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Uptake of carbamazepine by cucumber plants – A case study related to irrigation with reclaimed wastewater

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ABSTRACT

Reclaimed wastewater is an important source of irrigation in semiarid and arid zones. Here we report data on carbamazepine (CBZ) uptake by cucumber plants in hydroponic culture and greenhouse experiments using different soil types irrigated with fresh water or reclaimed wastewater. Data obtained from the hydroponic culture experiments suggest that CBZ is mainly translocated by water mass flow, and thus it is concentrated and accumulated to the largest extent in the mature/older leaves. Carbamazepine concentration in cucumber fruits and leaves was negatively correlated with soil organic matter content. The concentrations of CBZ in the roots and stems were relatively low, and most CBZ in the plant (76–84% of total uptake) was detected in the leaves. A greenhouse experiment using fresh water and reclaimed wastewater spiked, or not, with CBZ at 1 μg L^{-1} (typical concentration in effluents) revealed that CBZ can be taken up and bioaccumulated from its background concentration in reclaimed wastewater. Bioaccumulation factor (calculated as the ratio of CBZ concentration in the plant to that in the soil solution) for the fruits (0.8–1) was significantly lower than the value calculated for the leaves (17–20).

This study emphasizes the potential uptake of active pharmaceutical compounds by crops in organic-matter-poor soils irrigated with reclaimed wastewater and highlights the potential risks associated with this agricultural practice.

1. Introduction

Due to the shortage of fresh water in semiarid and arid zones, reclaimed wastewater is becoming an important source of irrigation. In the past decade, there have been a number of reports of wastewater effluents containing active pharmaceutical compounds (PCs) (Ternes, 1998; Tixier et al., 2003; Clara et al., 2004; Miao et al., 2005; Spongberg and Witter, 2008; Calisto and Esteves, 2009; Kummerer, 2009a; Segura et al., 2009), some of which have also been detected in the waterways at many sites (Kolpin et al., 2002; Zhang et al., 2008b). Pharmaceutical compounds are of potential concern because they are highly adsorbable, resulting in a tendency to accumulate in soils, sediments and tissues (Díaz-Cruz et al., 2003; Drillia et al., 2005; Hari et al., 2005; Loffler et al., 2005; Williams and Adamsen, 2006). Studies have shown that PCs concentrations in the drinking water are low (typically <1 ng L^{-1}) and not harmful to humans (Boxall et al., 2004; Kummerer, 2009b). On the other hand, the concentrations found in effluents used for irrigation are higher, up to the μg L^{-1} range in some cases (Tixier et al., 2003; Chefetz et al., 2008; Siemens et al., 2008), evoking concern of potential health risks from long-term low-level PC exposure or PCs biomagnification through the food-chain (Redshaw et al., 2008). The presence of PCs and their metabolites is also a concern for aquatic organisms (Jones et al., 2002; Ferrari et al., 2003).

Introduction of PCs into the environment via irrigation of agricultural fields is a highly relevant route of exposure in semiarid zones, where reclaimed wastewater is an important source of irrigation water (Kinney et al., 2009). Once in the soil, and depending on the physicochemical properties of the PC and soil characteristics (e.g., pH and organic matter content), PCs might be retained by the soil or mobilized by percolating water (Burkhardt and Stamm, 2007). Highly mobile PCs have the potential to leach to the groundwater, whereas strongly sorbed PCs can accumulate in the top soil layer (Chefetz et al., 2008). In the soil, PCs may affect the microbial community and may be taken up by plants (Thiele-Bruhn, 2003).

The concern addressed in this study is PCs' potential to enter the food-chain through irrigation of edible plants with reclaimed wastewater. In this study we target carbamazepine (CBZ), a drug used for the treatment of epilepsy, trigeminal neuralgia, bipolar affective disorder and acute mania. It is considered an environmentally recalcitrant compound which exhibits only limited removal efficiency in municipal wastewater-treatment plants (Ternes, 1998; Zhang et al., 2008a). Therefore, CBZ has been detected in

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the groundwater, surface water, reclaimed wastewater, and even drinking water (Kummerer, 2001; Tixier et al., 2003; Chefetz et al., 2008). Ecotoxicological studies suggest that CBZ has low acute toxic effects, with a predicted no-effect concentration of 0.42 μg L^{-1} (Ferrari et al., 2003), but its chronic effects require cautious attention (Zhang et al., 2008a).

Several studies have recently demonstrated that plants can take up PCs from the growth media when the PCs are introduced either by spiking of the medium or irrigation water, or by sludge application (Kumar et al., 2005; Boxall et al., 2006; Dolliver et al., 2007; Redshaw et al., 2008; Farkas et al., 2009; Herklotz et al., 2010). In this study we evaluated the uptake potential of refractory PC, i.e. CBZ, by cucumber plants and its fractionation into edible and other plant organs, as affected by soil characteristics and the quality of the irrigation water (fresh water vs. reclaimed wastewater). The study was performed in three experimental steps: (1) in hydroponic culture to verify CBZ phytotoxicity, and uptake and fractionation among vegetative organs; (2) in a pot experiment to examine the effect of solid matrix on the uptake and translocation of CBZ to different plant organs, including the edible fruits; and (3) in a second pot experiment to evaluate the effect of irrigation-water quality (fresh water vs. reclaimed wastewater) and the origin of CBZ (spiked vs. wastewater origin) on its uptake and bioaccumulation.

2. Materials and methods

Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide, 98%) was purchased from Sigma–Aldrich (Rehovot, Israel). Chemical and physical properties of CBZ are presented in Table 1.

2.1. Hydroponic culture experiment

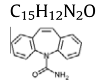
Cucumber (*Cucumis sativus* L., cv. Safi) seeds (Hazera Genetics Ltd., Berurim, Israel) were germinated on $CaSO_4$-saturated germination papers at 25 °C. Seedlings (7 d old, with two cotyledons and ~2 cm root length) were transferred to a continuously aerated nutrient solution under a 16/8-h day/night cycle in a temperature-controlled chamber at 25 ± 1 °C and 20 ± 1 °C day/night, 43–85% uncontrolled relative humidity, and a photosynthetically active radiation (PAR) intensity of 70 μmol m^{-2} s^{-1} at leaf height (1:1 Osram 36 W/77 Fluora and 36 W/840 cool-white fluorescent tubes). The 16-h irradiance per day equaled about 20–30% of the PAR commonly found in greenhouses on a daily basis. Ten seedlings were grown in each 3-L container in an aerated nutrient solution with the following macroelemental composition (mM): K_2SO_4, 0.7; KCl, 0.1; $Ca(NO_3)_2$, 2.0; $MgSO_4$, 0.5; KH_2PO_4, 0.1, and the following micronutrient composition (μM): Fe-EDDHA, 10; $MnSO_4{\cdot}H_2O$, 0.5; $ZnSO_4{\cdot}7H_2O$, 0.5; $CuSO_4$, 0.2; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.01; H_3BO_3, 10. The solution pH was buffered to about 7.0 with $CaCO_3$ (0.5 g L^{-1}). Fe-EDDHA was applied as commercial Sequestrene-138 (Ciba-Geigy, Basel, Switzerland) and all other salts were of analytical grade.

After an additional 7 d, when the first real leaf was fully expanded, each plant was transferred separately to a 1-L darkened glass jar containing the above nutrient solution spiked with CBZ in a wide concentration range of 0, 1, 10, 100, 1000, 10 000 and 100 000 μg L^{-1}. Five replicates were employed for each treatment. The whole nutrient solution was changed once every 4 or 5 d. Before each nutrient replacement, the solution volume was measured and solution was withdrawn from each jar for CBZ analysis. At the end of the experiment, 22 d after plant transfer to the glass jars, plants were decapitated 5 mm above the stem base and xylem sap, which continued to bleed from the stem stump, was collected for up to 60 min by glass pipette. The collected sap was frozen and kept at −20 °C until analyzed. The whole plant was then separated into roots, cotyledon leaves, stem, and fully expanded leaves. The shoot apexes with the youngest leaves were included with the stem fractions. The leaves were further separated into mature, semi-mature and young leaves. All plant fractions were fresh-weighed, rinsed with deionized water, left to drain, frozen, and kept at −20 °C until extraction. All glassware and containers were carefully treated by washing with dichloromethane, methanol and acetone or by heating to 400 °C for 5 h before use to eliminate any CBZ contamination.

2.2. Greenhouse experiments

Two pot experiments were conducted in a greenhouse during the summer (May to September) with natural full sunlight and a daily temperature range of about 23–42 °C. To examine the effects of soil characteristics, three soils with large ranges of organic matter content and soil texture were used and irrigated with fresh water (tap water, Rehovot, Israel) with or without (control) CBZ spiking at a concentration of 25 μg L^{-1}. Cucumber seeds were directly seeded in 5-L pots with either a commercial peat-based horticultural mixture (hereafter referred to as peat mixture), or with sandy loess or clay soil (Terra Rossa). Major soil properties are presented in Table 2. After germination and full expansion of the first true leaf, plants were thinned to one plant per pot. The pots were irrigated to maintain a water content of about 50–60% of the pot's water-holding capacity. The irrigation water contained (mg L^{-1}): N, 60 (1:1 NO_3^-:NH_4^+); P, 26; K, 50, and micronutrients (Fe, Mn, Zn, Cu, and Mo) from fertilizer solution commonly used in

Table 1
Selected chemical and physical properties of carbamazepine.

Chemical formula	$C_{15}H_{12}N_2O$
Structure	
Molecular weight (g mol^{-1})	236.27
Aqueous solubility (mg L^{-1})	125.0 ± 2[a]
log K_{ow}	2.45[b]

[a] Maoz and Chefetz (2010).
[b] Hanna et al. (1998).

Table 2
Major properties of soils and water.

	Sandy soil	Clay soil	Peat mixture
Texture			
Clay (%)	9	43	NA[a]
Silt (%)	6	25	NA
Sand (%)	85	32	NA
Organic matter content (%)	1.2 ± 0.06	6.5 ± 0.31	32.6 ± 0.62
$CaCO_3$ content (%)	8.9 ± 2.75	30.0 ± 1.54	ND[b]
Hygroscopic water content (%)	2.2 ± 0.84	6.0 ± 0.02	45.4 ± 1.72
Specific surface area (m^2 g^{-1})	67 ± 8.1	213 ± 7.5	81 ± 35.3
pH	7.37	7.46	6.11

	Fresh water	Reclaimed wastewater
Total suspended solids (TSS; mg L^{-1})	NA	<2
Biochemical oxygen demand (BOD; mg L^{-1} O_2)	NA	18
Chemical oxygen demand (COD; mg L^{-1} O_2)	NA	48
pH	8.2	7.7
Electric conductivity (dS m^{-1})	0.9	1.8
Carbamazepine (μg L^{-1})	ND	2.99

[a] Not applicable.
[b] Not detected.

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commercial vegetable production in greenhouses. Ten pots (replicates) were used for each treatment.

To examine the effect of water quality, cucumber seedlings were planted in 5-L pots with the same sandy loess soil and irrigated by either fresh water or reclaimed wastewater, with or without CBZ spiking at 1 μg L^{-1}. The reclaimed wastewater was obtained from Ayalon wastewater-treatment plant, which is fed mainly by wastewater from the city of Ramla, Israel and used for irrigation of nearby agricultural land. In this water, we detected an indigenous CBZ concentration of about 3 μg L^{-1}; the analysis protocol is reported in our previous study (Chefetz et al., 2008). Major properties of the irrigation water are presented in Table 2. The reclaimed wastewater was collected twice a week to minimize water alteration. For each water type, five pots (replicates) were irrigated with CBZ-spiked water and five served as controls (non-spiked).

Fruits were harvested according to the commercial criterion of size, i.e. at a length of about 12 cm, weighed, washed and kept at −20 °C until extraction. At the end of each experiment, when plants were about 3 months old, the whole aboveground plant was cut, separated into fractions as described previously, fresh-weighed, rinsed with deionized water, left to drain, frozen, and kept at −20 °C until extraction. Immediately after plant cutting, xylem sap was collected as described previously and frozen until analysis.

To estimate the average concentration of CBZ available to the roots, soils were irrigated to 60% of pot water-holding capacity with the corresponding CBZ concentration (0, 1 or 25 μg L^{-1}) and equilibrated for 1 h at 25 °C. Then, water content was doubled by adding deionized water; after an additional 1 h equilibration, the solution was extracted by vacuum filtration. Carbamazepine was measured in the extract and the concentration at 60% water-holding capacity was calculated. Bioaccumulation factor was calculated as the ratio between CBZ in the plant biomass (fresh weight) and its concentration in the soil solution.

2.3. Extraction and LC–MS analysis

Plant samples (0.5 g of fresh leaves or stems, 1 g of freeze-dried fruits) were extracted with 12 mL of methanol, assisted by ultrasonic probe (3.5 min, 50 kHz). After extraction, the mixture was agitated (30 min, 300 RPM), centrifuged and filtered (0.2-μm Teflon filters). Then, 330 μL of deionized water was added to a 660-μL aliquot of the extract and spiked with 10 μL of $D_2{}^{13}C$-labeled CBZ (Toronto Research Chemicals, Ontario, Canada). The labeled CBZ was used as an internal standard for the LC–MS analysis. Calibration was performed using CBZ spiked into cucumber-extracted matrix.

Chromatographic analysis was performed using the Agilent 1200 Rapid Resolution LC system (Agilent Technologies Inc.). Separation of CBZ was achieved under isocratic conditions (methanol/water 70:30 with 0.05% acetic acid) using a Thermo Gold Seal C18 HPLC column (2.1 × 100 mm, particle size 1.9 μm). The LC was coupled with the Agilent 6410 triple quad mass selective detector equipped with an electrospray ionization ion source. The mass spectrometer was operated in positive ionization mode, and ion source parameters were as follows: capillary voltage, 3000 V; drying gas, N_2 at 10 L min^{-1}; temperature, 350 °C; nebulizer pressure, 240 000 Pa; nitrogen (99.999%) was used as the collision gas. The LC–MS system was controlled and data analyzed by MassHunter software (Agilent Technologies Inc.). Quantitative analysis of CBZ was performed in multiple reaction monitoring (MRM) mode. MRM parameters for CBZ were: fragmenting voltage, 110 V; collision energy, 15 eV and MRM transitions, 237 → 194 (240 → 197 for CBZ $D_2{}^{13}C$); collision energy, 35 eV and MRM transitions, 237 → 194*m*/*z* (240 → 181 for CBZ $D_2{}^{13}C$). In all analyses, LOD and LOQ were 70 and 40 ng L^{-1}, respectively.

2.4. Data analysis

Statistical analysis (All Pairs, Tukey Kramer, $p = 0.05$) was performed by JMPIN software, version 4.0.4. (SAS Institute Inc., Cary, NC). Concentration and uptake data are presented as average ± standard deviation. Uptake data are presented per fresh mass basis.

3. Results and discussion

The uptake of CBZ by cucumber plants was evaluated in hydroponic culture to test for toxicity and uptake and distribution among plant organs, and in pot experiments with commercial-size cucumber plants to examine the effects of solid matrix and of irrigation-water quality.

3.1. Hydroponic culture

Cucumber plants were grown for 22 d in medium containing CBZ at several different concentrations. Statistical analysis showed no significant differences in cucumber biomass (both for the total plant and for specific organs) grown in spiked versus non-spiked media up to a CBZ concentration of 1000 μg L^{-1}. Phytotoxicity effects of CBZ were only observed for plants exposed to higher levels of CBZ (>10 000 μg L^{-1}). These latter plants exhibited about 50% reduction in total biomass weight, reduced length of the primary roots, modified shape and number of secondary roots, and reduced number and size of mature leaves. Phytotoxicity has also been observed at a high concentration (>5000 μg L^{-1}) of the antibiotic enrofloxacin introduced to cucumber plants (Migliore et al., 2003). Uptake level of 457 and 547 μg kg^{-1} of enrofloxacin and CBZ, respectively, were not toxic. To avoid phytotoxicity effects on the cucumber plants in our experiments and to examine uptake and fractionation, CBZ concentration was maintained below 1000 μg L^{-1}.

Detailed biomass and uptake data for cucumber plants exposed and not exposed (control) to 100 μg L^{-1} CBZ are presented in Table 3. Carbamazepine was not detected in any of the unexposed (blank) samples. For the hydroponic solution containing initial CBZ concentration of 100 μg L^{-1}, the CBZ concentrations in the xylem sap and nutrient solution during harvesting were 65.9 ± 28.4 and 76.1 ± 8.9 μg L^{-1}, respectively. These two values are statistically similar, suggesting that CBZ uptake can be considered passive and is not restricted by root membranes. The CBZ concentration in the leaves corresponded with leaf age – the highest level was exhibited in the cotyledon leaves (2354 μg kg^{-1}). With the true leaves, the concentration of CBZ was significantly lower in the youngest (top) leaves (462 μg kg^{-1}). Carbamazepine concentrations in the stem and roots were significantly lower than those observed in the leaves. This may suggest that CBZ is mainly translocated by water mass flow, and thus it is concentrated and accumulated to the largest extent in the mature/older leaves. Our finding of higher bioaccumulation of CBZ in the leaves than in the roots is similar to data reported for uptake of CBZ by ryegrass irrigated by urine (Winker et al., 2010), but contradicts data reported by Herklotz et al. (2010) for cabbage. In the latter report, the bioaccumulation factor for the tested PCs was <1 for the leaf/stem section of the cabbage, while much higher concentrations were detected in the plant's roots. This can be explained by the globe-like shape of the cabbage, with most of the evaporation being from the external leaves. Thus, when the whole cabbage is analyzed, the higher concentration of CBZ in the exterior leaves

Table 3
Harvest and uptake data (average ± standard deviation) for cucumber plants grown for 22 d in a nutrient solution exposed (or not) to 100 μg L^{-1} carbamazepine (CBZ). Uptake data are expressed for fresh biomass.

		Roots	Stems	Leaves			
				Cotyledon	Lower	Upper	Top
Mass (g)	Exposed samples	18.5 ± 0.5	6.3 ± 0.7	1.2 ± 0.1	14.9 ± 0.9	9.3 ± 0.9	3.4 ± 0.9
	Blank samples	16.2 ± 2.7	5.8 ± 0.8	1.0 ± 0.2	13.9 ± 0.7	9.3 ± 2.5	2.7 ± 1.6
CBZ concentration	(μg kg^{-1} fresh biomass of exposed samples)	163.4 ± 27.9	136.1 ± 10.2	2354.1 ± 59.9	957.0 ± 89.8	1086.7 ± 89.6	462.4 ± 116.2
	(μg $organ^{-1}$ in exposed samples)	2.5 ± 0.4	0.6 ± 0.3	1.8 ± 0.5	12.7 ± 1.2	9.9 ± 1.0	1.5 ± 0.5

is diluted by the low concentration in the internal parts (leaves and stems).

3.2. Greenhouse experiment I: effects of soil properties

Carbamazepine (25 μg L^{-1}) was introduced to plants grown on soils with varying organic matter content via irrigation with fresh water. This concentration is higher than that reported for CBZ in effluents, i.e. a median concentration of 2.1 μg L^{-1} with a maximum concentration of 6.3 μg L^{-1} (Ternes, 2001). This relatively high concentration was used in order to obtain CBZ concentration of about 0.6 μg L^{-1} in the organic-matter-rich (i.e., peat mixture) soil solution. In this experiment, we evaluated the uptake and bioaccumulation of CBZ in the fruits and the uptake and translocation into lower, middle and top leaves for plants grown in the peat mixture. Leaf-weight data indicated no effect of CBZ on plant growth in any of the medium types: Terra Rossa (clay) soil, loess (sandy) soil or peat mixture.

For the peat mixture, there were no significant differences in CBZ concentration in the mature, semi-mature and young leaves: 289.6 ± 167.1, 220.8 ± 62.6, and 351.6 ± 18.0 μg kg^{-1} fresh weight, respectively. With this medium, the concentration of available CBZ in the soil solution was 0.57 ± 0.06 μg L^{-1}. Based on these values, the average CBZ bioaccumulation factor in the leaves was about 500. In the hydroponic culture experiment, the average bioaccumulation factor for CBZ in the leaves (lower and upper leaves) was much lower (about 13). The significantly higher bioaccumulation factor obtained in the pot experiment is probably related to the longer period of the greenhouse experiment (~60 d vs. 22 d for the nutrient solution experiment) which resulted in larger leaf size and higher evaporation, and due to the different growing conditions (higher radiation intensity, higher temperature, and lower relative humidity in the greenhouse).

The bioaccumulation factors for cucumber leaves in our study were higher than those calculated for CBZ in ryegrass (Winker et al., 2010) and cabbage (Herklotz et al., 2010). Our bioaccumulation factors were also higher than those reported for the accumulation of several veterinary medicines by lettuce (Boxall et al., 2006). However, it is important to note that our calculation is based on direct measurements of the available CBZ concentration in the soil solution while in the abovementioned studies, this factor was based on total applied PC or calculated concentrations (based on adsorption parameters). The difference in bioaccumulation factor might be explained also by the physicochemical nature of the PC, by the way it is introduced into the matrix (via irrigation water or by single application) and by the nature and properties of the plant.

Carbamazepine concentrations in the commercial-size cucumber fruits were measured in plants grown in all three media (Table 4). The concentrations were 25.6, 17.1, and 6.4 μg kg^{-1} fresh weights for the sandy soil, clay soil, and peat mixture, respectively. Concentrations of CBZ in the different soils' aqueous phase were: 13.98, 2.73 and 0.57 μg L^{-1}, respectively. The concentration in the soils' aqueous phase was, as expected (Chefetz et al., 2008), negatively correlated with the level of organic matter in the soils (Table 2). Thus, it is shown that the amount taken by the plants was governed by the available CBZ in the soil solution which was in turn controlled by the soil-CBZ interactions. The bioaccumulation factors calculated for the fruits were: 1.8 ± 0.3, 6.3 ± 1.1 and 11.4 ± 5.9 for the sandy soil, clay soil and peat mixture, respectively. For the peat mixture, the bioaccumulation factor calculated for the leaves was about 40 times higher than that for the fruits. This supports the transpiration-derived mass flow translocation, since transpiration via the leaves is significantly larger than that through the fruits. The bioaccumulation factors of the fruits in the three soils lie within a relatively narrow range, and we thus assume that the unrestricted uptake and the transpiration-derived mass flow translocation were similar in all systems.

Another interesting observation is that the concentration of CBZ detected in the fruits that developed at an earlier stage of the growing season (4.11 ± 0.88 μg kg^{-1} fresh weight) was lower than that detected in fruits developing three weeks later, toward the end of the season (8.74 ± 0.87 μg kg^{-1} fresh weight). This might be related to the increasing concentration of CBZ in the soil solution (due to continuous loading of the soil adsorption complex) and increasing evaporation towards the end of the growing period. Both mechanisms would increase the CBZ concentration in the soil solution.

3.3. Greenhouse experiment II: effects of water quality

Cucumber plants were grown in loess (sandy) soil (Table 2) and irrigated with fresh water or reclaimed wastewater spiked, or not, with 1 μg L^{-1} CBZ. In this experiment, we used a concentration of CBZ that is typical to effluents, reclaimed wastewater and contaminated groundwater (Tixier et al., 2003; Clara et al., 2004; Miao et al., 2005; Chefetz et al., 2008). It is important to note that the reclaimed wastewater used in this experiment is actually used by farmers, mainly for corn and cotton irrigation. Carbamazepine concentrations in the cucumber roots, stems, leaves, fruits and xylem sap are presented in Table 5. Carbamazepine was not detected in

Table 4
Carbamazepine (CBZ) concentrations (average ± standard deviation) detected in greenhouse experiment where cucumber plants were irrigated with fresh water spiked with CBZ at 25 μg L^{-1}.

Growth media	Irrigation water (μg L^{-1})	Soil solution (μg L^{-1})	Fruits (μg kg^{-1} fresh biomass)	Bioaccumulation factor
Sandy (loess)	25	13.98 ± 0.4	25.6 ± 3.1	1.8 ± 0.3
Clay	25	2.73 ± 0.3	17.1 ± 1.2	6.3 ± 1.1
Peat mixture	25	0.57 ± 0.1	6.4 ± 2.6	11.4 ± 5.9

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Table 5
Carbamazepine (CBZ) concentration (average ± standard deviation) detected in cucumber plants grown in sandy (loess) soil.

Water type	Irrigation water (μg L^{-1})	Soil solution[b] (μg L^{-1})	Xylem sap (μg L^{-1})	Leaves (μg kg^{-1} fresh biomass)[c]	Stems (μg kg^{-1} fresh biomass)	Roots (μg kg^{-1} fresh biomass)	Fruits (μg kg^{-1} fresh biomass)	Total uptake (%)[d]
Fresh water: spiked	1.15	1.04 ± 0.07	0.33 ± 0.66	18.5 ± 1.6	1.4 ± 0.5	3.5 ± 1.6	1.2 ± 1.6	13.7 ± 1.8
Reclaimed wastewater: not spiked	2.99 ± 1.95[a]	1.19 ± 0.12	0.52 ± 0.40	20.4 ± 2.7	1.1 ± 0.1	2.0 ± 0.4	1.0 ± 0.3	4.9 ± 0.9
Reclaimed wastewater: spiked	4.14 ± 1.95	1.96 ± 0.02	1.34 ± 0.85	39.1 ± 5.0	1.9 ± 0.4	4.5 ± 2.1	2.1 ± 0.5	6.8 ± 0.9

[a] Based on five samples taken during the growing season.
[b] At the end of the experiment.
[c] Correction factors to convert concentration per fresh weight (as given) to dry weight basis (as calculated according to the water content of each tissue) are: 6.1 for leaves, 8.2 for stems, 6.0 for roots and 13.7 for the fruits.
[d] Percent from total applied CBZ in irrigation water.

the fruits and/or in the xylem sap of the cucumbers irrigated with non-spiked fresh water; some residual CBZ was detected in the roots and leaves, but the level was below the limits of quantification. Similar to observations in the hydroponic culture system, the concentrations of CBZ in the roots and stems were relatively low; most of the CBZ was concentrated in the leaves. This trend was even more pronounced when the uptake data were calculated per total mass of the different organs. In all treatments, the total amount of CBZ in the leaves made up 76 to 84% of the total taken amount. The bioaccumulation level in the roots and stems varied between 1 and 3%, and the total amount in the fruits was 11, 18 and 22% of the total uptake in plants irrigated with spiked fresh water, non-spiked reclaimed wastewater and spiked reclaimed wastewater, respectively. The maximum uptake of CBZ was obtained for the fresh water system; in this case about 13% of the total applied CBZ was detected in the biomass. In the reclaimed wastewater systems, the uptake amount was lower (Table 5) suggesting enhanced accumulation of CBZ in the solid phase of the soil. The CBZ concentration in the fruits (μg kg^{-1} fresh weight) was similar to its concentration in the corresponding soil solution (μg L^{-1}). This relationship was also observed when the loess soil was irrigated with 25 μg L^{-1} CBZ (see previous section).

Carbamazepine concentrations in the xylem sap (Table 5) were lower than those in the irrigation water or in the soil solution while in the hydroponic culture experiment these concentrations were statistically similar ($p = 0.05$). These results may suggest minor discrimination of CBZ during mass flow in the soil or in the root uptake system under soil conditions (where external matric tension may affect water uptake route and may increase the symplastic over apoplastic water uptake).

The concentration observed in the plants irrigated with the spiked reclaimed wastewater was higher than in all other treatments. The CBZ concentration in the cucumber leaves in plants that were irrigated with spiked fresh water was similar to that in plants irrigated with non-spiked reclaimed wastewater. These data suggest similar CBZ concentrations were available in the two systems. Independent analyses showed that the concentration of CBZ in the non-spiked reclaimed wastewater varied between 1.1 to 5.3 μg L^{-1} (average data are presented in Tables 2 and 5). This resulted in about twofold higher CBZ concentrations in the soil solution in the case of irrigation with spiked reclaimed wastewater containing both spiked and "native" CBZ. Our finding of similar uptake for the spiked fresh water and non-spiked reclaimed wastewater suggests that the bioavailability of "native" CBZ was lower than that of the spiked CBZ. This suggests that the possible binding of CBZ to dissolved organic matter (Maoz and Chefetz, 2010) reduces the free CBZ concentration in the soil solution and therefore affect (reduce) uptake.

Carbamazepine concentration in the soil solution of the spiked fresh water irrigated soil at the beginning of the experiment was 0.71 ± 0.03 μg L^{-1}. At the end of the experiment (i.e., after irrigation of each pot with about 9 L), the soil's aqueous phase concentrations increased (1.04 ± 0.07 μg L^{-1}). We suggest that the higher concentrations observed at the end of the experiments are mainly due to CBZ adsorption and accumulation in soil solid phase. Assuming constant adsorption affinity (K_d), the equilibrium CBZ concentration in the soil solution would increase with time. This may explain our finding that CBZ concentration in fruits that developed toward the final stage of the growing period was higher than that found in fruits developed early in the season (data for plants grown in the peat mixture).

Based on the applied CBZ amount and the total uptake, assuming minimal loss of CBZ during the pot experiment (minimal leaching, evaporation and degradation), adsorption affinities were calculated. The carbon normalized K_d values (K_{OC}) were 250 L kg^{-1} for the fresh water irrigated soil and 500–560 L kg^{-1} for the reclaimed wastewater irrigated soils. These values are within a range of values reported for CBZ adsorption by soils and sediments (Williams and Adamsen, 2006; Stein et al., 2008; Yamamoto et al., 2009). Moreover, the higher values obtained for the reclaimed wastewater irrigated soils is similar to the trend reported by Chefetz et al. (2008) for CBZ adsorption from fresh water versus secondary treated wastewater.

The bioaccumulation factors calculated for the fruits in this experiment were: 1.1 ± 0.22, 0.8 ± 0.4 and 1.1 ± 0.2 for the plants irrigated with spiked fresh water, reclaimed wastewater and spiked reclaimed wastewater, respectively. These values are similar to those calculated for cucumber plants grown in the same soil and irrigated with fresh water spiked with 25 μg L^{-1} CBZ. This suggests that the uptake potential of CBZ is mainly dependent on the available concentration in the soil solution and not on the concentration applied in the irrigation water. The bioaccumulation factors calculated for the leaves were: 17.9 ± 2.9, 17.1 ± 4.1 and 20.0 ± 2.8 for the plants irrigated with spiked fresh water, reclaimed wastewater and spiked reclaimed wastewater, respectively. These values are higher than those calculated for the fruits, similar to the trend observed for the plants grown in the peat mixture.

4. Conclusions

Our data and recent reports (Herklotz et al., 2010; Winker et al., 2010) demonstrate that CBZ can be taken up by crops and bioaccumulate. However, our data also show, for the first time, that CBZ and probably other PCs, can be taken up when introduced to plants via irrigation with reclaimed wastewater at indigenous levels (i.e.,

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1–3 μg L^{-1} for CBZ). In this study, we estimated the amount of CBZ that can be consumed through cucumbers contaminated with CBZ. Based on our data (Table 5), consumption of 200 g of cucumber fruits a day would result in uptake of about 200 ng CBZ daily. This daily dose is extremely low compared to the minimum therapeutic CBZ dose (70 mg d^{-1} for a 70-kg body weight).

Evaluation of the human-health-related risk of CBZ in crops is behind the scope of this paper. For the best of our knowledge there are no reports for risk assessment for PCs via consumption of contaminated crops. Several studies have recently reported that human health risks from exposure to PCs in water are not expected (Bruce et al., 2010). However, the daily intake considered in these reports was 60 ng d^{-1} (Webb et al., 2003). Our study indicates that introduction of PCs through the food-chain pathway is within the same magnitude or even higher than via drinking water. Therefore the combined effects should be investigated especially in areas using intensive irrigation of crops with reclaimed wastewater. Further, the significantly higher uptake in leaves versus fruits obtained in this study may imply a need for greater concern in crops such as lettuce, whose edible parts are the leaves.

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