Inverse modeling of the biodegradation of emerging organic contaminants in the soil-plant system

Hurtado, Carlos; Trapp, Stefan; Bayona, Josep M.

Published in:
Chemosphere

Link to article, DOI:
10.1016/j.chemosphere.2016.04.134

Publication date:
2016

Document Version
Peer reviewed version

Link back to DTU Orbit

Citation (APA):
Inverse modeling of the biodegradation of emerging organic contaminants in the soil-plant system

Carlos Hurtado\textsuperscript{a}, Stefan Trapp\textsuperscript{b,*}, Josep M. Bayona\textsuperscript{a}

\textsuperscript{a}Environmental Chemistry Department, IDAEA–CSIC, Jordi Girona 18-26, E-08034 Barcelona, Spain

\textsuperscript{b}Technical University of Denmark, DK-2800 Kongens Lyngby, Miljøvej bd 113, Denmark

* Corresponding author. Tel. +45 45251622, Fax +45 45932850, e-mail stt@env.dtu.dk
DTU Environment, Bygningstorvet bd. 115, DK-2800 Kgs. Lyngby, Denmark
Abstract

Understanding the processes involved in the uptake and accumulation of organic contaminants into plants is very important to assess the possible human risk associated with. Biodegradation of emerging contaminants in plants has been observed, but kinetical studies are rare. In this study, we analyse experimental data on the uptake of emerging organic contaminants into lettuce derived in a greenhouse experiment. Measured soil, root and leaf concentrations from four contaminants were selected within the applicability domain of a steady-state two-compartment standard plant uptake model: bisphenol A (BPA), carbamazepine (CBZ), triclosan (TCS) and caffeine (CAF). The model overestimated concentrations in most cases, when no degradation rates in plants were entered. Subsequently, biodegradation rates were fitted so that the measured concentrations were met.

Obtained degradation kinetics are in the order, BPA < CAF ≈ TCS < CBZ in roots, and BPA ≈ TCS < CBZ << CAF in leaves. Kinetics determined by inverse modeling are, despite the inherent uncertainty, indicative of the dissipation rates. The advantage of the procedure that is additional knowledge can be gained from existing experimental data. Dissipation kinetics found via inverse modeling is not a conclusive proof for biodegradation and confirmation by experimental studies is needed.

Keywords: dissipation; kinetics; plants; model; emerging organic contaminants.
1. Introduction

Pharmaceuticals, biocides and drugs as well as other chemicals from human use reach sewer systems and are partially removed during conventional wastewater treatment processes (Halling-Sørensen et al., 1998). By irrigation with reclaimed water, or sewage sludge amendment, these chemical residues may reach agricultural soils. Uptake into crops can lead to human exposure to such chemicals (Hospido et al., 2010). In the European Union, the environmental risk from pharmaceutical products is assessed only for veterinary drugs (EMA, 2011), and only few pharmaceuticals and drugs are regularly monitored according with the Watch List of the Water Framework Directive (Directive 2008/105/EC). Then, human exposure to emerging organic contaminants (EOCs) relies partly on scientific studies, and an increasing number of studies on their uptake into vegetables is reported (Wu et al., 2015; Miller et al., 2016).

Prosser and Sibley (2015) found no human health hazards from the plant uptake of the “majority of pharmaceuticals and personal care products”. However, Malchi et al. (2015) stated that “current data are insufficient to support a comprehensive human health risk assessment” of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, or reclaimed water irrigation. Due to the high number of compounds potentially present in reclaimed water (Calderón-Preciado et al., 2011; Loos et al., 2013; Luo et al., 2014), prediction tools for pre-screening of chemicals and priority setting for safety assessments are of high value (Polesel et al., 2015). Prosser et al. (2014) examined the ability of two prediction models to estimate the uptake of
pharmaceuticals and personal care products (PPCPs) into plants from sludge-amended soils. Predictions of plant uptake of PPCPs within one order of magnitude near the experimental results were achieved for some of the investigated compounds. Polesel et al. (2015) developed and tested a simulation tool for fate prediction from human pharmaceuticals down the drain through a sewage treatment plant and via sludge amendment and irrigation to agricultural fields and crops. However, simulations were performed disregarding degradation in plants. To reduce discrepancies between model predictions and measurements, the authors stressed the need for more measured input parameters (e.g., $K_d$) and kinetics of biotransformation in plant tissues.

For polar compounds, efficient translocation in xylem of plants can be expected (Trapp, 2007; Dettenmaier et al., 2009), leading to accumulation in leaves, if no losses occur. Biodegradation has been identified as among the most relevant dissipation processes of chemicals from plants (Fantke et al., 2012; Jacobsen et al., 2015), but is often unknown or uncertain and depends on a number of factors, such as species and temperature (Fantke and Juraske, 2013; Fantke et al., 2014; Jacobsen et al., 2015). Methods to measure metabolism in soil and plants have been developed early, typically employing the use of $^{14}$C-labeled compounds to close the mass balance (Trapp et al., 1990; Kästner et al., 2014). There are also OECD guidelines for pesticide metabolism in crops to elucidate the degradation pathway available (i.e. OECD Tests Nr. 501, 502). The drawback is that studies with hot labels are expensive, and safety issues arise. These safety issues can be
solved by using stable isotopes ($^{13}$C and $^{15}$N), but require IRM-MS equipment, if isotopically labeled compounds are available at all.

An alternative method to assess biodegradation that has rarely been attempted is the use of inverse modeling. Hereby, predictable loss due to physical-chemical processes (volatilization, translocation, dilution) is contrasted with measured dissipation. The difference is contributed to biodegradation. This method cannot prove degradation but can help to quantify loss processes (Jacobsen et al., 2015).

The kinetics of biodegradation affects the relation between concentrations in plants and soil. First-order degradation kinetics, either in soil or in plants, will change the slope of the trend line (lower for degradation in plants, higher for degradation in soil), but the relation will remain linear. In a study with lettuce grown under controlled conditions and irrigated with water containing eight emerging organic contaminants (EOCs), Hurtado et al. (2016) obtained mostly linear correlations between watering concentrations and concentrations measured in roots and leaves. Besides hydrophobicity ($\log D_{OW}$) of chemicals, their persistence was identified as a key determinant for plant uptake and accumulation of the EOCs.

In this study, we supplemented a standard plant uptake model (Rein et al., 2011) with different degradation kinetics for soil and plant. The model was parameterized to simulate the uptake experiments of emerging organic contaminants into lettuce performed by Hurtado et al. (2016). Degradation rate constants in soil were derived from the measured concentrations, while rates in leaves and roots were fitted,
based on the difference between the model prediction (without degradation) and the measured data. The resulting rates were compared to data from literature.

2. Materials and methods

2.1. Experimental section

Experiments were conducted in a glass greenhouse located in Viladecans (Barcelona, Spain) as described in Hurtado et al. (2016). Briefly, lettuce (*Lactuca sativa*) was planted in pots in a mixture of perlite and sand (2:1 v/v, approx. 1.2 kg) and watered with Hoagland nutrient solution (Hoagland and Arnon, 1950) diluted 1:1 with rain water. A dose of 50 mL of irrigation water was applied to each experimental unit per day. The number of daily irrigations was regulated to keep water in the soil below field capacity, thereby preventing leachate production.

After 40 days, EOCs were added to soil. Five treatments consisted of direct application of 0, 14, 35, 70 and 140 μg of eight EOCs per experimental unit in eight applications during 28 days. Taking into account the soil substrate mass in each experimental unit, this corresponds to an average nominal initial concentration in the substrate of 0, 11.7, 29.2, 58.3 and 116.7 μg kg⁻¹ dw. After 28 days, substrate, roots and leaves of lettuces were separated and analyzed. The data used in this study can be found in the SI and are also reported in Hurtado et al. (2016).

The EOCs measured in the experimental study were bisphenol A, caffeine, carbamazepine, ibuprofen, propranolol, sulfamethazine, triclosan and tonalide. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), except
tonalide from Ventós (Sant Just Desvern, Spain). The extraction of EOCs from vegetal tissue and substrate and the analytical parameters are listed in Hurtado et al. (2016). The properties of the compounds are listed in Table 1.

Table 1. Properties of the compounds added in the experiment by Hurtado et al. (2016). All values were obtained using ACD Advanced Chemistry Development (2010), ACD/i-lab 2.0. Toronto, 2010.

<table>
<thead>
<tr>
<th>EOC</th>
<th>Molar mass (g mol⁻¹)</th>
<th>pKa values</th>
<th>Speciation (z)</th>
<th>Neutral log K_{OW}</th>
<th>log D_{OW} at pH 6.4</th>
<th>log K_{AW}</th>
<th>log K_{HSA}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphenol A (BPA)</td>
<td>228.29</td>
<td>9.7, 10.5</td>
<td>0/-1/-2</td>
<td>3.46</td>
<td>3.46</td>
<td>-9.43</td>
<td>3.57</td>
</tr>
<tr>
<td>Caffeine (CAF)</td>
<td>194.19</td>
<td>NA</td>
<td>0</td>
<td>0.11</td>
<td>0.11</td>
<td>-8.83</td>
<td>2.53</td>
</tr>
<tr>
<td>Carbamazepine (CBZ)</td>
<td>236.27</td>
<td>NA</td>
<td>0</td>
<td>2.23</td>
<td>2.23</td>
<td>-7.20</td>
<td>3.74</td>
</tr>
<tr>
<td>Ibuprofen (IBU)</td>
<td>206.28</td>
<td>4.3</td>
<td>0/-1</td>
<td>3.63</td>
<td>1.53</td>
<td>-5.21</td>
<td>4.42</td>
</tr>
<tr>
<td>Propranolol (PROP)</td>
<td>259.34</td>
<td>9.5</td>
<td>1/0</td>
<td>2.69</td>
<td>0.13</td>
<td>-10.49</td>
<td>3.54</td>
</tr>
<tr>
<td>Sulfamethazine (SMT)</td>
<td>278.33</td>
<td>3.1, 7.2</td>
<td>1/0/-1</td>
<td>0.31</td>
<td>0.25</td>
<td>-11.33</td>
<td>4.1</td>
</tr>
<tr>
<td>Tonalide (TON)</td>
<td>258.40</td>
<td>NA</td>
<td>0</td>
<td>5.71</td>
<td>5.71</td>
<td>-2.04</td>
<td>4.71</td>
</tr>
<tr>
<td>Triclosan (TCS)</td>
<td>289.54</td>
<td>8.8</td>
<td>0/-1</td>
<td>5.21</td>
<td>5.21</td>
<td>-4.08</td>
<td>4.81</td>
</tr>
</tbody>
</table>

NA: Not applicable; z is charge number (valence); K_{OW} (L L⁻¹) is the partition coefficient octanol to water for the neutral molecule; D_{OW} (L L⁻¹) is the apparent partition coefficient of the neutral and ionic molecules at pH 6.4 (soil pH); K_{AW} (L L⁻¹) is the partition coefficient air to water for neutral molecules (known as dimensionless Henry’s Law constant) and K_{HSA} (L mol⁻¹) is the adsorption to human serum albumin (as predictor for the adsorption to proteins).

2.2. Model section

The plant uptake model is based on the commonly used "standard model" for plant uptake (Legind and Trapp, 2009; Legind et al., 2011; Rein et al., 2011; Trapp,
Modifications were introduced to consider different degradation kinetics. This version of the model is primarily designed for neutral compounds. As long as the fraction of ionic molecules is small, ionization only slightly affects the outcome when measured \( K_d \)-values are used. PROP, IBU and SMT were not included in the plant uptake simulations because the ionization prohibits the use of this model version. TON was excluded because of its high volatility. In a separate approach, Michaelis-Menten degradation kinetics in roots and leaves was calculated, but for mathematical reasons with initial (constant) concentration in soil.

The underlying differential equation for the change of concentration in roots \( (C_R, \text{mg kg}^{-1}) \) with time \( t \) (d) is

\[
\frac{dC_R}{dt} = \frac{Q}{M_R} \times \frac{C_S}{K_d} - \frac{Q}{M_R \times K_{RW}} \times C_R - k_{growthR} \times C_R - \text{degradation} \tag{1}
\]

where \( R \) is index for roots, \( Q \) is the transpiration \( (\text{L d}^{-1}) \), \( M \) is the plant mass \( (\text{kg}) \), \( C_S \) is the concentration of chemical in soil \( (\text{mg kg}^{-1}) \), \( K_d \) is the distribution coefficient between substrate and pore water \( (\text{L kg}^{-1}) \), \( K_{RW} \) is partition coefficient roots to water \( \text{(and xylem sap)} (\text{L kg}^{-1}) \), and \( k_{growthR} \) is the growth rate of roots \( (\text{d}^{-1}) \).

The differential equation for the change of concentration in leaves \( (C_L, \text{mg kg}^{-1}) \) with time, neglecting uptake of chemical from air, is

\[
+ \text{translocation from roots - loss to air - dilution by growth - degradation}
\]
\[
\frac{dC_L}{dt} = \frac{Q}{M_L \times K_{RW}} \times C_R - \frac{A_L \times g \times 1000 \, L \, m^{-3}}{K_{LA} \times M_L} \times C_L - k_{\text{growth}L} \times C_L - \text{degradation} \quad (2)
\]

where \( L \) is index for leaves, \( A \) is area (m²), \( g \) is conductance (m d⁻¹) and \( K_{LA} \) is partition coefficient between leaves and air (L kg⁻¹).

\[a) \text{Coupled dynamic differential equation system with first-order degradation}\]

The concentrations in soil, roots and shoots are calculated from a system of coupled ordinary differential equations that form a triangular matrix and are solved analytically.

The concentration in soil is considered time-dependent, with

\[C_S(t) = C_S(0) \times e^{-k_1 t} \quad (3)\]

The loss rate from soil \( k_1 \) (matrix element 1) was calculated from the measured initial and final concentrations at time \( t \), \( C_S(t) \), assuming first-order loss due to degradation, plant uptake and volatilization:

\[k_1 = \frac{\ln \frac{C_S(0)}{C_S(t)}}{t} \quad (4)\]

The transfer rate from soil to roots is

\[k_{12} = \frac{Q}{M_R K_d} \quad (5)\]
The rate $k_2$ is the sum of all loss terms (to shoots, dilution, degradation) from roots (d$^{-1}$)

$$k_2 = \frac{Q}{M_R \times K_{RW}} + k_{growthR} + k_R$$  

(6)

$k_R$ is the 1st order degradation rate that is to be fitted.

The rate $k_3$ (d$^{-1}$) is the sum of all loss terms (to air, dilution, degradation) from leaves

$$k_3 = \frac{A_r \times g \times 1000 L m^{-3}}{K_{LA} \times M_L} + k_{growthL} + k_L$$  

(6)

$k_L$ is the 1st order degradation rate that is to be fitted.

The transfer rate from roots to leaves is

$$k_{23} = \frac{Q}{M_L K_{RW}}$$  

(7)

The analytical solution for the concentration in roots (matrix element 2) is

$$C_R(t) = \frac{k_{12} \times C_s(0)}{k_2 - k_1} \times (e^{-k_1 t} - e^{-k_2 t})$$  

(8)

and for leaves (matrix element 3) is

$$C_L(t) = k_{12} k_{23} C_S(0) \left\{ \frac{e^{-k_1 t}}{(k_2 - k_1)(k_3 - k_1)} + \frac{e^{-k_2 t}}{(k_1 - k_2)(k_3 - k_2)} + \frac{e^{-k_3 t}}{(k_1 - k_3)(k_2 - k_1)} \right\}$$  

(9)
This model resembles the cascade model presented and tested by Rein et al. (2011) and applied by Legind et al. (2011) and Prosser et al. (2014).

b) Michaelis-Menten degradation kinetics in roots and leaves

Enzymatic reactions often follow the Michaelis-Menten kinetics

\[
\text{degradation} = \frac{v_{\text{max}} \times C}{K_M + C} \quad (10)
\]

where \(v_{\text{max}}\) is the maximal enzymatic removal in roots or leaves (mg kg\(^{-1}\) d\(^{-1}\)) and \(K_M\) (mg kg\(^{-1}\)) is the concentration at which removal is half \(v_{\text{max}}\). With Michaelis-Menten type kinetics, the shape of the trendline between concentrations in soil and plant is no longer linear. This kinetics has been observed for the degradation of cyanide by plants (Larsen et al., 2004; Yu et al., 2004) and for the exclusion of salt NaCl and NaF from roots (Trapp et al., 2008; Clausen et al., 2015). The assumption of steady-state was made to allow for a closed analytical solution, and requires constant concentration in soil \(C_S(0)\). The steady-state leads to a quadratic equation which was solved using Vieta's formulas (Larsen et al., 2004; Trapp et al., 2008).

The comparison of experimental values from different studies with different concentration levels is done by calculation of root concentration factor (RCF) and leaf concentration factor (LCF) which are defined as

\[
\text{RCF} = \frac{C_R(t_2)}{C_S(t_1)} \quad (11)
\]
Here, $t_1$ and $t_2$ stand for the time when the concentrations were measured. While $t_2$ (the time when the concentration in root and leaf is determined) typically refers to the time of harvest, there is no standard for $t_1$, and initial, nominal or final (at harvest) concentrations have been used for the calculation of RCF and LCF.

Model input data

Where available, input data for the plant properties were taken from the experiment. As shown recently, plant properties can significantly affect the outcome of the model simulations (Trapp, 2015). The experiment was carried out in a greenhouse in Spain, but in the winter period. Growth was moderate (growth rate 0.05 d$^{-1}$), and the ratio of transpiration to plant mass was relatively low (Table 2).

Table 2. Input data for the simulation of the uptake experiment with lettuce. Data shown are for an individual pot. Displayed is the data set for an experiment with carbamazepine (experiment number 17). Data source Hurtado et al. (2016)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>value</th>
<th>unit</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>distribution coefficient</td>
<td>$K_d$</td>
<td>0.72</td>
<td>L kg$^{-1}$</td>
</tr>
<tr>
<td>total loss rate</td>
<td>$k_1$</td>
<td>0.0895</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>water content roots</td>
<td>$W_R$</td>
<td>0.898</td>
<td>L kg$^{-1}$</td>
</tr>
<tr>
<td>lipid content roots</td>
<td>$L_R$</td>
<td>0.025</td>
<td>kg kg$^{-1}$</td>
</tr>
</tbody>
</table>
3. Results

Dry weight and water content of substrate, root and leaf for the different experimental units can be found in Table S1. Final concentrations in the three compartments can be found in Tables S2, S3 and S4.

Bioconcentration Factors derived from experimental data with lettuce

Root concentration factor (RCF, kg kg\(^{-1}\) dw) and leaf concentration factor (LCF, kg kg\(^{-1}\) dw) were calculated as the slopes of the linear regression of the concentration in plant versus either the initial (nominal) or the final concentration in the soil substrate. Figure 1 shows the RCFs and LCFs of CBZ and IBU. RCFs obtained for
CBZ were rather similar when initial or final concentrations of CBZ in soil were used to establish the regression. The slope, interpreted as RCF, was 10.4 kg kg\(^{-1}\) dw with the initial and 9.6 kg kg\(^{-1}\) dw with the final substrate concentration, however, the y-intercept went from -45 μg kg\(^{-1}\) dw to +92 μg kg\(^{-1}\) dw. Also the LCF of CBZ changed very little, from 17.6 to 15.1 kg kg\(^{-1}\) dw (but with high Y-intercept of 268 μg kg\(^{-1}\) dw). Conversely, for IBU the slope of the RCF regression changed from 2.0 to 9.4 kg kg\(^{-1}\) dw and from 0.20 to 0.94 kg kg\(^{-1}\) dw for the LCF with initial or final soil concentrations, and Y-intercepts were negative. The slopes for the other compounds can be seen in Figure S1 (RCF) and S2 (LCF). All compounds showed good uptake into roots with RCF > 1 kg kg\(^{-1}\) dw. Translocation to leaves was highest for CBZ and lowest for TCS. Most slopes increased when the final substrate concentration was used for the regression.
**Figure 1.** Root and leaf bioconcentration factors (RCFs and LCFs) of carbamazepine (CBZ) and ibuprofen (IBU). The solid and dashed lines represent the linear regression of the root and leaf concentration on the applied initial and the final soil concentration.

**Modeling**

The data obtained in the experiments (Table 2), such as dry weights at harvest and transpiration, were used to simulate plant uptake with the standard plant uptake model with degradation as described above (Eqs. 1-9). The loss rates from soil ($k_i$)
were calculated from the nominal initial concentration and the final measured concentration assuming exponential (1st order) decay (Table 3). Except one case (CAF, lowest applied amount), the loss rate from soil of all studied EOCs decreased when the initial concentration increased. For example, for CBZ the loss rates from soil were 0.090, 0.037, 0.015 and 0.006 d\(^{-1}\) for the four treatments (11.7, 29.2, 58.3 and 116.7 µg kg\(^{-1}\) dw). For IBU, the rates were 0.100, 0.094, 0.067 and 0.055 d\(^{-1}\) for the same treatments.

Degradation rates in roots and leaves were determined by fitting simulated concentrations in plants to the measured ones. Figure 2 shows an example for the simulations with fit. The simulation of BPA succeeds without added degradation in roots or leaves, but only when dissipation from soil is considered. For CAF, on the other hand, the simulation improves when a fast degradation rate in leaves is assumed. The results of the linear model appear curved due to the changing degradation rates in soil. Judged from the correlation between calculated and average measured concentrations, the Michaelis-Menten kinetics in this case is closer to the measured result. In all cases, however, omitting degradation in soil (labeled as 00 in Fig. 2) leads to drastic overestimation. Fitted 1st-order dissipation rates of selected EOCs are shown in Table 3b. The fastest first-order dissipation rate from roots was fitted for CBZ (0.35 d\(^{-1}\)). Dissipation from roots also affects leaves, but nonetheless a rapid dissipation rate of CAF from leaves was required to meet the measured data. The adjusted parameters for the Michaelis-Menten kinetics in roots and leaves can be found in Table 3c. Fitted \(v_{\text{max}}\) was higher in roots than in leaves, except for CAF. The values have to be taken with care.
because the two parameters cannot be verified independently, but also because the fitted degradation must replace partly the missing dissipation from soil which, for mathematical reasons, could not be considered in the simulation with Michaelis-Menten kinetics.

Figure 2. Simulated and measured concentrations in a) top left: CAF roots, b) top right: CAF leaves, c) bottom left: BPA roots; d) bottom right: BPA leaves. Solid points represent measured values; 00: no degradation in soil or plant; 1st: 1st order
degradation in soil, roots and leaves; MM: Michaelis-Menten degradation in roots and leaves.

Table 3a. Calculated first order degradation rates in soil, $k_{soil}$, (d$^{-1}$) for the four different treatments of selected EOCs (in brackets: standard deviation, n = 4).

<table>
<thead>
<tr>
<th>Treatment (µg kg$^{-1}$ dw)</th>
<th>BPA</th>
<th>CAF</th>
<th>CBZ</th>
<th>IBU</th>
<th>PROP</th>
<th>TCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7</td>
<td>0.039 (0.030)</td>
<td>0.038 (0.008)</td>
<td>0.109 (0.040)</td>
<td>0.104 (0.010)</td>
<td>0.088 (0.024)</td>
<td>0.008 (0.010)</td>
</tr>
<tr>
<td>29.2</td>
<td>0.038 (0.008)</td>
<td>0.060 (0.004)</td>
<td>0.058 (0.048)</td>
<td>0.099 (0.013)</td>
<td>0.080 (0.023)</td>
<td>0.001 (0.012)</td>
</tr>
<tr>
<td>58.3</td>
<td>0.031 (0.005)</td>
<td>0.044 (0.012)</td>
<td>0.017 (0.004)</td>
<td>0.073 (0.015)</td>
<td>0.076 (0.034)</td>
<td>0.001 (0.020)</td>
</tr>
<tr>
<td>116.7</td>
<td>0.029 (0.011)</td>
<td>0.022 (0.006)</td>
<td>0.001 (0.009)</td>
<td>0.059 (0.005)</td>
<td>0.063 (0.032)</td>
<td>0.001 (0.008)</td>
</tr>
</tbody>
</table>

Table 3b. Fitted first-order degradation rates in roots and leaves of selected EOCs.

<table>
<thead>
<tr>
<th></th>
<th>BPA</th>
<th>CAF</th>
<th>CBZ</th>
<th>TCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{root}}$ (d$^{-1}$)</td>
<td>0.00</td>
<td>0.05</td>
<td>0.35</td>
<td>0.10</td>
</tr>
<tr>
<td>$k_{\text{leaf}}$ (d$^{-1}$)</td>
<td>0.00</td>
<td>1.50</td>
<td>0.05</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3c. Adjusted Michaelis-Menten parameters $v_{max}$ (mg kg$^{-1}$ d$^{-1}$) and $K_M$ (mg kg$^{-1}$) for enzymatic degradation in kinetics equation of selected EOCs.

<table>
<thead>
<tr>
<th></th>
<th>BPA</th>
<th>CAF</th>
<th>CBZ</th>
<th>TCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_{\text{max root}}$</td>
<td>0.01</td>
<td>6.0</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>$K_M \text{ root}$</td>
<td>0.10</td>
<td>5.0</td>
<td>0.45</td>
<td>1.00</td>
</tr>
<tr>
<td>$v_{\text{max leaf}}$</td>
<td>0.0003</td>
<td>7.0</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>$K_M \text{ leaf}$</td>
<td>0.1</td>
<td>5.0</td>
<td>0.0001</td>
<td>none</td>
</tr>
</tbody>
</table>

4. DISCUSSION
Bioconcentration factors

Bioconcentration factors (BCF) are defined as concentration ratio between organism and surrounding medium. However, the calculations can be done in various ways. Some authors calculate BCFs from the concentration in the irrigation water, others from the concentration in soil and some from the concentration in the soil solution. Moreover, in some studies the nominal concentration is used while others use the final concentration in soil at harvest to calculate the BCFs. In this study, BCFs were derived as slope of the regression line so measurements at all concentrations contributed simultaneously, without contribution of background (Y-intercept), and with both initial (nominal) and final concentration (Figures SI1, SI2).

For CBZ there were no big differences in the BCF when it was calculated with initial or final substrate concentration (Figure 1). On the other hand, for IBU the difference was almost 5 times (2.0 to 9.4 g g\(^{-1}\) dw). Thus, it is important to consider that dissipation from soil or substrate will affect the BCF.

For most of the studied EOCs, experimental BCFs can be found in the literature (Table 4). The reported values show large variance and are generally far higher in hydroponics. In comparison to BCFs derived from experiments with soil, our values are at the higher end, probably due to the lower adsorption capacity of perlite and sand, compared to soil organic matter.

The half-lives (DT50) in the perlite and sand mixture are slower than those derived in soil. The perlite and sand mixture was chosen as a substrate because in laboratory studies we observed that there were lower interactions than when using
soil. The uptake simulations were done with measured $K_d$-values, thus, the difference in adsorption does not affect the simulations. But the substrate for this experiment lacked of organic matter, which can be used as main substrate by bacteria degrading co-metabolically EOC. This may explain why most loss rates from our substrate were lower than rates found in soil (Fent et al., 2003; Langdon et al., 2012; Matsumura et al., 2015). For example, DT50 of bisphenol A in soil have been determined from 0.5 to 7 days (Cousins et al., 2002, Ying et al., 2005 and Xu et al., 2009), while we found half-lives from 17.8 to 23.9 days. BPA dissipation is related to bacteria in soil and it dissipates faster in more aerobic environments (Fent et al., 2003).
Table 4. Experimental values of bioconcentration factors and dissipation times in soils reported and in this study.

<table>
<thead>
<tr>
<th>EOC</th>
<th>Experiment Type</th>
<th>Plant</th>
<th>Tissue</th>
<th>BCF literature</th>
<th>BCF in this study (kg kg(^{-1}) dw)</th>
<th>DT50 (d) in literature</th>
<th>DT50 (d) this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>Hydroponics</td>
<td>Dracaena(^a)</td>
<td>Stem</td>
<td>170 L kg(^{-1}) dw</td>
<td>Leaf 1.3 – 2.7 Root 2.6 – 4.5</td>
<td>0.5 – 7(^{i,k})</td>
<td>17.8 - 23.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Root</td>
<td>110 L kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lettuce, collard(^b)</td>
<td>Leaf</td>
<td>7 - 66 L kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Root</td>
<td>4339 - 9587 L kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAF</td>
<td>Soil</td>
<td>Cucumber, tomato, sweet potato, carrot(^c,d)</td>
<td>Leaf</td>
<td>0.4 – 17 L kg(^{-1}) dw</td>
<td>Leaf 1.2 – 0.94 Root 3.5 – 5.3</td>
<td>1.5 – 3(^{m})</td>
<td>11.6 - 31.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fruit</td>
<td>0.4 – 5.3 L kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber, tomato(^c)</td>
<td>Root</td>
<td>0.1 – 0.8 kg kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sweet potato, carrot(^d)</td>
<td>Root</td>
<td>4.4 – 6.9 kg kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBZ</td>
<td>Soil</td>
<td>Soybean, radish, cucumber, tomato, sweet potato(^c,d,e,i)</td>
<td>Leaf</td>
<td>0.6 – 425 kg kg(^{-1}) dw</td>
<td>Leaf 15 – 17.6 Root 9.9 – 10.4</td>
<td>60 – 533(^n,o)</td>
<td>6.4 - 693</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fruit</td>
<td>0.4 – 27 kg kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soybean, radish, sweet potato, carrot(^d,e,i)</td>
<td>Root</td>
<td>0.1 – 8.3 kg kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber, tomato(^c)</td>
<td>Fruit</td>
<td>0.4 – 27 kg kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBU</td>
<td>Hydroponics</td>
<td>Typha, phragmites, iris, juncus(^g)</td>
<td>Root</td>
<td>7 – 201 L kg(^{-1})</td>
<td>Leaf 0.2 – 0.94 Root 2.0 – 9.4</td>
<td>1 – 6(^p)</td>
<td>6.7 - 11.7</td>
</tr>
<tr>
<td>PRO</td>
<td>Soil</td>
<td>Radish, ryegrass(^l)</td>
<td>Leaf</td>
<td>0.01 – 11.9 kg kg(^{-1}) dw</td>
<td>Leaf 1.1 – 2.9 Root 3.2 – 6.7</td>
<td>&gt; 40(^l)</td>
<td>7.9 - 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Root</td>
<td>1.2 kg kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Root</td>
<td>1.2 kg kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCS</td>
<td>Soil</td>
<td>Radish, ryegrass(^l)</td>
<td>Leaf</td>
<td>0.1 – 38 kg kg(^{-1}) dw</td>
<td>Leaf 0.15 – 0.24 Root 4.4 – 6.9</td>
<td>18 (^n)</td>
<td>86.6 - 693</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Root</td>
<td>0.12 kg kg(^{-1}) dw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carrot</td>
<td>Leaf</td>
<td>0.18 kg kg(^{-1}) dw</td>
<td>Leaf 2.7 – 5.9 kg kg(^{-1}) dw</td>
<td>50 – 133(^i)</td>
<td></td>
</tr>
<tr>
<td>TON</td>
<td>Soil</td>
<td>Carrot, barley, meadow</td>
<td>Root</td>
<td>0.50 – 1.74 kg kg(^{-1}) dw</td>
<td>Leaf 2.7 – 5.9 kg kg(^{-1}) dw</td>
<td>50 – 133(^i)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Saiyood et al. (2010), \(^b\)Dodgen et al. (2013), \(^c\)Goldstein et al. (2014), \(^d\)Malchi et al. (2014), \(^e\)Wu et al. (2010), \(^f\)Carter et al. (2014), \(^g\)Zhang et al. (2016), \(^h\)Macherius et al. (2012), \(^i\)Xu et al. (2009), \(^j\)Cousins et al. (2002), \(^k\)Ying et al. (2005), \(^l\)Lin et al. (2010), \(^m\)Hendel et al. (2006), \(^n\)Monteiro et al. (2009), \(^o\)Walters et al. (2010), \(^p\)Löffler et al. (2005), \(^q\)Ying et al. (2007), \(^r\)Walters et al. (2010), \(^s\)Chen et al. (2014).
Degradation

Dissipation rates from soil in Table 3a were calculated from initial nominal and final measured substance concentrations in the substrate. Rates decreased with increasing concentrations. Such kinetics, i.e. decreasing (pseudo) first-order rates with increasing substrate concentrations, can occur by enzymatic degradation when the half-saturation concentration $K_M$ of the reaction is within the range of occurring concentrations and when at the same time the amount of enzymes is constant. Thus, co-metabolic (non-growth) degradation by microbes living in the substrate or near and in the roots, but also degradation by roots itself can lead to this kinetics. At higher soil concentrations, PROP, IBU, BPA and CAF had the highest dissipation rates, while CBZ and TCS showed the lowest dissipation. As it can be seen in Table 4, several studies suggest that CBZ is relative persistent with half-lives in soil (DT50) over 60 days. Moreover, TCS and its metabolites were found in soil still four years after the application with biosolids (Macherius et al., 2014). The other compounds are more labile to degradation. For example, metabolites of BPA have been found in soil such as 4-hydroxyacetophenone, 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid (Dodgen et al., 2014). Hurtado et al. (2016) determined the concentrations of test chemicals both in the bulk soil, and in the vicinity of roots. Only TCS showed an enrichment in the soil around roots, underlining its persistence. Low concentrations in the soil near roots were found for PROP and IBU, which also had the highest loss rates from soil (Table 3a). This
makes it likely that degradation is enhanced by roots. Furthermore, enantiomeric
fractionation of IBU was observed, with an enrichment of the S-enantiomer.

*Inverse modeling*

Inverse modeling can be a powerful tool to determine missing processes or rate
constants and is often used for model calibration. Jacobsen et al. (2015) used the
technique to find missing *in-planta*-degradation of pesticides, and the principle
applied here is similar: model predictions are compared to measured
concentrations, and the difference is contributed to dissipation by degradation. This
method is of course highly uncertain because both model simulation and
experiment have their own uncertainties, and unknown dissipation processes can
lead to reduced uptake. It is therefore a positive sign that the model in no case
underestimated the experimental concentrations, and that often only a rather small
dissipation rate was sufficient (Table 3).

Two degradation kinetics were evaluated: first-order kinetics and *Michaelis-
Menten*. It is noteworthy to mention that the first-order kinetic has only one
parameter to adjust (*k*) while *Michaelis-Menten* has two (*v_max* and *K_M*). Moreover,
mathematical constraints did not allow considering the (known) dissipation from
soil. The first-order fit is therefore preferable in our case.

Measured root and leaf concentrations of BPA were very close to those predicted,
thus, no degradation rates were fitted (Table 3b). We did not find degradation
studies of BPA in plants for comparison. A large difference between predicted CAF
concentrations in leaves and measured values required to fit the *k_leaf* to 1.50 d⁻¹.
Dettenmaier et al. (2009) derived the transpiration stream concentration factor (TSCF) with a pressure chamber experiment of several organic compounds and reported that polar neutral compounds such as CAF should be taken up rapidly by roots and translocated to the leaves. On the other hand, Goldstein et al. (2014) and Wu et al. (2014) reported that CAF was translocated to leaves less than CBZ due to polar interactions. In the experiments of Hurtado et al. (2016), similar behavior was observed, and the concentrations predicted for leaves were far above the measured ones. Both phenomenon reduced translocation or rapid transformation of CAF in leaves can lead to this discrepancy. In the literature, no degradation rates have been reported for CAF in plants. Regarding CBZ, transformation products (TPs) have been reported both for soil and plants, such as 10,11-epoxy carbamazepine, 10,11-dihydroxycarbamazepine or 10,11-dihydro-10,11-dihydroxy-carbamazepine (Goldstein et al., 2014; Malchi et al., 2014). Malchi et al. (2014) reported that in soil, CBZ parent compound was dominant (90%) and in leaves it depended on the species (potato or carrot). Goldstein et al. (2014) reported similar TPs and similar percentages in cucumbers and tomatoes. Metabolites formed in soil can also be taken up by plants, which makes it difficult to differentiate where exactly the degradation occurred.

Recently, Pietrini et al. (2015) detected 11 TPs of IBU in *Lemna gibba* L. plant extracts when plants were exposed to 1 mg L$^{-1}$ of IBU. In microalgae reactors, IBU and CAF were rapidly biodegraded (Matamoros et al. 2016), while CBZ appeared to be recalcitrant. In this case, the $S$-enantiomer of IBU was degraded faster. TCS was metabolized in carrot and horseradish to conjugates, and the final amount of
conjugates was five times higher than that of TCS (Macherius et al., 2012b). With the fitted degradation rate in roots of 0.1 d\(^{-1}\), such a ratio would be reached after 18 days, it is thus reasonable. In horseradish, 33 metabolites of TCS were detected, hereof 23 identified (Macherius et al., 2014).

**CONCLUSIONS**

Biodegradation of emerging contaminants in plants has been observed in many cases but kinetics data are rare. Inverse modeling may provide a way to obtain this missing information. Rates determined by inverse modeling are, despite the inherent uncertainty, indicative of the dissipation rates. In the present study, degradation kinetics was in the order BPA < CAF ≈ TCS < CBZ for roots, and BPA = TCS < CBZ << CAF for leaves. There are indications that the high rate for CAF could also compensate for less translocation than predicted.

In soil, decreasing first-order dissipation rates with increasing concentration were observed. Co-metabolic degradation can explain this kinetics. The dissipation rates were in the order TCS < BPA ≈ CAF < PROP ≈ IBU ≈ CBZ for the lowest initial concentration, and TCS ≈ CBZ < CAF ≈ BPA < IBU ≈ PROP at the highest applied dose.

The shape of the BCF-curve (the ratio of concentration in plant to soil) and the Y-intercept gives information on the type of degradation kinetics. A negative Y-intercept can be obtained from (rapid) enzymatic degradation in plants. Finally, the use of inverse modeling provides additional knowledge in the biodegradation of
chemicals that can be taken up and further translocated in plants. This can be very helpful to assess where biodegradation takes places and this method can be used to study further metabolism in plant.

Dissipation kinetics found via inverse modeling is not a conclusive proof for biodegradation and confirmation by experimental studies (for example, by determination of metabolites or by studies with labeled compounds) is needed.

**AUTHOR INFORMATION**

Phone: +45 4525 1622, e-mail: stt@env.dtu.dk

---

26
Acknowledgments

Authors thank to the Spanish Ministry of Economy and Competitiveness (MINECO) for funding (CGL2011-24844 and AGL2014-59353-R). C.H. acknowledges a predoctoral fellowship (BES-2012-055067) from MINECO and a short-stay grant (EEBB-I-15-10049) to be able to travel to Denmark.

The authors would like to acknowledge the financial support provided by COST-European Cooperation in Science and Technology, to the COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS).

Disclaimer

The content of this article is the authors' responsibility and neither COST nor any person acting on its behalf is responsible for the use, which might be made of the information contained in it.
References


Figure Captions

Figure 1. Root and leaf bioconcentration factors (RCFs and LCFs) of carbamazepine (CBZ) and ibuprofen (IBU). The solid and dashed lines represent the linear regression of the root and leaf concentration on the applied initial and the final soil concentration.

Figure 2. Simulated and measured concentrations in a) top left: CAF roots, b) top right: CAF leaves, c) bottom left: BPA roots; d) bottom right: BPA leaves. Solid points represent measured values; 00: no degradation in soil or plant; 1st: 1st order degradation in soil, roots and leaves; MM: Michaelis-Menten degradation in roots and leaves.