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Insights into the Uptake Processes of Wastewater-Borne Pharmaceuticals by Vegetables

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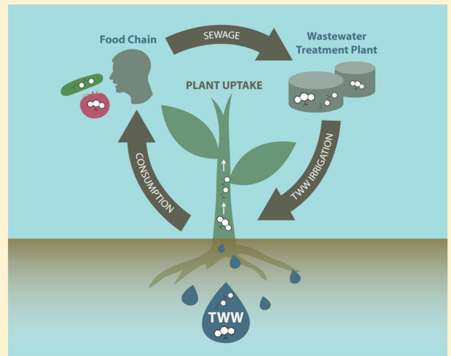
S *Supporting Information*

ABSTRACT: An increasing number of reports on plant uptake of pharmaceutical compounds (PCs) have been recently published, raising concerns of human exposure through dietary intake. In this study, PC uptake and translocation were evaluated in cucumber and tomato plants to elucidate the effects of PC physicochemical properties, soil type, and irrigation-water quality. Nonionic PCs were taken up and accumulated at higher levels in plants grown in soils of lower organic matter and clay content. While the concentration of most PCs in cucumber and tomato leaves were of similar order, their concentrations in the tomato fruit were much lower than in the cucumber fruit. This is related to differences in fruit physiology. Our data suggest that irrigation with treated wastewater reduces the bioavailability of acidic PCs for uptake by cucumber plants as compared to fresh water irrigation. This study sheds light on factors affecting the uptake of PCs by crops irrigated with treated wastewater, the governing role of PCs' physicochemical properties along with the physiological nature of the plant, soil properties and water quality that together determine uptake, translocation, and accumulation within plant organs. Occurrence of metabolites in plant suggests that PC metabolism has to be evaluated to reveal the total uptake.



INTRODUCTION

The growing demand for water in arid and semiarid regions exceeds the supply of renewable fresh water (FW) resources. Thus, treated wastewater (TWW) is becoming an important source of water for irrigation. For example, in Israel, more than 85% of TWW is used for crop irrigation; in Spain, ~71% and in California, ~46% of reclaimed wastewater is utilized in agriculture.[1] Thus, TWW makes up 50% of the total irrigation-water use in Israel, 17% in Spain, and only 6% in California. Organic pollutants, including endocrine-disrupting compounds, active pharmaceutical compounds (PCs), and other synthetic compounds have been detected in streams across the US receiving inputs of TWW.[2] Similar findings have been reported for river water receiving TWW in Europe[3−5] and China.[6] PCs have also been detected in TWW used for irrigation in different countries.[4,7−9]

In the past few years, there has been an increase in the number of publications on plant uptake of PCs, showing that once in the agricultural environment, organic pollutants and various PCs have the potential to be taken up by crops.[9−17] Boxall et al.[11] reported uptake at high levels of certain veterinary medicines with a range of lipophilicity in lettuce leaves and carrot roots. Another study reported that bioaccumulation of neutral PCs by cabbage and Wisconsin Fast Plants is higher than that of positively charged PCs under hydroponic conditions.[12] Accumulation of PCs at higher levels in plant roots than in aboveground compartments was reported for barley and carrots grown in soils spiked with PCs.[13] Distribution in the aboveground organs has also been observed, with higher bioaccumulation in leaves than in fruit.[9]

The proportion of an organic compound taken up by plant roots and its transportation route within the plant depend largely on its physicochemical properties.[18] Lipophilicity and charge play major roles in transport following root uptake.[19,20] Thus, the octanol−water partition coefficient (K_{ow}) has been suggested as a reliable predictor of uptake behavior.[19] However, for weak acidic and weak basic compounds which can be ionized based on the pH of the soil solution as well as the pH of the plant compartment, K_{ow} cannot be used as a suitable parameter to predict uptake. Uptake of weak acids increases as the pH of the external solution decreases, due to higher diffusion of the undissociated molecule across the cell membranes. Due to the higher pH inside the apoplast, dissociation occurs, and the anion cannot easily pass back through the lipophilic membrane, thus causing the molecule to accumulate within the cell. This process is known as ion-trapping.[21] Positively charged compounds are likely to bind to the negatively charged cell walls, reducing their translocation in the plant. Lipophilic compounds, due to their ability to

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partition into the lipophilic cell structure, can easily cross the plasma membrane of root hairs, cortex, and stele cells.[18,22]

It is difficult to draw general conclusions on the effects of soil and water properties on uptake, translocation, and bioaccumulation of PCs, because the data available in the literature are for different plant-growth methods (hydroponic, pot, culture media, field), different plants and PCs, and different methods of PC application. Thus, the aim of this study was to evaluate and quantify uptake and translocation of PCs in a systematic study; we used cucumber and tomato plants grown in different soils and exposed to different PCs representing a wide range of physicochemical properties at their indigenous concentrations in TWW.

EXPERIMENTAL SECTION

To examine plant uptake and translocation of PCs, greenhouse experiments were conducted using two test plants (cucumbers and tomatoes) grown in soils that varied in their organic matter and clay contents and irrigated with FW and TWW containing PCs with a wide range of physicochemical properties. Detailed information about the used PCs is listed in the Supporting Information Section and in Table S1.

Greenhouse Experiments. Uptake was studied in a greenhouse using cucumbers (*Cucumis sativus*) and tomatoes (*Solanum lycopersicum*) as model plants. To examine the effect of soil characteristics, three soils with a range of organic matter contents and soil textures were collected from three sites in Israel: sandy soil from Rehovot, aeolian sand from Besor, and alluvial soil from Beit Oren (Table S2). The soils were sieved through a 5 mm sieve, air-dried, and packed into 10-L pots. The pots were equipped with 1-m rock wool extensions that were designed to apply ~10-cBar suction at the bottom of the pots to eliminate saturated and poorly aerated conditions in the root zone. Three seedlings (Hishtil, Israel) were planted in each pot. After 10 days, the plants were thinned to one plant per pot. The two plant species were grown in consecutive years during the summer months (May−August), first cucumbers and then tomatoes, without replacing the soil (each pot received the identical irrigation treatment in the following season). The ambient daily temperature ranged from 23 to 42 °C. Plants were irrigated daily using four different types of water: FW, TWW, and FW or TWW spiked with a cocktail of PCs. Irrigation water was sampled 9 times throughout each growing season. Concentrations of the PCs in the irrigation water and water-quality analyses are presented in Table S3. Each treatment was conducted in four replicates. Nutrients were added by spiking the irrigation water with a fertilizer solution commonly used in commercial vegetable production, Shefer 5−3−8 (containing 5% N, 1.31% P, and 6.64% K) for the cucumber experiment and Or 4−2−6 (containing 4% N, 0.87% P, and 4.98% K) for the tomato experiment at a volumetric ratio of 1:1,000.

Harvesting and Analysis. Cucumber and tomato fruits were harvested daily once they reached commercial size (~100 g for cucumber, ~60 g for tomatoes). At the end of the growing season, leaves and soil samples were collected from all pots. All plant samples, fruits and leaves, were rinsed in deionized water, and all samples, plant material and soils, were kept at −20 °C until analysis. Detailed information about plant material, soil, and water analyses is presented in the Supporting Information.

Data Analysis. Statistical analysis (All Pairs, Tukey HSD, $p < 0.05$) was performed using JMP software, version 7.0.1. (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

All the studied PCs besides clofibric acid were present in the TWW used for irrigation, whereas the FW was free of PCs (Table S3). All studied PCs were detected in cucumber and tomato plant organs (fruits and/or leaves) in at least one soil−irrigation treatment combination (Figures 1−4). In general, all PCs that were detected in the fruits were also detected in the leaves; however, not all PCs that were detected in the leaves were detected in the fruits, suggesting the involvement of different translocation mechanisms. While the concentrations of PCs in both cucumber and tomato leaves were of similar magnitude for most of the PCs (Figures 1 and 3), their concentration in tomato fruit (Figure 4) was lower than in cucumber fruit (Figure 2). Our data also showed differences in accumulation of nonionic versus ionic PCs in the plants as a result of soil and water characteristics. The following discussion emphasizes the differences in plant uptake, linking these differences to the physicochemical properties of the studied PCs (Table S1), plant physiology, and the properties of the soil and irrigation water.

Nonionic PCs. Based on their physicochemical properties, charge and lipophilicity, nonionic PCs can easily cross membranes, moving from xylem to phloem and thus being transported predominantly in the direction of the transpiration stream and accumulating mostly in the leaves.[23] The nonionic PCs that were introduced into the plants in this study were caffeine, carbamazepine, lamotrigine, and sulfapyridine. Carbamazepine was detected in the leaves at a concentration that was at least 1 order of magnitude higher than that of the other nonionic PCs. Lamotrigine exhibited the second highest concentration in cucumber leaves, followed by caffeine, while in tomato plants, lamotrigine and caffeine were presented at similar concentrations. Sulfapyridine was detected at relatively low concentrations in the cucumber plants and was not detected at all in the tomato plants (Figures 1 and 3).

This order of concentrations emphasizes the effect of the PCs' hydrophobicity on their uptake by plants. Compounds with log K_{ow} of 2.45 (carbamazepine) and 2.57 (lamotrigine) were taken up at higher concentrations than the somewhat hydrophilic sulfapyridine and caffeine (log K_{ow} of 0.35 and −0.07, respectively), in agreement with Briggs et al.,[19] Hsu et al.,[22] and Burken and Schnoor.[24] The significantly ($p < 0.05$) higher accumulation of carbamazepine compared to lamotrigine (leaf concentrations were ~5 times higher in cucumber plants; ~100 times higher in tomato plants), despite quite similar log K_{ow} values and molecular masses (236 and 256 Da, respectively), might be explained by the partially ionized (+1) state of lamotrigine as the pH at the outside boundary of the apoplast drops to values close to its pK_a (5.34). In its ionic state lamotrigine permeability through root membranes is largely reduced. Rhizoplane acidification due to NH_4^+ rather than NO_3^- plant nutrition is well established, and in accordance we found that in the cucumber plants, fertilized with the NH_4^+-rich fertilizer Shefer 5−3−8 (32% of the N supplied as NH_4^+), lamotrigine uptake was restricted compared to carbamazepine. Lamotrigine uptake was even more restricted in the tomato plants, which may result from rhizosphere acidification due to excess cations over anions uptake. After taken up, in the cytosol and symplast with their typical higher pH (~7.2), lamotrigine is neutral again, but it can be partially ionized in certain plant compartments, such as vacuoles, intercellular spaces, and xylem vessels which have a pH of 5−6. Within these plant

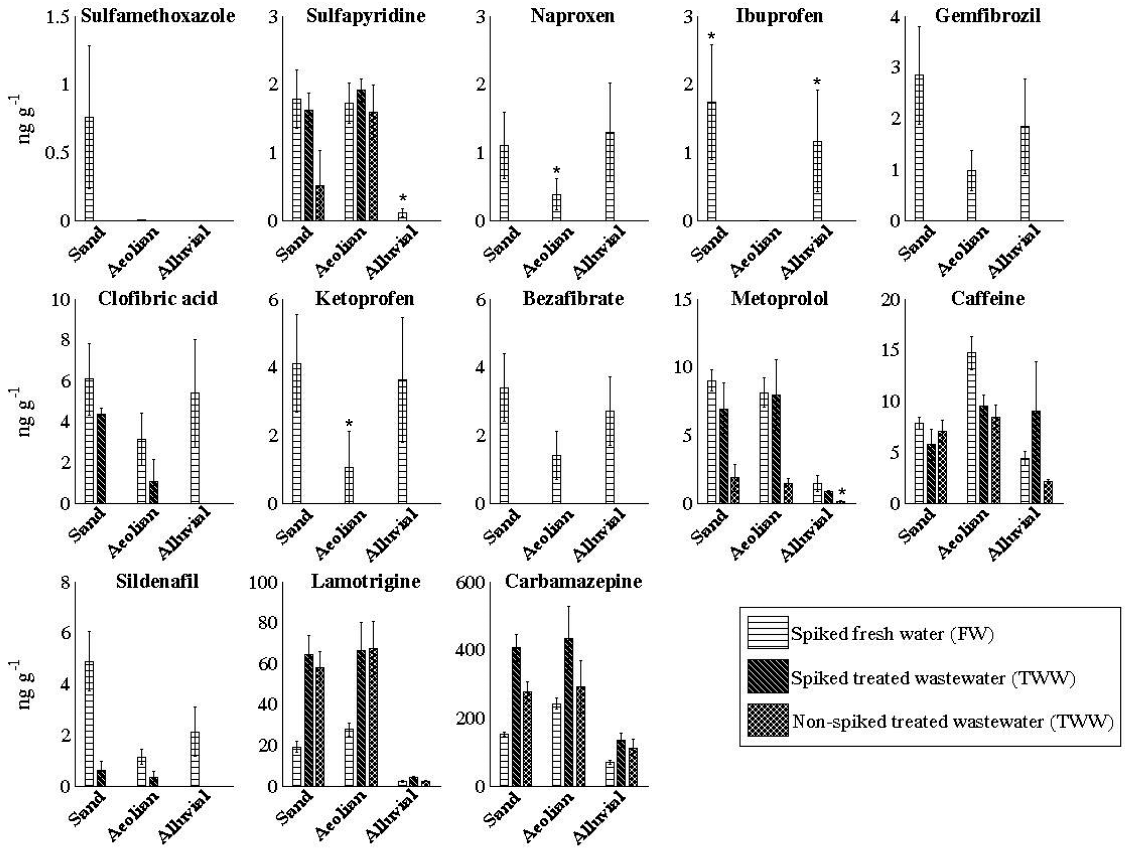

Figure 1. Concentrations of pharmaceuticals in leaves of cucumber plants (ng g^{-1} dry weight) grown in sandy soil (Sand), aeolian soil (Aeolian), or alluvial soil (Alluvial) and irrigated with spiked fresh water (FW) or spiked or nonspiked treated wastewater (TWW). Mean values and standard error are presented (n = 9 for spiked FW, n = 4 for spiked and nonspiked TWW). Bars marked with * indicate concentrations between LOD and LOQ.

compartments, lamotrigine may be up to 70% ionized (at pH 5) and thus can be trapped as an ion in the vacuoles or bind to the negatively charged cell walls. Its translocation to the aboveground plant organs is then largely reduced. Sulfapyridine, caffeine, lamotrigine, and carbamazepine were detected in the cucumber fruit, whereas in the tomato fruit caffeine and carbamazepine were the only nonionic PCs detected (Figures 2 and 4). This might be related to differences in fruit physiology. Cucumber fruit transpire water through their stomata,[25] creating a driving force for water and solute flow into the fruit, as opposed to tomato fruit, which lack stomata.[26,27]

Carbamazepine is neutral within a wide range of pH and is therefore translocated more readily in the plant and accumulated at a higher concentration in transpiring organs (i.e., leaves; Figure S6). Shenker et al.[9] reported a similar level of carbamazepine in cucumber leaves cultivated in loess soil irrigated with 1 μg L^{-1} of this PC. Wu et al.[17,28] reported carbamazepine concentrations of 24, 29, and 3 ng g^{-1} in cucumber, iceberg lettuce, and spinach leaves, respectively, grown under hydroponic conditions, where carbamazepine was introduced at 0.5 μg L^{-1}. Wu et al.[29] reported a concentration of 3.4 ng g^{-1} in soybean leaves grown in sandy soil and irrigated with 10 μg L^{-1} of carbamazepine. The difference in these reports of carbamazepine uptake and accumulation can be attributed to many factors, such as differential water consumption due to specific experimental conditions, as well as inherent differences between the studied plants. Even when comparing similar or identical crops, factors such as growing period, climatic conditions, and irrigation regime may produce accumulation discrepancies in the plants. Wu et al.[30] reported carbamazepine concentrations of 400 ng g^{-1} in shoots and low concentrations in the fruit of tomato plants grown in soils in which biosolids had been applied. These results are in agreement with our study, supporting the assumption that carbamazepine is translocated by the transpiration stream and accumulates at significantly higher levels in leaves than in other organs.

Similar to carbamazepine, significantly higher concentrations of lamotrigine in cucumber leaves than in fruits were observed in the aeolian soil for all irrigation treatments and in spiked and nonspiked TWW irrigated sandy soil, while lamotrigine was not detected in tomato fruit (Figure S7). We found no previous publications on lamotrigine uptake by plants, precluding any direct comparisons. We therefore compared our results for lamotrigine (which contains a triazine ring) to uptake of other triazines[31] with similar physicochemical properties, such as the herbicide atrazine (log $D_{(pH>3.4)}$, 2.53; pK_a, 1.7). Accumulation in plants irrigated with TWW containing the herbicide was observed and substantial accumulation in leaves relative to fruits indicated translocation predominantly in the direction of the transpiration stream. These results coincide with our findings for lamotrigine.

Compared to carbamazepine and lamotrigine, caffeine was present at relatively low concentrations in the cucumber leaves, and its concentration in the fruits was slightly and in most cases not significantly lower than in the leaves (Figures 1, 2).

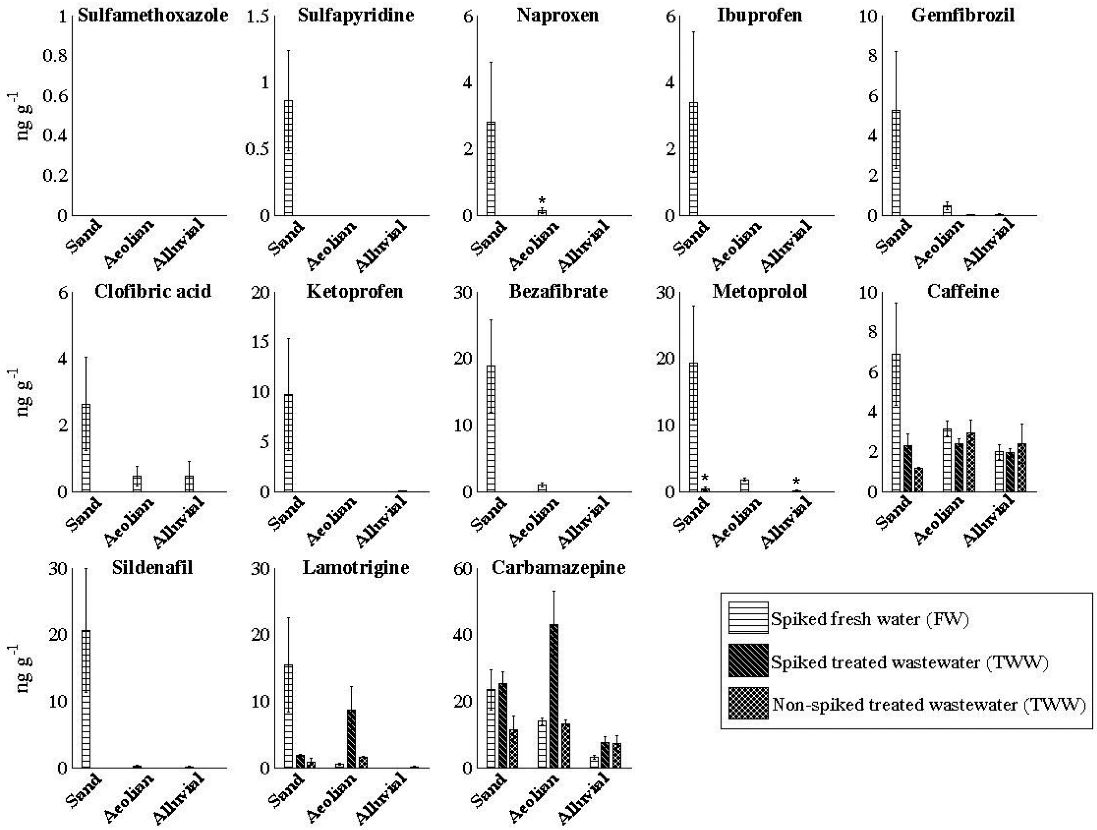

Figure 2. Concentrations of pharmaceuticals in fruit of cucumber plants (ng g^{-1} dry weight) grown in sandy soil (Sand), aeolian soil (Aeolian), or alluvial soil (Alluvial) and irrigated with spiked fresh water (FW) or spiked or nonspiked treated wastewater (TWW). Mean values and standard error are presented (n = 9 for spiked FW, n = 4 for spiked and nonspiked TWW). Bars marked with * indicate concentrations between LOD and LOQ.

Caffeine is a highly water-soluble compound that can easily be translocated in the soil with water mass flow due to its nonionic state. Its uptake is, however, largely restricted due to its rather hydrophilic characteristic (log K_{ow} = −0.77), resulting in a lower ability to partition into the lipophilic cell structure; thus, it accumulated to lower concentrations than the more hydrophobic compounds. In the tomato plants, caffeine was found at relatively low concentrations and was not detected in all treatment combinations. Caffeine has been previously reported to be taken up by cucumber plants[17] but at relatively lower levels than found in our study. These differences may be related to growing technique (hydroponic cultivation versus soil cultivation in our study) and growth duration (21 days versus ∼3 months in our study).

Sulfapyridine (pK_a = 8.4) mainly existed in its nonionic form at the studied soils' pH and exhibited less lipophilic characteristics (log K_{ow} = 0.35) than carbamazepine and lamotrigine. Accordingly, it accumulated less in the cucumber leaves (up to 1.9 ng g^{-1}; Figure 1). The lower accumulation of this PC might also result from its ∼10 times lower concentration in the bulk soils and their solutions (Figures S2 and S1, respectively), suggesting higher degradability in the soil environment. Sulfapyridine was detected in the cucumber leaves in most of the treatment combinations, while in the cucumber fruit, it was only found in sandy soil irrigated with spiked FW and its concentration was significantly lower than in the leaves. Sulfapyridine was not detected in tomato plants, yet it was found at low concentrations in the spiked FW-irrigated soil solution (Figure S3) and in the bulk soils in all irrigation treatments, especially in the alluvial soil (Figure S4). This soil is relatively rich in organic matter and clay (Table S2), implying that either of these phases may play an important role in sulfapyridine binding to the soil, reducing uptake potential by the plant roots. Supporting this assumption, Thiele[32] reported that sulfapyridine adsorbs strongly to the soil through its organic matter and that the adsorption depends heavily on soil organic matter (SOM) concentration. Similar to carbamazepine and lamotrigine, sulfapyridine was taken up and accumulated in the leaves at a significantly higher concentration than that found in the fruit in the aeolian soil, in the spiked FW alluvial soil and in the spiked TWW sand, indicating in-plant translocation with the transpiration stream.

As discussed above, uptake of nonionic PCs is governed by their lipophilicity; however, the nature of the soils was found to play a significant role in affecting uptake, mainly via controlling bioavailability of the PCs. Except for caffeine in alluvial soil irrigated with spiked TWW, the nonionic PCs exhibited significantly higher concentrations in the leaves of plants grown in soils containing a low level of SOM and low clay content (i.e., sandy and aeolian soils) as compared to the alluvial soil (Table S2). Adsorption of nonionic and polar PCs, such as carbamazepine, sulfapyridine, lamotrigine, and caffeine, through polar interactions[7,33−35] may largely reduce the concentration of these compounds in the soil solution, thus limiting plant uptake. This is shown by comparing the concentration in the bulk soil (i.e., total amount of PC) to the concentration in the soil solution (i.e., readily available PC) in the alluvial soil. The concentration in the bulk soil of the

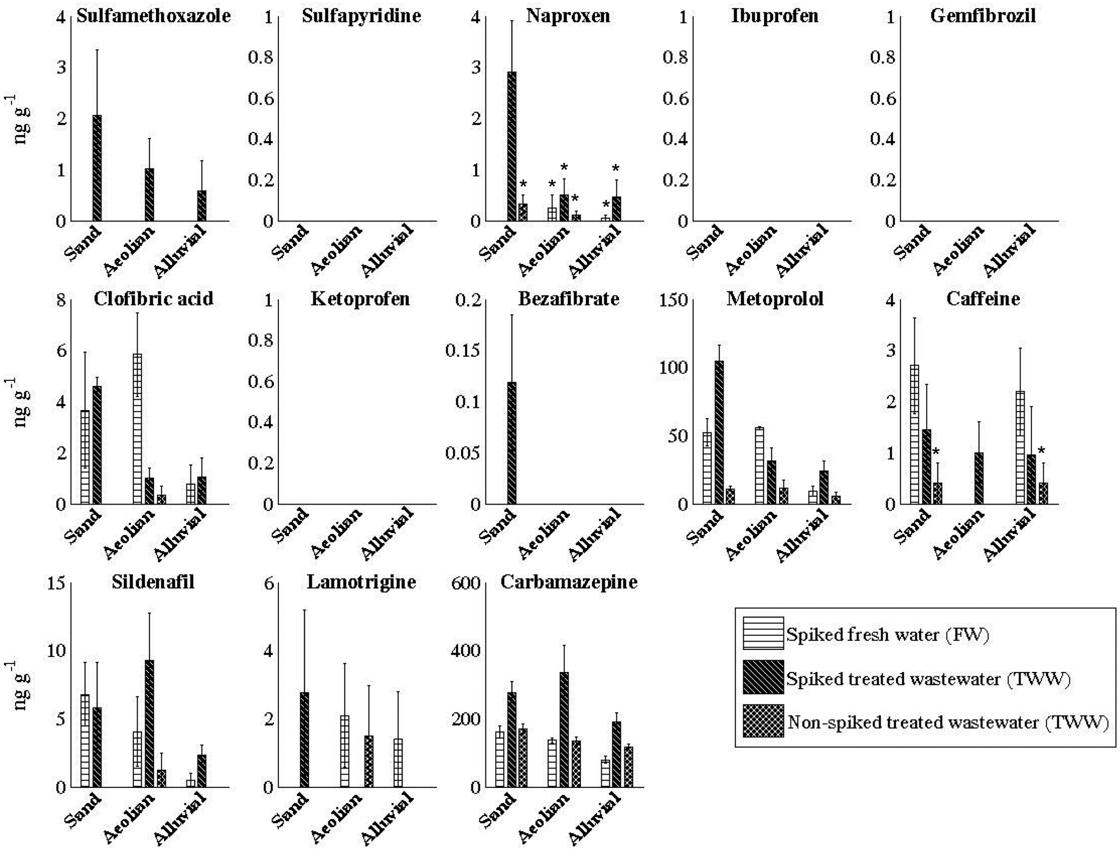

Figure 3. Concentrations of pharmaceuticals in leaves of tomato plants (ng g^{-1} dry weight) grown in sandy soil (Sand), aeolian soil (Aeolian), or alluvial soil (Alluvial) and irrigated with spiked fresh water (FW) or spiked or nonspiked treated wastewater (TWW). Mean values and standard error are presented (n = 9 for spiked FW, n = 4 for spiked and nonspiked TWW). Bars marked with * indicate concentrations between LOD and LOQ.

nonionic PCs in the different treatments was significantly higher and at least 10 time the concentration in the soil solution (Figures S1−S4).

The lower plant uptake of PCs in the alluvial soil was mostly pronounced for caffeine, carbamazepine, and lamotrigine (Figures S5−S7). For caffeine the bioaccumulation factors (the leaves to bulk soil concentration ratio) for both crops were 0.1 for the alluvial soil and 2 for the sandy and aeolian soils (Figure S5). For carbamazepine the bioaccumulation factor was 6 for the alluvial soil and 47 and 38 for the sandy and aeolian soils, respectively (Figure S6). For lamotrigine a similar trend was observed but only for the cucumber plants with a bioaccumulation factor of 0.2 for the alluvial soil and 24 and 49 for the sandy and aeolian soils, respectively (Figure S7).

Ionic PCs. The ionic PCs introduced to the plants in our study were metoprolol (positively charged) and the negatively charged PCs: bezafibrate, clofibric acid, gemfibrozil, ibuprofen, ketoprofen, naproxen, and sulfamethoxazole. Sildenafil changes its charge from positive to neutral to negative based on pH. The concentrations of all these ionic compounds in the plant material were usually much lower than those of the nonionic PCs, in agreement with the lower permeability of cell membranes to ionic organic compounds. Among the ionic compounds, the positively charged metoprolol was detected in the cucumber leaves at relatively higher concentrations compared to the negatively charged PCs (Figure 1), probably because of its attraction to the negative charge in the protoplast. Significantly higher concentrations of metoprolol were found in the leaves of plants grown in soils containing a low level of SOM and clay (i.e., sandy and aeolian soils) as compared to the alluvial soil. This is explained by the higher concentration of this PC in the alluvial soil (Figures S2 and S4), where it most likely was loosely bound to the negatively charged clay minerals and organic matter. Metoprolol concentrations in the tomato leaves were significantly higher in all soil treatments compared to those found in the cucumber leaves. Although metoprolol was not detected in all soils (Figures S1−S4), when comparing concentrations in the soil solution and bulk soil for the two crops, higher concentrations were observed in the soils from the tomato experiment. This accumulation in the soils increased the available concentration for plant uptake, resulting in higher accumulation in the tomato plants.

All of the weakly acidic PCs exhibited similar physicochemical properties (Table S1) and therefore demonstrated similar uptake behavior and trends. These PCs may penetrate the root by water mass flow but to a lesser extent than neutral compounds, since they are repelled by the negatively charged cell walls. Of the anionic PCs included in this study, the pK_a values of sulfamethoxazole (5.6), ibuprofen (4.9), gemfibrozil (4.7), ketoprofen (4.5), and clofibric acid (3.2) suggest their partial existence as nonionic species in the rhizoplane, which allows their dissolution into the membranes and release to the cytosol. Once in the cytosol, with its higher pH (~7.2), these weak acids retain their anionic state, and, due to the lower permeability of the membranes to anions, they may be trapped as ions and accumulate in the cytosol.[21] The ionic trapped PCs

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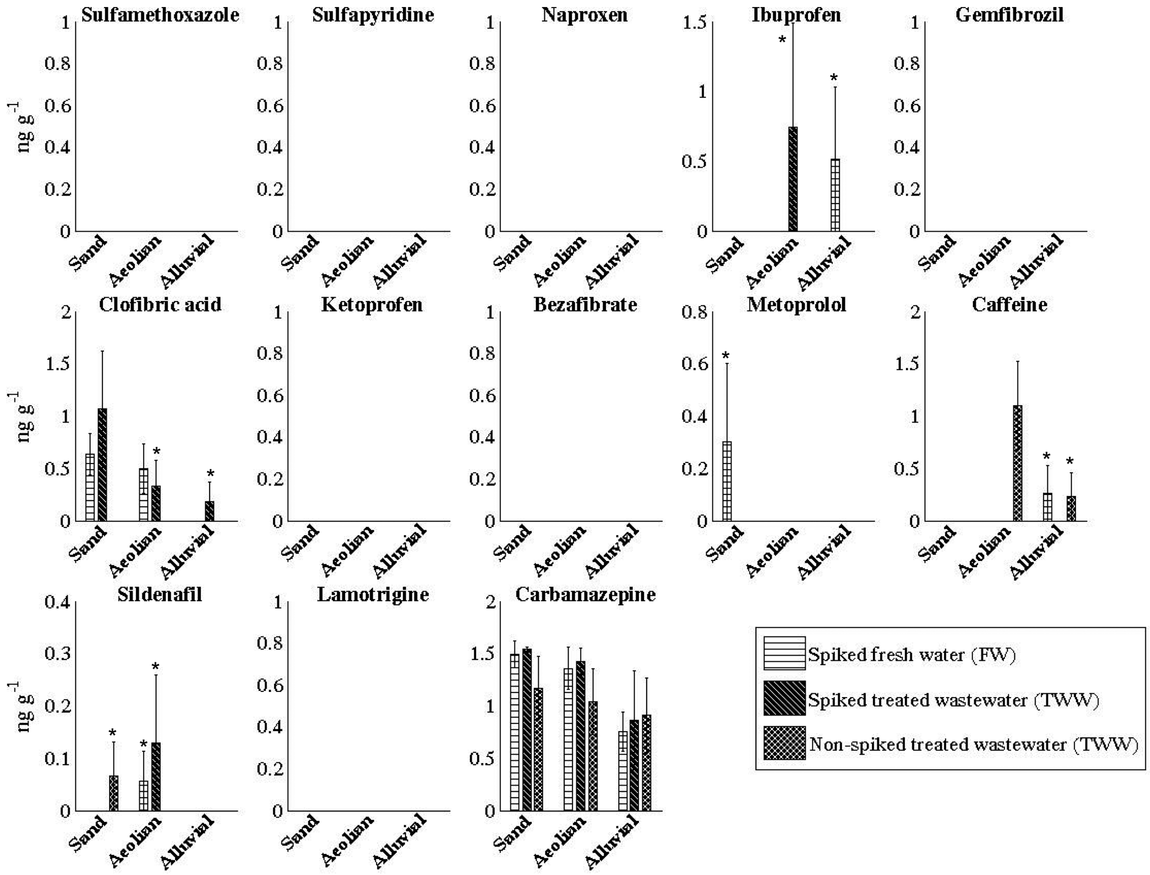

Figure 4. Concentrations of pharmaceuticals in fruit of tomato plants (ng g^{-1} dry weight) grown in sandy soil (Sand), aeolian soil (Aeolian), or alluvial soil (Alluvial) and irrigated with spiked fresh water (FW), or spiked or nonspiked treated wastewater (TWW). Mean values and standard error are presented (n = 9 for spiked FW, n = 4 for spiked and nonspiked TWW). Bars marked with * indicate concentrations between LOD and LOQ.

are expected to preferentially being translocated in the phloem rather than the xylem, as previously suggested for herbicide movement in plants.[23] In accordance with symplastic translocation, and as opposed to the nonionic PCs, the concentrations of bezafibrate, ketoprofen, and naproxen were significantly higher in the fruit than in the leaves.

Sildenafil exhibits several charge species within the soil and plant pH range. At the soil pH (7.5–7.9), almost 60% of the compound is negatively charged, 30% is neutral, less than 5% is positively charged, and around 5% is present as zwitterion. This explains why sildenafil was taken up at higher levels than the weakly acidic PCs but at lower levels than the nonionic PCs. Once in the plant, in the intercellular spaces and xylem with pH ranging from 5 to 6, sildenafil is mostly positively charged (~70–97%), and thus its translocation is hindered, similar to metoprolol and lamotrigine. When entering the cytosol (pH ~ 7.2), sildenafil may be retained by the ion-trap mechanism.

Another important result is that clofibric acid, ibuprofen, naproxen, bezafibrate, and ketoprofen were not detected in most of the soils (Figures S1, S2). Based on our previous study,[36] naproxen, bezafibrate, and ibuprofen were found to be rapidly degraded in TWW-irrigated soils, we assume that the above-mentioned PCs were biodegraded rapidly in the soils during the growing season.

For the weakly acidic PCs, water quality has been found to affect plant uptake. Although the concentrations of these PCs were similar in the spiked FW and in the spiked and nonspiked TWW, they were detected in the spiked FW irrigated cucumber plants but not in the spiked or nonspiked TWW irrigated plants. The cucumber plants irrigated with spiked TWW were found to take up mostly nonionic and positively charged PCs and not the acidic ones. We assume that polar interactions between the weakly acidic PCs and the DOM present in TWW (Table S3) might restrict the bioavailability of these PCs. Such interaction was reported for the ionized naproxen that was found to bind to DOM fractions with positively charged functional groups.[37] These interactions can lead to the formation of water-soluble complexes that are not available for uptake and/or cosorption to solid phases in the soil, which also reduces their concentration in the soil solution.

In the cucumber fruit the negatively charged PCs were accumulated at higher levels in the sandy soil irrigated with spiked FW. The sandy soil has low water-holding capacity, which caused the plants grown in this soil to experience water-deficiency-induced stress.[38] Plants experiencing stress tend to expedite fruit growth, resulting in the increased transport of organic compounds from the source (leaves) to the sinks (shoot, fruit, and roots).[39,40] Phloem-loading of these compounds reduces the water potential, causing water to flow from the xylem into the phloem, thus creating a driving force that moves solutes, including these PCs from the leaves to the fruits. In the case of weak acids, the higher bioaccumulation in the fruit could be explained by phloem mobility, which depends heavily on the compounds' physicochemical properties, such as lipophilicity and pK_a. As opposed to the cucumber fruit, in the tomato fruit the only ionic PCs detected were ibuprofen, clofibric acid, metoprolol, and sildenafil (Figure 4). The different accumulation in the two fruits might derive from the differences in fruit transpiration, as mentioned above.

In-Plant Metabolism. To assess whether the parent PC concentration data reflect the actual uptake of these compounds we chose carbamazepine as an analyte and quantified its main metabolites (10,11-epoxide-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine) in leaves and fruits of both plants grown in aeolian soil irrigated with spiked TWW. While carbamazepine exhibits low soil biodegradability,[36] in the leaves of both plants the parent compound comprised only 50% of the three analyzed species (Figure 5). In this organ, the second dominant metabolite was

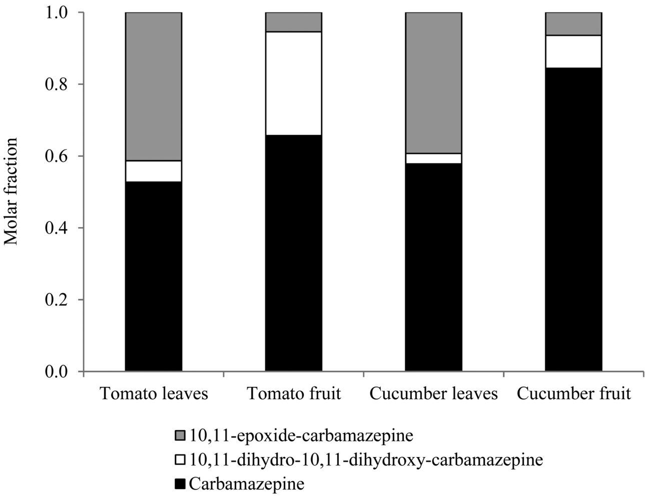

Figure 5. Distribution of carbamazepine and its metabolites 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine in tomato and cucumber leaves and fruits grown in aeolian soil (Aeolian) irrigated with spiked treated wastewater.

10,11-epoxide-carbamazepine, while 10,11-dihydro-10,11-dihydroxy-carbamazepine made up only a minor fraction (3−6%). Similar to leaves, carbamazepine was dominant in the fruits of both plants; however, a larger portion as compared to the leaves was found as 10,11-dihydro-10,11-dihydroxy-carbamazepine. These data clearly show that carbamazepine (and probably other PCs) is metabolized in the plant after been taken up. Carbamazepine is most likely metabolized by cytochrome P450 monooxygenase enzymes that are known to be responsible for the metabolism of a variety of herbicides, insecticides, and organic pollutants in plants.[15,41−43] These observations highlight the need to follow metabolism of PCs in plants.

Environmental Implications. In this report, we demonstrate plant uptake of a relatively large spectrum of PCs under realistic agricultural and environmental scenarios. Among the possible affecting factors (soil properties, water quality, and the nature of the PC), the physicochemical nature of the PC seems to be the major governing factor in determining uptake. Moreover, we found that crops grown in soils with low SOM and clay contents are at greater risk for PC uptake and accumulation at higher concentrations. Nonionic PCs were found to accumulate at higher concentrations in the leaves, supporting the assumption that nonionic PCs are transported predominantly in the direction of the transpiration stream and accumulating in the leaves. The ionic PCs, which are repelled by the negatively charged cell walls and cytosol, exhibited lower uptake. Ionic PCs may be trapped in the phloem, resulting in lower concentrations in the leaves and potentially higher accumulation in the fruit. Another conclusion is related to the physiology of the plant: the better the fruit's ability to transpire water, the higher the risk of PC accumulation within the fruit. Our data on the occurrence of carbamazepine metabolites suggest that PC metabolism in plants has to be evaluated to reveal the actual uptake.

■ ASSOCIATED CONTENT

Supporting Information

Information about used pharmaceuticals, properties of the studied soils, irrigation water characteristics, concentrations of the pharmaceuticals in the soil solutions and the bulk soils and correlations between concentration in fruit versus leaves and leaves versus bulk soil. 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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