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Uptake of Organic Emergent Contaminants in Spath and Lettuce: An In Vitro Experiment

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Supporting Information

ABSTRACT: Although a myriad of organic microcontaminants may occur in irrigation waters, little attention has been paid to their incorporation in crops. In this work, a systematic approach to assess the final fate of both ionizable and neutral organic contaminants taken up by plants is described. In vitro uptake of triclosan (TCS), hydrocinnamic acid (HCA), tonalide (TON), ibuprofen (IBF), naproxen (NPX), and clofibric acid (CFA) were studied in lettuce (*Lactuca sativa* L) and spath (*Spathiphyllum* spp.) as model plants. After 30 days incubation, analyte depletion from the culture medium was 85−99% (lettuce) and 51−81% (spath). HCA, NPX, and CFA exhibited the highest depletion rate in both plant species. Lettuce plant tissue analysis revealed an accumulation of all compounds except for HCA. These compounds reached a peak in tissue concentration followed by a sudden drop, probably due to the plant detoxification system and analyte depletion from the culture medium. Kinetic characterization of the uptake and detoxification processes was fitted to a pseudo-first-order rate. Compounds with a carboxylic group in their structure exhibited higher uptake rates, possibly due to the contribution of an ion trap effect. Molecular weight and log K_{ow} played a direct role in uptake in lettuce, as proven by the significant correlation of both properties to depletion and by the correlation of molecular weight to kinetic uptake rates.

KEYWORDS: *Plant uptake, emerging organic contaminants, K_{ow}, molecular weight, plant tissue*

INTRODUCTION

Hydrological cycles and freshwater resources are constrained by global changes, for example, increases in water demand, increases in drought frequency, or limited renewable freshwater resources that are moreover threatened by pollution.[1,2] Consequently, the use of reclaimed water for crop irrigation is viewed as an excellent sustainable water source.[3] Indeed, since agricultural activities consume approximately 70% of all freshwater resources, and since only 1% of the bulk water used is reclaimed water, water reuse could have a greater impact in this sector.

However, wastewater treatment plant (WWTP) effluents are one of the main sources of emerging organic contaminants in the aquatic environment.[4−6] In fact, they are either not removed at all or only partially removed in wastewater reclamation. In addition to reclaimed water, treated sewage sludge is also commonly used worldwide in agriculture, and for the latter, land application is largely the most used method of disposal.[7] Following irrigation with reclaimed water or treated sewage sludge disposal, crops are exposed to a myriad of pharmaceuticals and other organic microcontaminants.[8] These compounds can either be mobilized and enter into the hydrological cycle, and then they can become adsorbed on the organic matter of the soil, or those that are recalcitrant enough can be taken up by plants and possibly enter into the food chain.[9−11] These contaminants can exert chronic toxic effects even at very low concentrations (nanograms per liter) because of their chemical properties, such as high membrane solubility, persistence, and bioactivity.[12]

Xenobiotics are expected to enter the plant through passive diffusion unless the compound exhibits a hormone-like structure, in which case active transport may be possible.[13] The most relevant uptake pathways are (i) root uptake,[14−17] (ii) vapor uptake from the surrounding atmosphere,[18,19] and (iii) contaminant diffusion through deposition on plant surfaces.[20] Once the xenobiotic has entered the plant, a posterior translocation, driven by the transpiration process, can take place. The extent of distribution within the plant will depend on the compound's physicochemical properties.[21]

One of the most useful chemical descriptors of organic contaminants for plant uptake is their K_{ow}. Uptake is greatest for compounds with a log K_{ow} in the range of 1−4.[22] In this regard, Briggs et al.[23] predicted the concentration of nonionic compounds in a plant transpiration stream from the concentration in soil solution and log K_{ow}. The maximum transpiration stream concentration factor was obtained for chemicals with a log K_{ow} of 1.8−3.1. Compounds with a log K_{ow} within the optimum range are thought to be hydrophobic enough to move through the lipid bilayer of membranes yet

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water-soluble enough to travel into the cell fluids.[24] If the compound is too hydrophilic, it will be unable to cross hydrophobic lipid membranes, while high lipophilicity will impair its efficiency to cross the endodermis, possibly due to adsorption on the lipid material.[25] In addition, if a compound dissociates in the physiologically relevant pH range, this will influence both uptake velocity and level.[26]

Although there are several studies involving the plant uptake of neutral microcontaminants such as polycyclic aromatic hydrocarbons (PAHs),[27−29] some pharmaceuticals,[12,30,31] and flame retardants,[32] little attention has been paid to the plant uptake of ionizable molecules. For example, Calderón-Preciado et al.[11] reported in a field study the uptake of ibuprofen and naproxen in *Medicago sativa* and *Malus domestica*.

Plants have developed multiple mechanisms to respond to alterations in their environment.[33] Continuous exposure to potentially toxic chemicals is one of these alterations. Such organic compounds cannot be exploited for nutritional purposes or as a source of energy, yet they are still taken up by plants.[34] Plants have developed detoxification systems for xenobiotic elimination, since catabolization is not possible. The cellular detoxification systems by which plants dispose of xenobiotics have been termed "green liver"[35] and comprise a network of enzymatic reactions. In addition to the intrinsic metabolic detoxification systems of plants, the presence of endophytic bacteria may alter xenobiotic degradation. Internal plant tissues may be colonized by these bacteria without showing external signs of infection or negative effects[36]

In this paper, an in vitro study was designed to assess the uptake of selected emerging organic contaminants, commonly identified in WWTP effluents, by lettuce *Lactuca sativa*, a commercial commodity, and spath, *Spathiphyllum* sp., an ornamental plant. The six compounds studied were four pharmaceuticals (clofibric acid, CFA; naproxen, NPX; ibuprofen, IBF; and triclosan, TCS) and two fragances (tonalide, TON; and hydrocinnamic acid, HCA). The target analytes were selected on the basis of their high occurrence and abundance in reclaimed waters[6,11] and due to their wide range of physical and key chemical properties in the uptake and translocation of xenobiotics in plants (e.g., log K_{ow}, Henry's law constant, dissociation constant, and molecular weight). Spath and lettuce plants were chosen as models because they have different growth rates and can be grown in a sterile medium spiked with emerging organic contaminants. Time series of both growing medium and vegetation were analyzed, along with suitable controls to calculate the uptake and depletion kinetics. Finally, the experimental kinetic constants were correlated with physicochemical properties of the selected contaminants.

MATERIALS AND METHODS

Reagents. Pharmaceuticals and fragrances [ibuprofen (IBF), clofibric acid (CFA), tonalide (TON), and hydrocinnamic acid (HCA)] were purchased from Sigma−Aldrich (Bornem, Belgium). Trimethylsulfonium hydroxide (TMSH) and triclosan (TCS) were obtained from Fluka (Buchs, Switzerland), and naproxen (NPX) was bought from Riedel-de Haën (Seelze, Germany). The remaining reagents used were analytical-grade (97−99% purity) and their detailed information is provided in Supporting Information.

Stock solutions of each individual compound were prepared in methanol or ethyl acetate at a concentration of 5000 mg·L^{-1}. All prepared standards were stored in the darkness at −20 °C and used to prepare the single and mixed working standards solutions.

Experimental Setup. The experiment consisted of four treatments (Figure 1): (A) plantlets grown in the presence of target analytes (CFA, NPX, IBF, TCS, HCA, and TON; Table 1); (B) plantlets grown in the absence of target analytes; (C) spiked culture medium without plantlets; and (D) spiked culture medium without plantlets in darkness. All treatments were incubated under the same conditions (23 °C under fluorescent lamps, 80 μmol·m^{-2}·s^{-1}). Treatments C and D were employed for control purposes. Both lettuce and spath samples were randomly sampled at the time intervals shown in Figure 1. For treatment A, nine individuals for lettuce and four for spath were taken at each sampling point (90 and 20 individuals total, respectively), while for treatments B−D, six individuals for lettuce and four for spath were taken (180 and 60 individuals total, respectively). Target analytes were spiked into the corresponding treatments to obtain a final concentration of 100 ng·mL^{-1}. Although this concentration was higher than those found in reclaimed waters (0.1−1 ng·mL^{-1}), it was chosen to ensure quantitation of the target analytes in plant material.

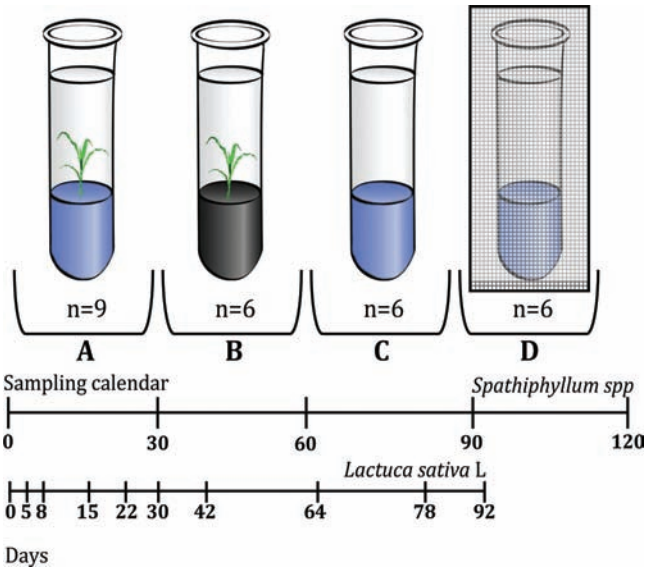
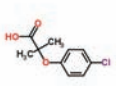
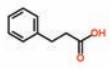
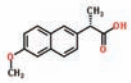
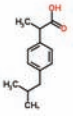
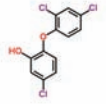
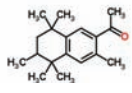


Figure 1. Experimental treatments to determine uptake behavior of target analytes through time: (A) plantlets grown in presence of target analytes, (B) plantlets grown in absence of target analytes, (C) spiked medium, and (D) spiked medium in darkness.

Germination and Plant Culture. Lettuce seeds were obtained from Utah State Crop Physiology Laboratory (Logan, UT). Seeds were disinfected in a flask containing a 2% chlorine aqueous solution and 50 μL of Tween 20. Seeds were stirred in the solution with a magnetic bar for 20 min and then washed three times with sterile distilled water. To initiate germination, seeds were placed in Petri dishes containing damp sterile filter paper and kept at 23 °C. The *Spathiphyllum* material used in the uptake assays was obtained through micropropagation from lateral buds. Plantlets were transferred to test tubes containing Murashige and Skoog culture medium, at a final pH of 5.5.[37] The test tubes appropriately identified with the corresponding treatment were placed in a growth chamber under the following conditions: 16 h light/8 h dark, 80 μmol·m^{-2}·s^{-1} at 23 °C.

Table 1. Physicochemical Properties of Target Analytes

Analyte	Estructure	MW	log K_{ow}	pK_a	Solubility, mg L^{-1}	Henry Law constant*
Clofibric acid (CFA), *2-(4-Chlorophenoxy)-2-methylpropanoic acid*		214	2.84	2.84	582.5	8.96 E-7
Hydrocinnamic acid (HCA), *3-phenylpropanoic acid*		150	1.84	4.66	5046	2.40 E-6
Naproxen (NPX), *(2S)-2-(6-methoxynaphthalen-2-yl) propanoic acid*		230	3.18	4.2	144.9	1.39 E-8
Ibuprofen (IBF), *2-(4-(2-methylpropyl)phenyl) propanoic acid*		206	3.79	4.31	41.05	6.20 E-6
Triclosan (TCS), *5-chloro-2-(2,4-dichlorophenoxy) phenol*		289	4.66	7.9	4.621	2.04 E-7
Tonalide (TON), *1-(3, 5, 5, 6, 8, 8-hexamethyl-6, 7 – dihydronaphthalen-2-yl)ethanone*		258	5.9	NA	0.239	1.73 E-3

*Dimensionless. NA: Not Applicable.

Extraction and Determination of Target Analytes. *Culture Medium.* The extraction of target analytes from the culture medium was carried out as follows. Test tubes containing the culture medium were heated in a water bath at 80 °C for 4 min and then vortexed until the medium was homogeneous. Afterward, 25 μL of HCl, surrogate standards (fenoprop and 2,2′-dinitrobiphenyl), and 2 g of NaCl were added to the test tube. The medium was thoroughly mixed. Ethyl acetate (10 mL) was then added, and the sample was mixed again. Test tubes were frozen at −20 °C before the organic phase was collected. This procedure was repeated twice. The raw extracts were evaporated to 2 mL under a nitrogen stream and percolated through a Na_2SO_4 column. Next, the extract was evaporated to approximately 20 μL under a gentle nitrogen stream, and 25 μL of triphenylamine solution (9.9 $\mu g \cdot mL^{-1}$) were added as an internal standard. The vial was reconstituted to 300 μL with ethyl acetate.

Methylation of the acidic carboxyl group in all samples was performed in the GC injector port at 270 °C by adding 10 μL of TMSH solution (0.25 $mol \cdot L^{-1}$ in methanol) to a 50 μL sample aliquot before injection. Derivatized samples were injected (1 μL volume) onto a Trace GC-MS (Thermo Scientific) in the electron impact mode (70 eV ionization energy) fitted with a 20 m × 0.18 mm i.d. × 0.18 μm film thickness Sapiens 5MS (Teknokroma, Barcelona, Spain). Helium was used as carrier gas (99.9995% purity) at a constant flow rate of 0.6 $mL \cdot min^{-1}$. The oven temperature was held at 65 °C for 1 min. It was then programmed at 15 $°C \cdot min^{-1}$ to 120 °C, at 6 $°C \cdot min^{-1}$ to 160 °C, at 9 $°C \cdot min^{-1}$ to 180 °C, at 6 $°C \cdot min^{-1}$ to 220 °C, and finally at 8 $°C \cdot min^{-1}$ to 315 °C, with the final temperature held for 7 min. Compound individual recoveries were carried out in a triplicate at a spiking level of 100 $ng \cdot L^{-1}$, and ranged from 82% (NPX) to 117% (IBF). Recoveries for fenoprop and 2,2′-dinitrobiphenyl were 87% and 98%, respectively.

Plant Tissue. The fresh lettuce biomass was extracted as previously reported;[38] details are provided in Supporting Information. Sample analysis was carried out by gas chromatography tandem mass spectrometry (GC-MS/MS). Compound individual recoveries were carried out in a triplicate at a spiking level of 100 $ng \cdot L^{-1}$, and ranged from 50% (CFA) to 106% (TON). Repeatability ranged from 1% (CFA) to 12% (TON). Limits of detection and quantification ranged from 0.001 to 4 $\mu g \cdot kg^{-1}$ fresh weight (FW).

Statistical Data Treatment. All statistical analyses were undertaken with the SPSS 15 package (Chicago, IL). Data were checked for normality (Kolmogorov−Smirnov test), followed by one-way analysis of variance (ANOVA) and the Bonferroni correction method in the case of multiple comparisons, and Pearson correlation to establish relationships between properties.

RESULTS AND DISCUSSION

Evaluation of Physiological Plant Parameters. Phytotoxicity, such as bleaching or necrotic spots, did not appear on the lettuce or spath, meaning that both plant species were tolerant to the target analytes at the studied concentration and compound combination.

Fresh weight and length of whole spath and lettuce plants were recorded prior to tissue processing so as to assess growth evolution and recognize possible phytohormonal effects on the plants. Spath appearance was markedly different between treatments. Both length and mass differences in the tissue were found to be statistically significant ($\alpha < 0.05$) by the Bonferroni

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correction method (Figure 2). For both parameters, spath grown in the presence of target analytes displayed significant differences compared to the control. The largest differences, 27% in tissue length and 64% in tissue mass, were found at 30 days. These differences declined with time and by the end of the experiment, at 120 days, stood at 11% and 15% respectively.

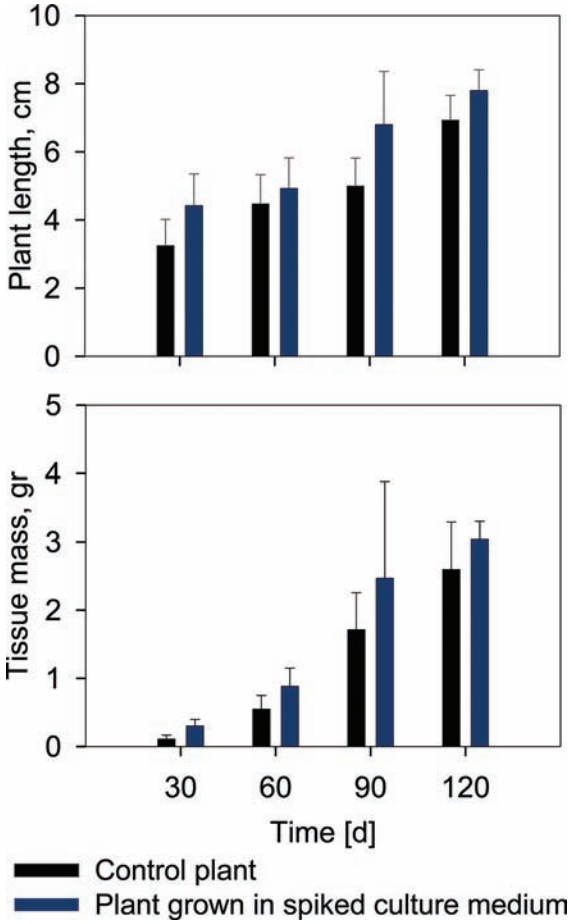

Figure 2. Physiological differences between treatments through time: (top) spath tissue mass (FW); (bottom) length in whole plants. Differences between treatments at all sampling times were significant ($\alpha < 0.05$).

This was not the case for lettuce, for which no statistically significant differences were found between treatments with regard to either tissue mass or length (not shown). Differences in size between treatments may have been masked by the apical dominance displayed by the lettuce plantlets, whereby predominant growth of the main shoot suppresses the outgrowth of auxiliary buds.[39]

Analyte Depletion from Culture Medium. Figure 3 shows target analyte behavior in the culture medium throughout the experiment for lettuce. Light control analysis showed a marked decline in concentration for TCS, TON and NPX, whose total respective masses had degraded to 49%, 51%, and 70% of the original content at 64 days. On the other hand, HCA, CFA, and IBF remained almost constant, with total mass decaying 4%, 7%, and 14%, respectively. All treatment tubes were sterile and therefore devoid of microorganism activity. Hence, the decline in analyte mass was solely attributed to photodegradation, as no significant changes in compound concentration were found in the control samples kept in darkness. This behavior was not unexpected, since photolysis of all three compounds has already been documented.[40−42]

Target analyte concentration in the culture medium in the presence of plants is shown for both spath (Figure S1.1 in Supporting Information) and lettuce (Figure 3). For spath, analyte mass in the medium was depleted by 75−80% at 60 days and 95−98% at 90 days, while in lettuce the decline in analyte mass reached 80−95% loss in 22 days and then became asymptotic until the end of the experiment. HCA was the fastest depleted analyte in both plant species, with 99% loss in 30 days. These results support previous reports claiming that, in addition to certain physicochemical properties, uptake also heavily depends on the plant species.[14,43] Kinetic behavior of the target analytes was obtained from the concentration data over time. All target analytes in both plant species conformed well to pseudo-first-order kinetic behavior. Thus, rate constants for photolysis and depletion were calculated from experimental data (Table 2). It should be stressed that although photo-

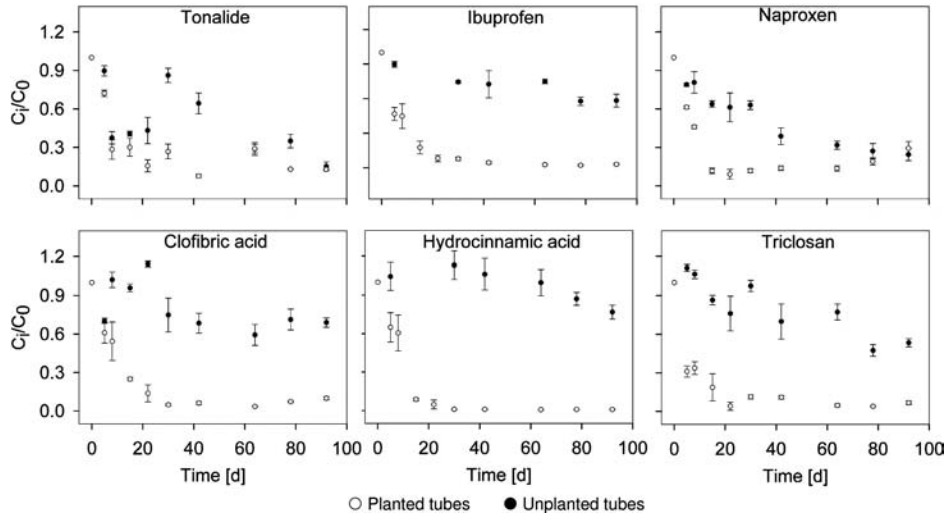

Figure 3. Normalized concentration of target analytes in culture medium through time for lettuce. For unplanted and planted tubes, n = 6 and 9, respectively. Error bars correspond to ±1 standard deviation.

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Table 2. Calculated Kinetic Constant Rates for Target Analytes

	spath		lettuce		
analyte	k_{photo}, d^{-1} (R^2)	$k_{depletion}$, d^{-1} (R^2)	$k_{depletion}$, d^{-1} (R^2)	k_{uptake}, d^{-1} (R^2)	$k_{metabolism}$, d^{-1} (R^2)
clofibric acid (CFA)	0.003 (0.76)	0.0409 (0.95)	0.103 (0.95)	0.184 (0.98)	0.106 (0.99)
hydrocinnamic acid (HCA)	0.004 (0.85)	0.0432 (0.94)	0.179 (0.97)	-	-
naproxen (NPX)	0.010 (0.96)	0.0253 (0.89)	0.117 (0.99)	0.101 (0.97)	0.208 (0.99)
ibuprofen (IBF)	0.002 (0.42)	0.0388 (0.97)	0.064 (0.87)	0.236 (0.99)	0.089 (0.93)
triclosan (TCS)	0.009 (0.44)	0.0315 (0.91)	0.081 (0.70)	0.077 (0.78)	0.154 (0.99)
tonalide (TON)	0.020 (0.93)	0.0224 (0.88)	0.050 (0.92)	0.087 (0.99)	0.125 (0.81)

degradation proved to be very effective in reducing the concentrations of TCS, TON, and NPX over the 64 days of the experiment, it had a smaller impact at 22 days, by which sampling time the analytes had been almost completely depleted from the culture medium, mainly due to lettuce uptake. The loss of analyte mass at 22 days was 7% for TON, 15% for TCS, and 27% for NPX. For the rest of the analytes, mass loss ranged from 1% to 3%. However, photodegradation would play a bigger role in the case of spath, which has the slower uptake rate of the two model plants.

The highest kinetic uptake rates in both plant species were found for HCA, as expected in light of its faster depletion. In spath, it was followed by CFA, while in lettuce it was followed by NPX. Interestingly, these three compounds may exert phytohormonal activity on the model plants: HCA is a documented growth inhibitor,[44] CFA is a putative auxin inhibitor,[45] and NPX is an inhibitor of abscisic acid biosynthesis.[46] This could mean that these compounds enter the plant not merely by diffusion but also through active transport. In addition, they exhibit log K_{ow} values within the optimum uptake range, that is, 1.8−3.1. However, since all compounds except TON have ionizable carboxylic or hydroxyl groups, D_{ow} was applied. D_{ow} is K_{ow} corrected for the effect of ionized groups on K_{ow}-dependent distribution. This is related to the acid−base coefficient (pK_a) of the compound and the medium pH. It can be calculated from the following equation: $D_{ow} = K_{ow}(1 + 10^{pH-pK_a})^{-1}$.[47] A significant correlation between compound depletion from the medium and D_{ow} was expected but was not found. Although the medium pH remained constant at 5.5 throughout the experiment period, it has been reported that plants exude organic and inorganic acids through the roots, which modifies the medium pH near the root surface (1−2 mm) over time.[43] These differences in the medium pH lead to inaccurate D_{ow} calculations since the ionized and neutral forms of the contaminants may occur at the same time in the medium and in the first millimeters surrounding the root surface, respectively. The latter behavior could explain the lack of correlation between D_{ow} and analyte depletion from the culture medium for both plants.

K_{ow} was used for correlation analysis, along with compound molecular weight, because the use of D_{ow} was deemed inappropriate due to its dependence on medium pH. Statistically significant negative correlations ($p < 0.05$) were found between rate constants of depletion and molecular weight and log K_{ow}, with Pearson coefficients of 0.69 and 0.90, respectively, in lettuce (Figure 4). However, spath depletion constants correlated only with log K_{ow}. The correlation between $k_{depletion}$ and molecular weight translates into a higher potential uptake for compounds with lower molecular weights, which is in accordance with previous studies,[48] while the second correlation taking place between $k_{depletion}$ and log K_{ow} shows

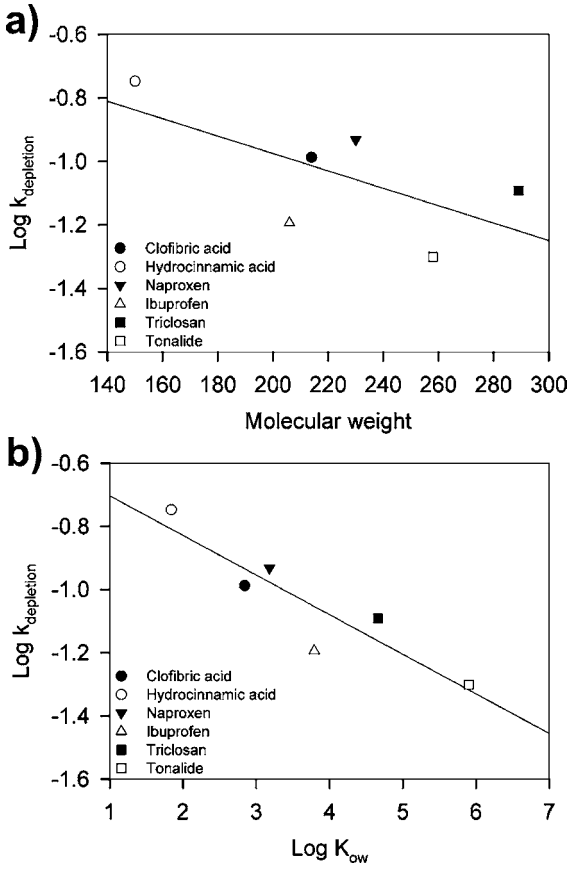

Figure 4. Statistical correlation ($p < 0.05$) between target analyte depletion from culture medium in lettuce: (a) molecular weight (R = 0.69) and (b) log K_{ow} (R = 0.90) for the studied compounds.

higher uptake potential for those compounds within the range of optimum log K_{ow} values.

It is important to note that, as stated before, root exudates contain released ions (i.e., H^+), inorganic and organic acids, proteins, and enzymes but mainly consist of carbon-based compounds. Enzymes, namely peroxidases and hydrolases,[49] could act on xenobiotics and lead to their degradation. Compound degradation due to exudates could be especially important in the final stages of the experiment, since exudation rates vary according to the plant developmental stage. Root exudation is positively correlated with root growth, which translates into a higher secretion of exudates in actively growing root systems.[50]

Uptake Kinetics in Plant Tissue. Since lettuce uptake of the target analytes was clearly faster than spath uptake, and lettuce is a food commodity of commercial value, further analyses were conducted on lettuce. Analyses were performed on tissue samples taken at 5, 8, 15, 22, and 64 days. All target

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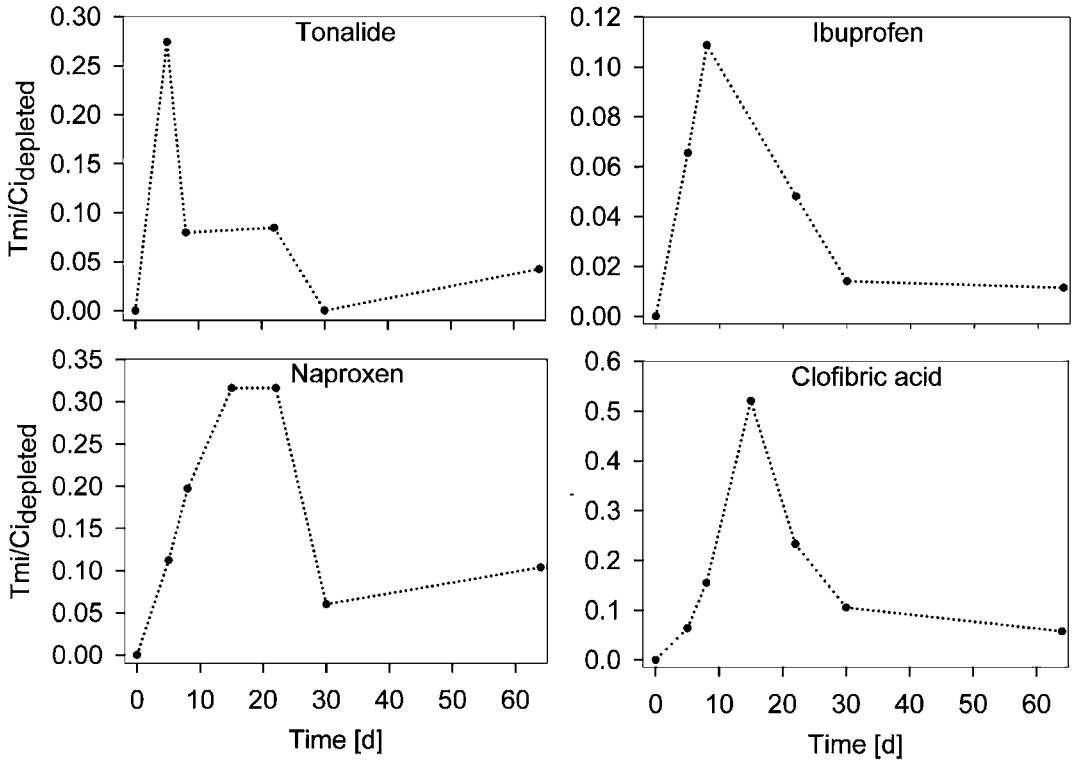

Figure 5. Normalized concentration of target analytes in lettuce tissue (whole plant), grown in spiked medium, through time. Each point in the graph corresponds to the quotient between the analysis of a tissue pool made of three plants (Tmi) and the depleted concentration from the medium culture (Cidepleted).

compounds except HCA were detected in their parent form at all sampling times. As HCA is a growth inhibitor, it may have a faster metabolization pathway, which would impair its free-form detection.

The determined levels of target analytes in the tissue are due to both plant translocation and lipophilic absorption in cell membranes. However, lipophilic absorption would be important only for TON, as its effect would be negligible for weak acids, which are predominantly in their ionic forms at the physiological pH range, 5.5−8.[26]

A maximum analyte concentration was observed in all cases from 15 to 22 days. Following this maximum, a sudden drop in the free-form compound concentration in tissue was detected. These results suggest the existence of a detoxification system for these compounds in lettuce. This behavior has already been reported in other studies involving acetaminophen uptake by Indian mustard[12] and phenanthrene uptake by ryegrass.[51]

The kinetic behavior of target analytes was obtained from plantlet tissue concentration data over time (Figure 5). The first plot segment, representing analyte incorporation into the tissue, was used to calculate the relative uptake rate. It should be noted that this segment also includes the detoxification rate, which was lower than the uptake rate at this stage of the experiment. Additionally, based on the second plot segment, the plant detoxification system was used to obtain the respective metabolic rate constant. Since the analytes had been almost completely depleted from the culture medium by this stage of the experiment, uptake was deemed negligible.

All target analytes for both detoxification and uptake behavior closely followed pseudo-first-order kinetics (R^2 > 0.81). Accordingly, rate constants for relative uptake and plant detoxification were obtained and are shown in Table 2. Interestingly, with regard to relative uptake rate constants, compounds whose structure includes a carboxylic group exhibited higher uptake rates. These compounds, which are weak acids with pK_a between 2.84 and 4.66, were in their neutral form because the culture medium, as noted above, should have a lower pH due to plant exudates. This facilitates their uptake by roots, which in turn could explain the higher uptake rates for these compounds. In addition, their translocation could be supported by an ion trap effect,[52] which depends on the chemical pK_a and log K_{ow} and the pH of the solution, cytoplasm, vacuole, and apoplast of the cell, as well as the permeability ratio between neutral and ionic molecules.[26] Nonionic acid can pass through the membrane, dissociate to the anion in the plant compartment with higher pH, the apoplast (pH ∼ 5.5), and then accumulate in the phloem (pH ∼ 8−8.5) as a result of ion trapping in this compartment.

Statistically significant negative correlations (p < 0.05) were found to exist between rate constants of uptake and molecular weight, R = 0.89. As stated before, this correlation translates into a higher uptake potential for compounds with lower molecular weights. Neither log K_{ow} nor log D_{ow} correlated significantly with uptake rate constants. Both molecular weight and hydrophilicity/hydrophobicity are properties related to diffusion and thus passive transport. Perhaps the lack of correlation with log K_{ow} and log D_{ow} suggests that these compounds may enter the plant by active transport, which would not be unexpected since these compounds exhibit both hormonelike structures and effects. For instance, the hormone-like structure 2,4-D, a phenoxy acid compound like CFA, is known to be taken up by active transport through influx carriers.[53] In addition, phloem mobility is optimal for compounds of intermediate hydrophobicity (log K_{ow} 1−3)

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and weak acidity (pK_a 3−6).[54] Those conditions are fulfilled by CFA and NPX (IBF has a slightly higher log K_{ow}), which would further enhance their uptake and translocation.

As for detoxification rate constants, the highest rate was displayed by NPX, while the lowest was displayed by CFA. Neither log K_{ow} nor log D_{ow} nor molecular weight correlated significantly with detoxification constant rates. No trend was apparent regarding the magnitudes of the constant rate of detoxification for the studied analytes.

However, all the compounds in this study except for TON are thought to be detoxified by glycosyltransferases, since these enzymes act on -OH, -NH_2, -SH, and -COOH functional groups in molecules. In contrast, tonalide could undergo glutathione conjugation, catalyzed by glutathione S-transferases, since the presence of conjugated double bonds or halogen functions triggers these enzymes.[55] It is also worth mentioning that these compounds are functionalized with -COOH and -OH groups suitable for their direct entrance at stage II,[34] which would increase their displayed detoxification rate constants.

In addition, the rates of chemical transformation and the types of metabolites formed will also depend strongly on the plant species.[34] It is thus necessary to examine the conjugates formed by the plant detoxification system more closely. For instance, glucose conjugation is a frequent inactivation pathway in plants that yields glycosylate compounds that may be better absorbed and more bioavailable through the food chains than their aglycon counterparts.[14] Although in this study we did not determinate metabolites or conjugates, the elucidation of xenobiotic metabolization pathways and conjugate formation could be undertaken with the use of isotopically labeled standards, which could allow the characterization of the xenobiotic behavior upon entrance to the plant. Study of conjugates is important, as these compounds could be further hydrolyzed in animal digestive tracts, rendering possibly toxic molecules.

The plant uptake and fate of neutral compounds have been frequently addressed. However, this is not the case with ionic compounds, for which numerous uncertainties remain. This study offers insight into plant uptake of both ionic and neutral organic contaminants via root in commercially relevant plant species. However, care should be taken in extrapolating the results obtained in this study since plants grown in vitro are mixotropic, meaning they are not fully dependent on photosynthesis, because of the low light intensity in which they develop, and carbohydrate content of the medium.[56] Nevertheless, since plant root is fully functional, the compound uptake behavior under these controlled conditions is a valid first step in understanding this complex process. The study of ionic compounds is particularly meaningful, because in a real environment, weak electrolytes have high accumulation and translocation potential due to their low lipophilic sorption to the soil matrix. This could translate to high bioavailability with simultaneous high accumulation due to the ion trap effect.[57]

■ ASSOCIATED CONTENT

Supporting Information

Additional text with details of reagents and plant tissue analysis; one table listing GC-MS/MS product ion information;.and one figure showing behavior of target analytes in the presence of spath. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

CFA, clofibric acid; HCA, hydrocinnamic acid; NPX, naproxen; TCS, triclosan; TON, tonalide

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