S., Ternes, T., Gibert, M., Olejniczak, K. Indirect human exposure to pharmaceuticals via drinking water. *Toxicol. Lett.*, **2003**, 142, 157–167.
- [54] Prosser, R.S., Sibley, P.K. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. *Environment International*, **2015**, 75 223–233.
- [55] Goldstein, M.; Shenker, M.; Chefetz, B. Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. *Environ. Sci. Technol.*, **2014**, 48, 5593–5600.
- [56] Malchi, T.; Maor, Y.; Tadmor, G.; Shenker, M.; Chefetz, B. Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. *Environ. Sci. Technol.*, **2014**, 48, 9325–9333.
- [57] Calderón-Preciado, D. Matamoros, V. Savé, R., Muñoz, P., Biel, C., Bayona, J.M. Uptake of microcontaminants by crops irrigated with reclaimed water and groundwater under real field greenhouse conditions. *Environ. Sci. Pollut. Res.*, **2013**, 20, 3629–3638.
- [58] Hurtado, C., Trapp, S., Bayona, J.M. Inverse modeling of the biodegradation of emerging organic compounds in the soil-plant system. *Chemosphere*, 2016, **156** 236–244.
- [59] Droge, S. T. J.; Goss, K. U. Development and evaluation of a new sorption model for organic cations in soil: Contributions from organic matter and clay minerals. *Environ. Sci. Technol.* **2013**, 47, 14233–14241.
- [60] Macherius, A., Eggen, T., Lorenz, W., Moeder, M., Ondruschka, J., Reentsma, T. Metabolization of the bacteriostatic agent triclosan in edible plants and its consequences for plant uptake assessment. *Environ. Sci. Technol.*, **2012**, 46 (19), 10797–10804.
- [61] Riemenschneider, C.; Seiwert, B.; Moeder, M.; Schwarz, D.; Reemtsma, T. Extensive Transformation of the Pharmaceutical Carbamazepine Following Uptake into Intact Tomato Plants. *Environ. Sci. Technol.* **2017**, 51, 6100–6109.
- [62] Tomson, T., Almkvist, O., Nilsson, B.Y., Svensson, J.O., Bertilsson, L. Carbamazepine-10,11-epoxide in epilepsy. A pilot study. *Arch. Neurol.* **1990**, 47, 888–892.

- [63] Jacobsen, R.E., Fantke, P., Trapp, S. Analyzing half-lives for pesticide dissipation in plants. *SAR/QSAR Environ. Res.*, **2015**, 26, 325–342.
- [64] Paltiel, O., Fedorova, G., Tadmor, G., Kleinstern, G., Maor, Y., Chefetz, B. Human exposure to wastewater-derived pharmaceuticals in fresh produce: A randomized controlled trial focusing on carbamazepine. *Environ. Sci. Technol.*, **2016**, 4476–4482.

Highlights:

- This study presents the results of a model for plant uptake of emerging organic contaminants in leaves and roots.
- The model considers the adsorption to proteins, speciation and phloem transport of weak acids.
- Input data were derived from an experimental study with lettuces irrigated with treated wastewater spiked with carbamazepine at different concentrations.
- Predicted carbamazepine concentrations in leaves and roots showed a good agreement between measured and calculated data.
- The simulation results of ibuprofen, ketoprofen, naproxen predicted in lettuces does not imply any risk to human health.