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Irrigation of Root Vegetables with Treated Wastewater: Evaluating Uptake of Pharmaceuticals and the Associated Human Health Risks

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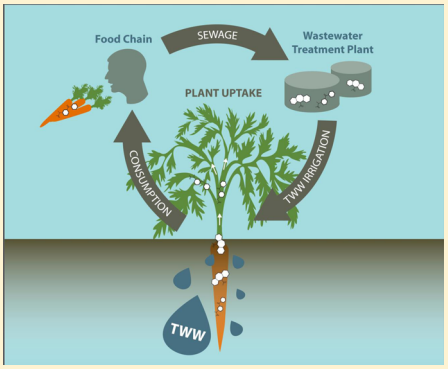
Supporting Information

ABSTRACT: To meet mounting water demands, treated wastewater has become an important source of irrigation. Thus, contamination of treated wastewater by pharmaceutical compounds (PCs) and the fate of these compounds in the agricultural environment are of increasing concern. This field study aimed to quantify PC uptake by treated wastewater-irrigated root crops (carrots and sweet potatoes) grown in lysimeters and to evaluate potential risks. In both crops, the nonionic PCs (carbamazepine, caffeine, and lamotrigine) were detected at significantly higher concentrations than ionic PCs (metoprolol, bezafibrate, clofibric acid, diclofenac, gemfibrozil, ibuprofen, ketoprofen, naproxen, sulfamethoxazole, and sildenafil). PCs in leaves were found at higher concentrations than in the roots. Carbamazepine metabolites were found mainly in the leaves, where the concentration of the metabolite 10,11-epoxycarbamazepine was significantly higher than the parent compound. The health risk associated with consumption of wastewater-irrigated root vegetables was estimated using the threshold of toxicological concern (TTC) approach. Our data show that the TTC value of lamotrigine can be reached for a child at a daily consumption of half a carrot (~60 g). This study highlights that certain PCs accumulated in edible organs at concentrations above the TTC value should be categorized as contaminants of emerging concern.



INTRODUCTION

Pharmaceutical compounds (PCs) comprise a wide range of chemicals including prescription and over-the-counter medications, veterinary drugs, diagnostic agents, and nutritional supplements. After ingestion or application, the PCs are excreted from the body as parent or metabolized compounds and enter the municipal sewage system. Recent studies have shown that conventional wastewater treatment does not fully eliminate PCs in the process; therefore PCs are present in treated effluents and sludge.[1−4]

PCs and their metabolites are introduced into the agricultural environment through application of biosolids and irrigation with treated wastewater. The fate of PCs in the agricultural environment has been extensively studied.[5−11] Moreover, during the past decade there have been a growing number of studies on PC uptake by plants and crops.[12−17] These studies can be divided into three main categories based on their experimental design: hydroponic, greenhouse, and field studies. Hydroponic experiments are useful in understanding the mechanism of uptake, but they do not manifest the complexity of an actual agricultural environment. Even if the experiments are conducted at environmentally relevant PC concentrations, they do not account for the importance of affecting factors in the real environment (e.g., the presence of soil organic matter or the effect of the wastewater's properties) and therefore do not represent the actual uptake of different PCs in the agro-environment. Greenhouse experiments can better estimate the actual PC uptake since they are conducted in soils. However, the data collected from the experiments are limited because they do not represent actual farming practices, or genuine soil, or ecological conditions typical for commercial agriculture farming. Only through field experiments can the actual potential uptake of PCs by crops be fully assessed and integrated into a database for risk assessment. Only a few studies reported the uptake of PCs by crops in field experiments. Two studies have focused on biosolid application,[12,18] and only one has examined the introduction of PCs via irrigation with surface water that had been mixed with treated wastewater.[19] In contrast to biosolids application, irrigation with PC contaminated water provides continuous introduction of PCs to the agriculture environment. This affects the fate of PCs in the soil[10,11,17] as well as their uptake by crops.

The use of treated wastewater for irrigation is on the rise worldwide and is not limited to arid zones.[20] Considering the ubiquity of PCs in treated wastewater,[3] it is highly important to evaluate their fate and particularly their uptake by crops under conditions that are as similar as possible to those of commercial

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agricultural farming. Therefore, the objective of this study was to investigate uptake of PCs by root vegetables (carrots and sweet potatoes) which may represent a worst case scenario of direct contact between the treated wastewater and the consumed crop. PCs exhibiting a wide range of physicochemical properties were introduced to crops through treated wastewater at their environmentally relevant concentrations. In addition, a risk assessment associated with the consumption of treated wastewater irrigated root vegetables was conducted based on the threshold of toxicological concern (TTC) approach. The TTC is a useful tool to estimate the safety of exposure to chemicals found at low concentration in foods and drinking water.[21,22]

EXPERIMENTAL SECTION

Pharmaceuticals. Bezafibrate, carbamazepine, diclofenac, gemfibrozil, ibuprofen, clofibric acid, sulfapyridine, ketoprofen, sulfamethoxazole, and 10,11-epoxycarbamazepine (all >97% purity) were purchased from Sigma-Aldrich Israel Ltd. (Rehovot, Israel). Caffeine (99%) was purchased from Bio Basic Inc. (Toronto, Canada), lamotrigine (>99%) from EnzoBiochem Inc. (New York, NY), metoprolol (99%) from LKT laboratories (St. Paul, MN), naproxen (99%) from Alfa-Aesar Inc. (Heysham, U.K.), sildenafil (99%) from Molekula Ltd. (Dorset, U.K.), and 10,11-dihydroxycarbamazepine from Santa Cruz (Heidelberg, Germany). Selected chemical and physical properties of the studied compounds are presented in Table S1. The following labeled PCs were purchased from Toronto Research Chemicals Inc. (Toronto, Canada): bezafibrate-D4, carbamazepine-^{13}C-D2, diclofenac-D4, naproxen-^{13}C-D3, gemfibrozil-D6, sulfamethoxazole-D4, caffeine-D9, lamotrigine-^{13}C3, (R)-metoprolol-D7, and sildenafil-D3. Ibuprofen-D3, sulfapyridine-phenyl-^{13}C6, ketoprofen-D3, and 10,11-epoxycarbamazepine-D2 were purchased from Sigma-Aldrich Israel Ltd.

Crops, Soils, and Growing Conditions. Two root crops were grown: carrot (*Daucus carota*) during the summer of 2011 and sweet potato (*Ipomea batatas*) during the summer of 2012. The crops were grown in lysimeters (100 cm height, 0.5 m^2 surface area) containing soils from three locations in the northwest Negev region of Israel (Sa'ad, Nir Oz and Ein Hashlosha). These soils are classified as Loessial Arid Brown Calcisol soils;[23] their properties are listed in Table S2. Lysimeters were irrigated with fresh water or treated wastewater. The experiment was performed in triplicate for each soil and water quality. Treated wastewater was provided by a conventional activated-sludge wastewater-treatment facility in the city of Kiryat Gat, Israel. The treated wastewater characteristics during the irrigation period were as follows: total suspended solids (TSS), 7.0−31.7 mg/L; biological oxygen demand (BOD), 22.7−28.1 mg/L; chemical oxygen demand (COD), 40−70 mg/L; pH, 7.7−8.4; and EC, 2.11−2.38 dS/m. To ensure exposure, PCs were added to the treated wastewater in the irrigation line during the growing period at environmentally relevant concentrations,[1,6,8,24] regardless of indigenous concentration in the treated wastewater. PCs were added to the irrigation line using a commercial dosing pump at a dilution rate of 1/500. In addition, three lysimeters with Ein Hashlosha soils were irrigated with treated wastewater without adding PCs to the irrigation line. These lysimeters were therefore exposed only to the indigenous levels of PCs. PC concentration in irrigation water was measured in the water emitted by the drippers in the lysimeters; concentration data are listed in Table S3.

To ensure healthy establishment of the young seedlings, all lysimeters were irrigated with fresh water for the first three weeks after sowing. Thereafter, each lysimeter was irrigated with its designated water type. Throughout the carrot growing season (100 days), each lysimeter was irrigated with 266 L using a drip irrigation system (seven drippers of 1 L/h). The irrigation scheme was dependent on evapotranspiration conditions and crop age. Two months after sowing, the number of carrots per lysimeter was reduced from 70 to 30−35. At harvest, a composite sample of 10 carrots and their leaves as well as soil samples (0−25 cm) were collected from each lysimeter. Throughout the sweet potato growing season (154 days) each lysimeter was irrigated with 305 L using the same drip-irrigation system. At harvest, a composite sample of five sweet potatoes, 20 leaves, and soil samples (0−25 cm) were collected from each lysimeter. Each plant sample was thoroughly rinsed with deionized water to remove soil particles, air-dried for a few hours, and stored at −20 °C until processing.

Sample Preparation, Extraction, and Analysis. Freeze-dried plant materials were ground to a fine powder with a planetary micro mill (Pulverisette 7, Fritsch, Idar-Oberstein Germany) and extracted using an accelerated solvent extractor (ASE350, Dionex, Sunnyvale, CA). Ground plant materials (1 g) were placed in 10 mL of extraction cells on top of 1 g of Florisil (Mg_2O_4Si, Alfa Aesar, Ward Hill, MA) and covered with an extra 1 g of Florisil. Glass-fiber filters (27 mm) were placed at the bottom of the cells. The packed cells were extracted in two static cycles (5 min) with 100% methanol at 80 °C under a constant pressure of 10.34 MPa. Freeze-dried soil samples (5 g) were similarly prepared and extracted with three static cycles (15 min) with acetonitrile/water (70:30, v/v) at 100 °C under a constant pressure of 10.34 MPa. Soil aqueous extracts were obtained by agitating soil samples (10 g) with deionized water (1:1, w/v) for 90 min, followed by centrifugation at 12 000*g* for 15 min. All extracts were evaporated to dryness and redissolved in 990 μL acetonitrile/water (30:70), spiked with 10 μL of a mixture of stable isotope labeled internal standards in acetonitrile, sonicated (37 kHz, 10 min), centrifuged at 17 000*g* for 20 min, and filtered (0.22 μm PTFE) prior to LC-MS analysis.

The final solutions were analyzed by an Agilent 1200 Rapid Resolution LC system (Agilent Technologies Inc., Santa Clara, CA) equipped with a Gemini C-18 column (150 × 2 mm, 3-μm particle size; Phenomenex, Torrance, CA, USA), coupled to an Agilent 6410 triple quadruple mass spectrometer with an ESI ion source (Agilent). A binary gradient of 1.5% acetic acid in deionized water and 0.05% acetic acid in acetonitrile was used for separation of the PCs. LC-MS limits of detection and quantification (LOD and LOQ, respectively) as well as mass transitions are listed in the Supporting Information (Tables S4 and S5, respectively). Recovery values for the plant compartments and the soil are listed in Table S6.

Risk Assessment. The health risks associated with PCs in crops were assessed using the TTC approach,[25−27] based on the Cramer classification tree.[28] TTC is useful to assess risks for substances present in food at low concentrations and lacking toxicity data.[21,22,27] TTC values and compound classification were determined using Toxtree software.[29] Using this method, compounds were classified as having genotoxic potential, or as one of three structural classes (I, II, and III). Class I compounds contain simple structures and are easily metabo-

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lized, thus presenting little toxicity concern. Class II compounds contain substances that may be more harmful than those in Class I, but they do not contain structural features that are suggestive of toxicity. Class III compounds contain reactive functional groups and present greater toxic concern. Potentially genotoxic compounds may interact with DNA and cause mutation to genetic code. Class III compounds (carbamazepine, caffeine, bezafibrate, clofibric acid, ketoprofen, naproxen, and metoprolol) have a TTC value of 1500 ng/kg of body weight per day. Potentially genotoxic compounds (10,11-epoxycarbamazepine, lamotrigine, sildenafil, sulfamethoxazole, and sulfapyridine) have a TTC value of 2.5 ng/kg body weight per day.[27] Consumption of a PC above the TTC value indicates a possible risk of exposure and demands specific toxicity analysis of the PC.

Data Analysis. PC concentrations are presented per dry sample weight (soil, plant). For the risk assessment analysis, PC concentration is given per crop fresh weight. Statistical analysis between treatments (All Pairs, Tukey HSD, $p < 0.05$) and comparison between the two means (Student's t $p < 0.05$) were performed using JMP software, version 10.0.0.1 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The average root yields of carrots and sweet potatoes per lysimeter were 2.5 ± 0.6 and 3.5 ± 1.8 kg, equivalent to 49.5 ± 12 and 70 ± 36 Mg/ha, respectively. These yields were similar to those obtained in commercial fields. No significant differences in carrot yields were obtained between the different applied irrigation water (fresh water, spiked or nonspiked treated wastewater) or between the different soils. For sweet potatoes, no significant differences in yields were obtained between the different water treatments for each soil.

PC Concentrations in Water, Soil, and Plant. Carrots and sweet potatoes were exposed to 14 different PCs at environmental concentrations via irrigation with treated wastewater (Table S3). PCs were not detected in plants irrigated with fresh water. For plants irrigated with treated wastewater, PCs that were taken up by the plants exhibited generally higher concentrations in leaves compared to roots (Figure 1). This trend was similar for both crops. Concentrations in the carrot leaves were in the following order: carbamazepine > lamotrigine > caffeine > bezafibrate ≥ clofibric acid ≥ sildenafil ≥ sulfapyridine ≥ metoprolol. For the sweet potato leaves, the order was caffeine > carbamazepine > lamotrigine ≥ clofibric acid ≥ bezafibrate ≥ sildenafil ≥ sulfapyridine. In the carrot roots, the order of PC concentrations was carbamazepine > lamotrigine > bezafibrate ≥ caffeine ≥ clofibric acid ≥ sildenafil ≥ gemfibrozil ≥ sulfapyridine ≥ sulfamethoxazole. In the sweet potato roots, the concentration order was caffeine > carbamazepine > lamotrigine > sulfamethoxazole ≥ gemfibrozil ≥ clofibric acid. Sulfamethoxazole was only detected in the roots, while metoprolol was only detected in the leaves. Since none of the tested PCs are volatile under the applied conditions and no direct contact occurred between the leaves and the irrigation water, the detection of the applied PCs in the leaves is related to their uptake by the roots and translocation within the plant.[16,30,31]

Nonionic PCs (carbamazepine, lamotrigine, and caffeine) were detected at significantly higher concentrations than ionic PCs in leaf, root, and soil samples. Nonionic organic molecules are able to cross cell membranes easily and thus have higher potential to be taken up by the roots.[32,33] Once in the root, these compounds tend to be translocated by the water flow driven by the water potential gradient and thus accumulate at a higher proportion in the leaves.[32,34] Lower concentrations of ionic PCs in the leaves may result from the much lower permeability of cell membranes to ionic compounds and due to adsorption to the soil and the cell wall. For PCs having pK_a values in the range of ca. 4.5−7.5, their charge may change along the soil-rhizoplane-cytosol-apoplast-symplast continuum due to the pH variation. Thus, a compound having negative charge in the soil may become uncharged in the rhizoplane and be taken up easily, and a compound having zero-charge in the cytosol might become positively charged in the vacuole, which will result in its trapping within the vacuole and limit its transport.[34]

At the time of harvest, only eight PCs were detected in the soils (Figure 1). Ionic PCs, except for sulfamethoxazole, were not detected in the soil's profile up to a depth of 75 cm (Figure S1), suggesting that these compounds were rapidly biodegraded and/or transformed in the soils.[10] For most PCs (caffeine, carbamazepine, lamotrigine, sildenafil, sulfapyridine, sulfamethoxazole, gemfibrozil, and metoprolol), the recovered amounts in the soils were several magnitudes higher than the amounts taken up by the studied plants (Figure S2). The maximum amount of PCs taken up by the plants was up to 3.5% of the amount accumulated in the soil, supporting that soils are the major sink determining the fate of PCs in agricultural environments.[35]

The distributions of the PCs between the soil aqueous extracts, roots, and leaves are presented in Figure 2. Data for the two crops show a similar trend of increasing PC concentrations in the leaves as PC concentrations in the root increase (Figure 2A). This suggests that translocation from root to shoots was similar in both crops and that the differences obtained in leaf concentrations (Figure 1) between the crops are related to uptake potential. In Figure 2B, the concentration ratio between leaves to soil aqueous extracts for the two crops exhibited a similar trend (concentration in the leaves increase with increasing concentration in the soil aqueous extracts) even though each crop exhibited a different trend of PC uptake. The differences obtained between the crops (Figure 2B and C) are most likely due to the lower lipid content in the sweet potato root compared to the carrot root (0.05%[36] and 0.6−1.9%,[37] respectively) and the different dynamics of root and plant development. Root bulking of the carrot is gradual, occurring throughout the season, whereas sweet potato bulking starts only after about 100 days after planting when irrigation and transpiration are reduced. In addition, sweet potatoes are fast growing and support much larger foliage biomass than carrots. These differences resulted in higher concentrations of the PCs in the roots and leaves of the carrot plant (Figure 1).

Nonionic PCs. In our system, the nonionic PCs were carbamazepine, lamotrigine, caffeine, and sulfapyridine. Carbamazepine, lamotrigine, and caffeine were detected at high concentrations in all plant and soil samples. It is important to note that the uptake order of these PCs did not correlate directly with their K_{ow} values, indicating that additional factors such as the compound's pK_a, pH, ionic strength, biodegradation, and sorption might affect uptake. The relatively high concentration of carbamazepine in the leaves supports that its translocation is governed by transpiration-derived mass flow.[16] Unlike carbamazepine, lamotrigine (weak base, pK_a 5.34) is partially ionized in the extracellular space and vacuole

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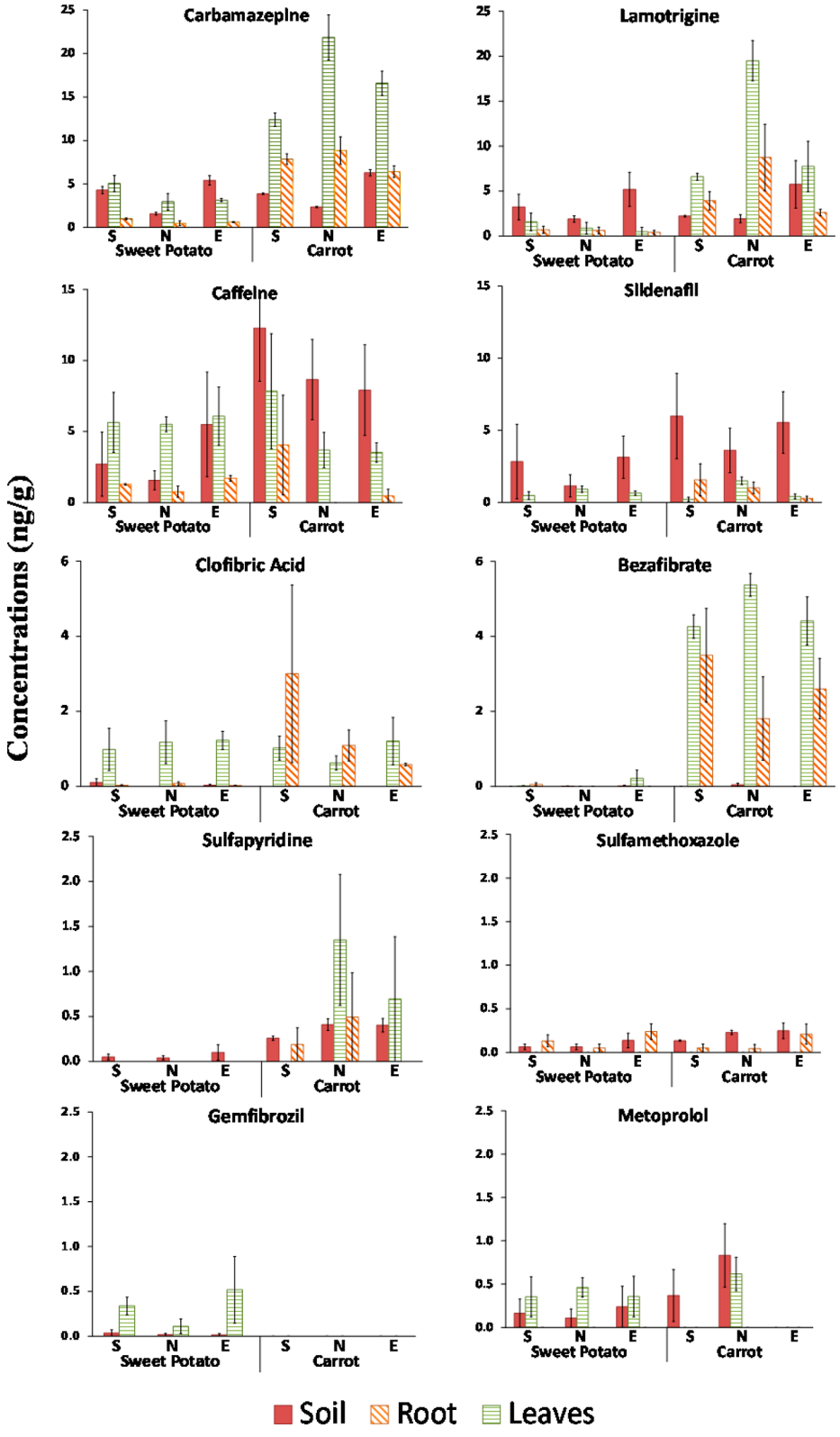

Figure 1. Concentration (ng/g) of pharmaceutical compounds (PCs) in the roots, leaves, and soils for crops irrigated with PCs-spiked treated wastewater (Table S3). Mean values and standard errors are presented (n = 3) for the different soil treatments (Sa'ad, S; Nir Oz, N; Ein Hashlosha, E).

(pH ~ 5.5) and is mostly nonionic in the soil and in the cytoplasm (pH ~ 7.5). At pH 5.5, ~40% of the lamotrigine is positively charged, which promotes its adsorption to negatively charged sites in the rhizoplane and cell walls and hinders uptake. The uncharged lamotrigine can easily cross the cell membranes, but once it is inside the cell it can be trapped as an anion in the vacuole.[9,34] Both processes result in lower

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lamotrigine translocation to the leaves in comparison with carbamazepine (Figure 2B).

Carbamazepine and lamotrigine were detected at similar concentrations in the corresponding soils for both crops (Figure 1). Concentrations of carbamazepine and lamotrigine were higher in soils containing higher organic matter (Ein Hashlosha) or clay (Sa'ad) contents. In these soils, the two compounds were taken up by the plant at a lower level, indicating the importance of PC−soil interactions determining uptake. The compound's ability to be taken up by the plant is based on three partitioning equilibria: between the soil solid phase and the soil solution, between the soil solution and the aqueous phase in the root, and between the soil solution and the solid phase of the lipophilic root.[32] Thus, the organic content of the soils, the type of organic matter, the clay content, and the different lipid contents in the roots have a significant impact on the fate of the compound.

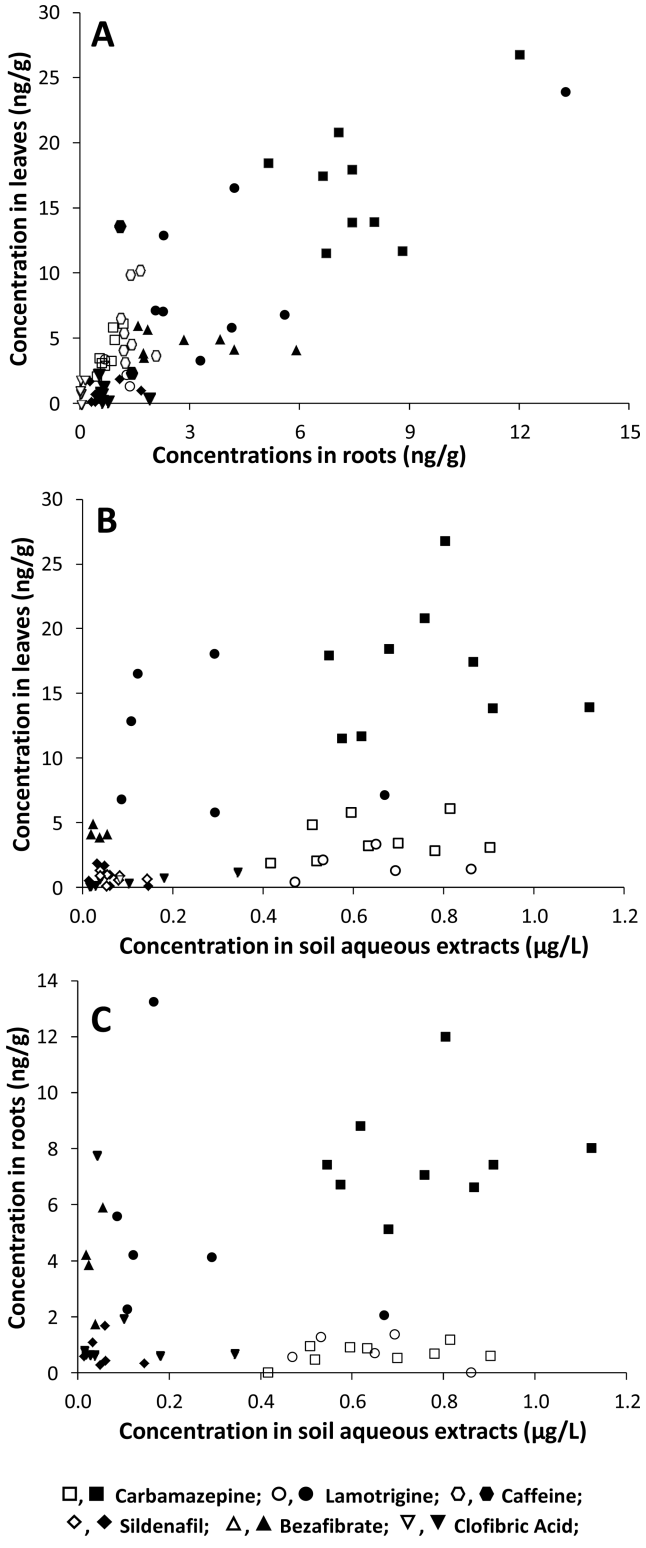

Figure 2. Concentrations of pharmaceutical compounds in the leaves versus roots (A), in the leaves versus soil aqueous extracts (B), and roots versus soil aqueous extracts (C). Filled symbols are data obtained for carrots; open symbols are data obtained for sweet potato.

Caffeine is a polar neutral compound which is rapidly taken up by the plant and translocated from roots to shoots.[38] In the sweet potato leaves and roots, caffeine was found at the highest concentration; in the carrot roots and leaves, caffeine concentrations were generally lower than carbamazepine and lamotrigine. The lower caffeine concentrations in carrots are most likely the result of the higher lipid content of the carrot roots which hinders the uptake of the hydrophilic caffeine. Sulfapyridine was detected at low concentrations in both carrot organs but was not detected in sweet potato roots and leaves (Figure 1). The lower concentration of sulfapyridine in the soils compared to the other nonionic compounds was likely due to high degradation rates in the soil environment. It is interesting to note that sulfapyridine was detected in all soils (Figure 1); however it was not detected in the soils' aqueous extract. This is likely due to strong chemisorption of sulfapyridine to the soil matrix.[39]

Ionic PCs. In our study, 10 ionic PCs were introduced with the irrigation water into the soils (Table S3): one positively charged PC (metoprolol), eight negatively charged PCs (bezafibrate, clofibric acid, diclofenac, gemfibrozil, ibuprofen, ketoprofen, naproxen, and sulfamethoxazole), and one with a varying charge (sildenafil). Four of the anionic PCs, ketoprofen, naproxen, ibuprofen, and diclofenac, were not detected in any plant organs of either crop or in the receiving soils. These nonsteroidal anti-inflammatory PCs are weak acids with pK_a values between 4.15 and 4.91. In the soil environment (pH ∼ 7.5), they are negatively charged and thus are repulsed by the negative charge of the cells of the root apoplast deterring uptake.[34] Another mechanism that might reduce uptake of these compounds is related to their rapid biodegradation rates in the soil. In an earlier work,[10] conducted in the same soils used in the current study, half-lives were reported to be less than 1 day for ibuprofen and diclofenac and 2−9 days for naproxen. The other anionic PCs, clofibric acid, gemfibrozil, and bezafibrate (lipid regulators), were detected in the leaves or roots of at least one of the crops. Clofibric acid was detected at low concentrations in the roots (<0.8 ng/g) and leaves (0.43−2.43 ng/g) of both crops. Gemfibrozil was detected only in sweet potato leaves (0.1−0.53 ng/g). Bezafibrate concentrations in carrot leaves (3.49−5.93 ng/g) and roots (1.50−5.91 ng/g) were the highest of all anionic PCs. Sulfamethoxazole was detected in the roots of both crops (0.05−0.24 ng/g). The low concentrations of the above-mentioned PCs is most likely the results of rapid degradation in the soils.[9]

Sildenafil was detected in the leaves of both crops, and in the carrot's roots. Sildenafil has two pK_a values, 6.4 and 7.4, and therefore in soils it can be found as positive, neutral, or negative species. This complicates the prediction of sildenafil uptake, translocation, and fate in the agro-environment. At the soil pH,

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sildenafil can be taken up as a neutral compound, but in the extracellular space or vacuole (pH ~5.5), it becomes positively charged and can be trapped as an adsorbed cation in the extracellular cell walls and as a cation in the vacuoles as suggested for other molecules.[34,40] The neutral form of sildenafil is more lipophilic and less soluble than the anionic form, and therefore more likely to interact with the soil organic matter similar to nonionic compounds.[41] This suggests why although soil concentrations of sildenafil, carbamazepine, and lamotrigine were similar (Figure 1), the uptake and translocation of sildenafil were significantly lower.

Metabolites of Carbamazepine. As carbamazepine was detected in all studied samples, we further analyzed two of its metabolites (10,11-epoxycarbamazepine and 10,11-dihydroxycarbamazepine) in the soil, soil aqueous extract, and plant organs. The metabolite 10,11-epoxycarbamazepine was detected in all roots (0.09−2.70 ng/g), leaves (14.78−65.81 ng/g), aqueous soil extract (0.04−0.11 ng/L), and soils (0.12−0.40 ng/g) of both crops. The metabolite 10,11-dihydroxycarbamazepine was detected in leaves (0.31−2.91 ng/g), aqueous soil extract (0.06−0.21 ng/L), and soils (0.05−0.09 ng/g) of both crops. 10,11-Dihydroxycarbamazepine was negligible in root samples. Although different soils exhibited various concentrations and plant uptake varied (Figure 1), the ratio of metabolites to parent compound was quite constant for the soil and each plant organ for both crops (Figure 3). In the soil, the parent compound was dominant (~90%), supporting low biodegradability of carbamazepine.[10] In the roots, the parent compound was also dominant (~90%), suggesting that carbamazepine is predominantly taken up by the plant as the parent compound and is probably not metabolized in the roots. In leaves of carrots and sweet potatoes, concentrations of metabolites were significantly higher compared to soil and root concentrations. The parent compound made up only 11 ± 2% of all carbamazepine species in sweet potato leaves and 28 ± 3% in carrot leaves, suggesting significant metabolism of carbamazepine in leaves. This hypothesis is supported by the elevated concentrations of carbamazepine metabolites observed in leaves of tomatoes and cucumbers.[42]

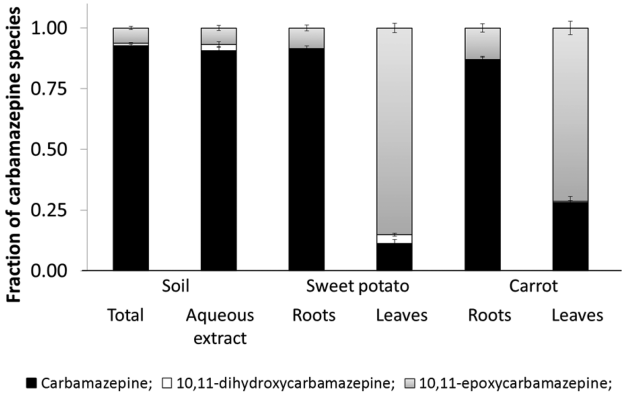

Figure 3. Distribution of carbamazepine and its metabolites, 10,11-epoxycarbamazepine and 10,11-dihydroxycarbamazepine, in the bulk soils, soil aqueous extracts, roots, and leaves of carrots and sweet potatoes. Mean values and standard errors are presented (n = 6).

The metabolism of PCs is categorized into phase I and phase II. Phase I processes modify PCs by incorporating reactive and polar groups into their substrates to activate a pharmacologically inactive compound or to decrease the reactivity of an active compound. Cytochrome P450 monooxygenase (CYP450), a phase I enzyme, is responsible for ~75% of the metabolic reactions and PC's bioactivation in the human body. Plant CYP450 enzymes are known to be responsible for the metabolism of a variety of herbicides, insecticides, and organic pollutants.[43,44] Carbamazepine was reported to be metabolized by CYP450 enzymes in the fungus *Pleurotus ostreatus*.[45] Thus, in our study it is also likely that carbamazepine is metabolized by CYP450 in the leaves of plants.

Exposure and Risk Assessment. Sweet potato is an important crop, with global production exceeding 100 million tons annually, of which the vast majority is produced in Asia.[46] Per capita consumption of sweet potato varies from country to country: 2.4 kg/y in the U.S.A., 1.9 kg/y in Israel, 0.3 kg/y in southern European countries, 13.5 kg/y in Caribbean countries, and 25.5 kg/y in China.[46] In several African countries, sweet potatoes are a vital component of the daily diet, and per capita consumption is as high as 73 to 89 kg/y.[46] In addition to the roots, the leaves of sweet potatoes are also consumed as vegetables in Southeast Asia and increasingly in Africa.[47,48] Carrot consumption is higher than that of sweet potatoes. In the U.S.A., per capita consumption is around 5.5 kg/y, in Europe it is 6−8 kg/y, and in Australia around 10 kg/y.[49]

The health risks associated with PCs in crops were assessed using the TTC approach, as is common for assessing the safety of chemicals that are found at low concentrations in food and lack toxicity data.[21,22] The TTC is a conservative estimate that is based on 5% of the level at which there are no observed adverse effects with an additional 10^{-6} uncertainty factor. This approach has been used to evaluate risks associated with 10,11-epoxycarbamazepine in drinking water.[21] The TTC values for a child (25 kg) are 62.5 and 37 500 ng/day for potentially genotoxic PCs and for class III PCs, respectively. For an adult weighing 70 kg, the TTC values are 175 and 105 000 ng/day for the two category PCs, respectively. Consumption of a PC above the TTC value should not be presumed to be toxic. It should however indicate a demand for specific toxicity analysis of the compound. The TTC approach takes into account chronic exposure to a mixture of compounds by population groups which are more susceptible in regards to risks (e.g., children, pregnant women, and elderly people).

To assess the risk of PCs in the irrigation water, three lysimeters with Ein Hashlosha soil were irrigated with nonspiked treated wastewater, as used by farmers for local agriculture. The uptake of PCs was measured at indigenous concentrations in treated wastewater used for irrigation (Table S3). It is important to note that most of the PCs in this study were not detected in any of the plants grown under this treatment. Table 1 presents the fresh weight concentrations of the PCs detected in the crops and the daily consumption required to reach TTC levels. Class III compounds such as carbamazepine and caffeine would require an adult to consume hundreds of kilograms of sweet potatoes or carrots daily to reach the TTC level. This is not a reasonable daily consumption estimate, and therefore we conclude that the presence of these compounds in treated wastewater does not pose an actual health risk to the consumer. However, the TTC levels of potentially genotoxic compounds such as the metabolite 10,11-epoxycarbamazepine and lamotrigine are reached at a much lower daily consumption (Table 1). The TTC level of lamotrigine would be surpassed for an adult (70 kg) by consuming two carrots a day (180 g carrot/day) and for a child (25 kg) by consuming half a carrot a day (60 g carrot/

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Table 1. Risk Assessment of Daily Exposure to Pharmaceutical Compounds Based on Concentrations in Carrot and Sweet Potato Grown in Ein Hashlosha Soil Irrigated with Non-Spiked Treated Wastewater Using the Threshold of Toxicological Concern (TTC) Approach[a]

	sweet potato roots	sweet potato leaves	carrot roots	carrot leaves
	fresh weight concentration (ng/g)			
carbamazepine	0.116	0.177	0.799	1.069
10,11-epoxycarbamazepine	0.013	0.708	0.244	4.130
caffeine	0.256	0.719	0.293	0.603
lamotrigine	n.d.	n.d.	0.983	0.416
	daily consumption (kg) by a 70 kg person to reach TTC			
carbamazepine	905.17	593.22	131.38	101.65
10,11-epoxycarbamazepine	13.58	0.25	0.71	0.04
caffeine	410.16	146.14	288.78	199.24
lamotrigine	n.d.	n.d.	0.18	0.42
	daily consumption (kg) by a 25 kg person to reach TTC			
carbamazepine	323.28	211.86	46.92	36.30
10,11-epoxycarbamazepine	6.25	0.09	0.25	0.02
caffeine	146.48	52.19	103.14	71.16
lamotrigine	n.d.	n.d.	0.06	0.15

[a]n.d., not detected.

day). Consumption of sweet potato leaves and carrot leaves by a child (25 kg) would surpass the TTC level of 10,11-epoxycarbamazepine at 90 g leaves/day and 25 g leaves/day, respectively. As mentioned before consumption of sweet potatoes leaves is common in parts of Asia and Africa. It is noteworthy that consumption above the TTC value should not be presumed to be toxic. It should, however, indicate a demand for specific toxicity analysis of the PC.

Before consumption, root vegetables are usually peeled, thus the effect of peeling was evaluated to estimate realistic exposure. In terms of amount, most of the root mass is in the core, the peel represents approximately 5% and 13% of the root fresh weight in sweet potatoes and carrots, respectively. In the sweet potato, only carbamazepine and lamotrigine were found at significantly higher concentrations in the peel than in the peeled root. Based on a mass balance, the amount of PCs removed by peeling the sweet potato represents approximately 7% of the total root carbamazepine and lamotrigine. In carrots, carbamazepine and caffeine were the only PCs that exhibited significantly higher concentrations in the peel than in the peeled root. Thus, by peeling, the amount of carbamazepine and caffeine is reduced by 25% and 32%, respectively. However for the genotoxic compounds, lamotrigine and 10,11-epoxycarbamzepine, no significant differences were found between the concentrations in the peel compared to the rest of the root. Thus, peeling has no effect on the TTC levels of these compounds. We therefore suggest that peeling would not reduce the risk associated with PCs present in carrots irrigated with treated wastewater.

Environmental and Health Implications. Results of this study demonstrate that PCs are taken up by root crops irrigated with treated wastewater. Our data show that the extent of PC uptake is influenced by the physicochemical properties of the compound, the physiological properties of the crop, and the soil properties. Weakly acidic PCs exhibited low or no bioconcentration in plants or soils. Nonionic PCs, carbamazepine, caffeine, and lamotrigine, were detected in all soil and plant samples with greater concentrations in the leaves than the roots. Higher concentrations of nonionic PCs were detected in crops grown in soils with low organic and clay contents. Thus, crops grown on sandy soils with poor soil organic matter present a greater risk for PC accumulation when irrigated with treated wastewater. This study demonstrates that carbamazepine metabolites in leaves could be several times higher than those of the parent compound and that measurements of only the parent compound might underestimate the bioconcentration of a PC.

Our data for most of the studied PCs demonstrate that the daily consumption of root crops irrigated with treated wastewater does not pose a health threat. However, for two compounds (lamotrigine and 10,11-epoxycarbamazepine) detected in carrot roots, carrot leaves, and sweet potato leaves (Table 1), a health risk was suggested. Hence, certain PCs that are taken up by plants and accumulate in edible organs at concentrations above the TTC value should be categorized as contaminants of emerging concern. These PCs should be studied in the future, and their exact level of toxicity should be determined, after which regulation of acceptable levels in treated wastewater for irrigation could be established.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Information regarding the studied PCs (Table S1), the properties of the soils (Table S2), concentration of PCs in the irrigation water (Table S3), detection and quantification limits (Table S4), LC-MS operational parameters (Table S5), extraction recovery data (Table S6), and figures describing PC distribution in the lysimeter profile (Figure S1) and total accumulation of PC per lysimeter (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Ratola, N.; Cincinelli, A.; Alves, A.; Katsoyiannis, A. Occurrence of organic microcontaminants in the wastewater treatment process. A mini review. *J. Hazard. Mater.* **2012**, *239*, 1−18.

(2) Paoletti, E.; Miège, C.; Choubert, J. M.; Ribeiro, L.; Eusèbe, M.; Coquery, M. Fate of pharmaceuticals and personal care products in wastewater treatment plants − Conception of a database and first results. *Environ. Pollut.* **2009**, *157* (5), 1721−1726.

(3) Fent, K.; Weston, A.; Caminada, D. Ecotoxicology of human pharmaceuticals. *Aquat. Toxicol.* **2006**, *76* (2), 122−159.

(4) Carballa, M.; Omil, F.; Lema, J. M.; Llompart, M.; García-Jares, C.; Rodríguez, I.; Gómez, M.; Ternes, T. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Res.* **2004**, *38* (12), 2918−2926.

(5) Fatta-Kassinos, D.; Kalavrouziotis, I. K.; Koukoulakis, P. H.; Vasquez, M. I. The risks associated with wastewater reuse and xenobiotics in the agroecological environment. *Sci. Total Environ.* **2011**, *409* (19), 3555−3563.
(6) Chefetz, B.; Mualem, T.; Ben-Ari, J. Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater. *Chemosphere* **2008**, *73* (8), 1335−1343.
(7) Williams, C. F.; McLain, J. E. T. Soil persistence and fate of carbamazepine, lincomycin, caffeine, and ibuprofen from wastewater reuse. *J. Environ. Qual.* **2006**, *41* (5), 1473−1480.
(8) Kinney, C. A.; Furlong, E. T.; Werner, S. L.; Cahill, J. D. Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. *Environ. Toxicol. Chem.* **2006**, *25* (2), 317−326.
(9) Dalkmann, P.; Broszat, M.; Siebe, C.; Willaschek, E.; Sakinc, T.; Huebner, J.; Amelung, W.; Grohmann, E.; Siemens, J. Accumulation of pharmaceuticals, *Enterococcus*, and resistance genes in soils irrigated with wastewater for zero to 100 years in central Mexico. *PLoS One* **2012**, *7* (9), e45397.
(10) Grossberger, A.; Hadar, Y.; Borch, T.; Chefetz, B. Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. *Environ. Pollut.* **2014**, *185*, 168−177.
(11) Dalkmann, P.; Siebe, C.; Amelung, W.; Schloter, M.; Siemens, J. Does long-term irrigation with untreated wastewater accelerate the dissipation of pharmaceuticals in soil? *Environ. Sci. Technol.* **2014**, *48* (9), 4963−4970.
(12) Sabourin, L.; Duenk, P.; Bonte-Gelok, S.; Payne, M.; Lapen, D. R.; Topp, E. Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. *Sci. Total Environ.* **2012**, *431*, 233−236.
(13) Calderón-Preciado, D.; Renault, Q.; Matamoros, V.; Cañameras, N.; Bayona, J. M. Uptake of organic emergent contaminants in spath and lettuce: an in vitro experiment. *J. Agric. Food Chem.* **2012**, *60* (8), 2000−2007.
(14) Karnjanapiboonwong, A.; Chase, D.; Cañas, J. E.; Jackson, W.; Maul, J. D.; Morse, A. N.; Anderson, T. Uptake of 17α-ethynylestradiol and triclosan in pinto bean, *Phaseolus vulgaris*. *Ecotoxicol. Environ. Saf.* **2011**, *74* (5), 1336−1342.
(15) Herklotz, P.; Gurung, P.; Vanden Heuvel, B.; Kinney, C. Uptake of human pharmaceuticals by plants grown under hydroponic conditions. *Chemosphere* **2010**, *78* (11), 1416−1421.
(16) Shenker, M.; Harush, D.; Ben-Ari, J.; Chefetz, B. Uptake of carbamazepine by cucumber plants–a case study related to irrigation with reclaimed wastewater. *Chemosphere* **2011**, *82* (6), 905−910.
(17) Wu, C.; Spongberg, A. L.; Witter, J. D.; Fang, M.; Czajkowski, K. P. Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water. *Environ. Sci. Technol.* **2010**, *44* (16), 6157−6161.
(18) Gottschall, N.; Topp, E.; Metcalfe, C.; Edwards, M.; Payne, M.; Kleywegt, S.; Russell, P.; Lapen, D. R. Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field. *Chemosphere* **2012**, *87* (2), 194−203.
(19) Calderón-Preciado, D.; Jiménez-Cartagena, C.; Matamoros, V.; Bayona, J. M. Screening of 47 organic microcontaminants in agricultural irrigation waters and their soil loading. *Water Res.* **2011**, *45*, 221−231.
(20) Sato, T.; Qadir, M.; Yamamoto, S.; Endo, T.; Zahoor, A. Global, regional, and country level need for data on wastewater generation, treatment, and use. *Agric. Water Manage.* **2013**, *130*, 1−13.
(21) Houeto, P.; Carton, A.; Guerbet, M.; Mauclaire, A. C.; Gatignol, C.; Lechat, P.; Masset, D. Assessment of the health risks related to the presence of drug residues in water for human consumption: Application to carbamazepine. *Regul. Toxicol. Pharmacol.* **2012**, *62* (1), 41−48.
(22) Larsen, J. Risk assessment of chemicals in European traditional foods. *Trends Food Sci. Technol.* **2006**, *17* (9), 471−481.
(23) Wieder, M.; Gvirtzman, G.; Porat, N.; Dassa, M. Paleosols of the southern coastal plain of Israel. *J. Plant Nutr. Soil Sci.* **2008**, *171* (4), 533−541.
(24) Jelic, A.; Fatone, F.; Di Fabio, S.; Petrovic, M.; Cecchi, F.; Barcelo, D. Tracing pharmaceuticals in a municipal plant for integrated wastewater and organic solid waste treatment. *Sci. Total Environ.* **2012**, *433* (1), 352−361.
(25) Linge, K.; Blair, P. Chemicals in reverse osmosis-treated wastewater: occurrence, health risk, and contribution to residual dissolved organic carbon. *J. Water Supply: Res. Technol.—AQUA* **2012**, *61* (8), 494−505.
(26) Blackburn, K.; Stickney, J. A.; Carlson-Lynch, H. L.; McGinnis, P. M.; Chappell, L.; Felter, S. P. Application of the threshold of toxicological concern approach to ingredients in personal and household care products. *Regul. Toxicol. Pharmacol.* **2005**, *43* (3), 249−259.
(27) Kroes, R.; Renwick, A. .; Cheeseman, M.; Kleiner, J.; Mangelsdorf, I.; Piersma, A.; Schilter, B.; Schlatter, J.; van Schothorst, F.; Vos, J. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chem. Toxicol.* **2004**, *42* (1), 65−83.
(28) Cramer, G. M.; Ford, R. A.; Hall, R. L. Estimation of toxic hazard—A decision tree approach. *Food Cosmet. Toxicol.* **1976**, *16* (3), 255−276.
(29) Patlewicz, G.; Jeliazkova, N.; Safford, R. J.; Worth, A. P.; Aleksiev, B. An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. *SAR QSAR Environ. Res.* **2008**, *19* (5−6), 495−524.
(30) Wu, X.; Ernst, F.; Conkle, J. L.; Gan, J. Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables. *Environ. Int.* **2013**, *60*, 15−22.
(31) Trapp, S. Bioaccumulation of polar and ionizable compounds in plants. In *Ecotoxicology Modeling*; Devillers, J., Ed.; Springer US: Boston, MA, 2009; Vol. *2*, pp 299−352.
(32) Briggs, G. G.; Bromilow, R. H.; Evans, A. A. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pestic. Sci.* **1982**, *13* (6), 495−504.
(33) Hsu, F. C.; Marxmiller, R. L.; Yang, A. Y. Study of root uptake and xylem translocation of cinmethylin and related compounds in detopped soybean roots using a pressure chamber technique. *Plant Physiol.* **1990**, *93* (4), 1573−1578.
(34) Trapp, S. Discussion articles plant uptake and transport models for neutral and ionic chemicals. *Environ. Sci. Pollut. Res.* **2004**, *11* (4), 33−39.
(35) Rosendahl, I.; Siemens, J.; Groeneweg, J.; Linzbach, E.; Laabs, V.; Herrmann, C.; Vereecken, H.; Amelung, W. Dissipation and sequestration of the veterinary antibiotic sulfadiazine and its metabolites under field conditions. *Environ. Sci. Technol.* **2011**, *45* (12), 5216−5222.
(36) Charoenpong, C.; Peng, A. C. Changes in β-carotene and lipid composition of sweet potatoes during storage. *Spec. Circ.—Ohio Agric. Res. Dev. Cent.* **1990**, 15−20.
(37) Macherius, A.; Eggen, T.; Lorenz, W.; Moeder, M.; Ondruschka, J.; Reemtsma, 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.
(38) Dettenmaier, E. M.; Doucette, W. J.; Bugbee, B. Chemical hydrophobicity and uptake by plant roots. *Environ. Sci. Technol.* **2009**, *43* (2), 324−329.
(39) Haham, H.; Oren, A.; Chefetz, B. Insight into the role of dissolved organic matter in sorption of sulfapyridine by semiarid soils. *Environ. Sci. Technol.* **2012**, *46* (21), 11870−11877.
(40) Coleman, J.; Blake-Kalff, M.; Davies, E. Detoxification of xenobiotics by plants: chemical modification and vacuolar compartmentation. *Trends Plant Sci.* **1997**, *2* (4), 144−151.
(41) Wang, Y.; Chow, M. S. S.; Zuo, Z. Mechanistic analysis of pH-dependent solubility and trans-membrane permeability of amphoteric compounds: application to sildenafil. *Int. J. Pharm.* **2008**, *352* (1−2), 217−224.
(42) Goldstein, M.; Shenker, M.; Chefetz, B. Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. *Environ. Sci. Technol.* **2014**, *48* (10), 5593−5600.

(43) Morant, M.; Bak, S.; Møller, B. L.; Werck-Reichhart, D. Plant cytochromes P450: tools for pharmacology, plant protection and phytoremediation. *Curr. Opin. Biotechnol.* **2003**, *14* (2), 151−162.
(44) Harvey, P. J.; Campanella, B. F.; Castro, P. M. L.; Harms, H.; Lichtfouse, E.; Schäffner, A. R.; Smrcek, S.; Werck-Reichhart, D. Phytoremediation of polyaromatic hydrocarbons, anilines and phenols. *Environ. Sci. Pollut. Res.* **2002**, *9* (1), 29−47.
(45) Golan-Rozen, N.; Chefetz, B.; Ben-Ari, J.; Geva, J.; Hadar, Y. Transformation of the recalcitrant pharmaceutical compound carbamazepine by *Pleurotus ostreatus*: role of cytochrome P450 monooxygenase and manganese peroxidase. *Environ. Sci. Technol.* **2011**, *45* (16), 6800−6805.
(46) Food and agriculture organization of the United Nations (FAOSTAT). 2013. FAOSTAT database. http://faostat3.fao.org/.
(47) Nagai, M.; Tani, M.; Kishimoto, Y.; Iizuka, M.; Saita, E.; Toyozaki, M.; Kamiya, T.; Ikeguchi, M.; Kondo, K. Sweet potato (*Ipomoea batatas L.*) leaves suppressed oxidation of low density lipoprotein (LDL) in vitro and in human subjects. *J. Clin. Biochem. Nutr.* **2011**, *48* (3), 203−208.
(48) Woolfe, J. A. *Sweet Potato: An Untapped Food Resource*; Cambridge University Press: Cambridge, MA, 1992.
(49) *USDA National Nutrient Database for Standard Reference*; U.S. Department of Agriculture, Agricultural Research Service: Columbus, OH, 2013. Nutrient Data Laboratory Home Page. http://www.ars.usda.gov/ba/bhnrc/ndl.