Ecotoxicology and Environmental Safety 74 (2011) 1336–1342

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Uptake of 17α-ethynylestradiol and triclosan in pinto bean, *Phaseolus vulgaris*

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ARTICLE INFO

Article history:
Received 11 November 2010
Received in revised form
14 March 2011
Accepted 20 March 2011
Available online 21 April 2011

Keywords:
Plant uptake
Bioconcentration factor (BCF)
PPCPs
EE2
Triclosan

ABSTRACT

Pharmaceuticals and personal care products (PPCPs) have emerged as a group of potential environmental contaminants of concern. PPCPs in soil may enter terrestrial food webs via plant uptake. We evaluated uptake of 17α-ethynylestradiol (EE2) and triclosan in bean plants (*Phaseolus vulgaris*) grown in sand and soil. The extent of uptake and accumulation of EE2 and triclosan in plants grown in sand was higher than in plants grown in soil. In sand (conditions of maximum contaminant bioavailability), bioconcentration factors (BCFs) of EE2 and triclosan in roots (based on dry weights) were 1424 and 16,364, respectively, whereas BCFs in leaves were 55 for EE2 and 85 for triclosan. In soil, the BCF of EE2 decreased from 154 in the first week to 32 in the fourth week while it fluctuated in leaves from 18 to 20. The BCF for triclosan in plants grown in soil increased over time to 12 in roots and 8 in leaves. These results indicate that the potential for uptake and accumulation of PPCPs in plants exists. This trophic transfer pathway should be considered when assessing exposure to certain PPCPs, particularly with the use of recycled wastewater for irrigation.

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1. Introduction

During the last decade, pharmaceuticals and personal care products (PPCPs), including antibiotics, synthetic and biogenic hormones, and veterinary medicines, have begun to emerge as a group of potential environmental contaminants of concern. Studies have demonstrated that PPCPs are not completely removed during wastewater treatment and are eventually discharged into aquatic environments (Singer et al., 2002; Pedersen et al., 2005); interest in the occurrence, fate, and toxicity of PPCPs in the terrestrial environment, including soil and sediment, has also significantly increased of late. Several studies indicate that PPCPs can be found in effluent, streams, biosolids, and sediment in the range of parts-per-trillion to parts-per-million (Daughton and Ternes, 1999; Heberer, 2002; Kolpin et al., 2002; Xia et al., 2005; Haggard et al., 2006; Kinney et al., 2008; Karnjanapiboonwong et al., 2010; McClellan and Halden, 2010). These contaminants can reach soil via agricultural runoff, the spreading of manure and sewage sludge (provided it meets regulatory requirements), and the application of treated wastewater to land.

Several studies have observed that some estrogens, such as 17α-ethynylestradiol (EE2) and estriol (E3), cause abnormal sexual development and reduced reproductive capacity in wildlife with continual exposure at concentrations as low as parts-per-trillion (Länge et al., 2001; Parrott and Blunt, 2005; Kidd et al., 2007). Additional studies have found that triclosan, the antimicrobial agent found in hand soaps and body washes, is a persistent contaminant suspected of producing antibiotic resistance in the environment (Neumegen et al., 2005; Waller and Kookana, 2009) as well as accumulating in the fatty tissues of organisms (Samsoe-Petersen et al., 2003; Balmer et al., 2004; Coogan and La Point, 2008; Fair et al., 2009).

PPCPs may be taken up, accumulated in, or induce phytotoxicity in vegetables, wetland macrophytes, and algae (Lai et al., 2002; Migliore et al., 2003; Kumar et al., 2005; Coogan et al., 2007; Dolliver et al., 2007; Kong et al., 2007; Redshaw et al., 2008; Liu et al., 2009; Stevens et al., 2009; Wu et al., 2010). For example, antibiotics such as oxytetracycline, enrofloxacin, chlortetracycline, and sulfamethazine are taken up by alfalfa, corn, lettuce, potato, onion, cabbage, and cucumber from manure-amended soil, agar medium, or nutrient solutions (Migliore et al., 2003; Kumar et al., 2005; Dolliver et al., 2007). In addition, some researchers have reported that triclosan and estrogens can be bioaccumulated and induce phytotoxicity in algae, rice, cucumber, and wetland plants (Lai et al., 2002; Coogan et al., 2007; Liu et al., 2009; Stevens et al., 2009). Results of these studies indicate that some PPCPs could enter terrestrial food webs through soil via the land application of wastewater and biosolids. Since residual concentrations of PPCPs from both human and agricultural uses can be found in soils, these compounds have the potential to be

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doi:10.1016/j.ecoenv.2011.03.013

taken up by plants and transferred to humans through consumption.

In this study, pinto beans, *Phaseolus vulgaris*, were used to determine uptake of EE2 and triclosan from sand and soil since legumes are commonly consumed and are known to have some sensitivity to contaminants (Fletcher et al., 1985). The results obtained are expected to provide information on the magnitude of uptake of PPCPs from soil into plants and if plants present a viable exposure route for PPCPs to terrestrial organisms, both of which could aid in understanding the fate of PPCPs in wastewater.

2. Materials and methods

2.1. Chemicals and reagents

EE2 (purity >98%) and triclosan (purity >97%) were obtained from Sigma-Aldrich (St. Louis, MO) and Fluka Chemie GmbH (Buchs, Switzerland), respectively. Relevant chemical properties of the test compounds are shown in Table 1. HPLC-grade acetonitrile was obtained from Fisher Scientific (Fair Lawn, NJ). Ultrapure water (>18 MΩ) was prepared by a Barnstead NANOpure infinity ultrapure water system (Dubuque, IA). Standard solutions of test compounds were prepared in acetonitrile.

2.2. Uptake experiments

Pinto bean, *P. vulgaris*, was selected to determine uptake of EE2 and triclosan from sand and soil. Seeds were washed, soaked in Milli-Q water for 4 h, and transferred into Petri dishes on moist Kimwipes® for germination at 25 °C. Seedlings were transferred to 125-mL glass amber jars containing 150 g of Ottawa sand (20–30 mesh, Fisher Scientific) or 120 g of sandy loam soil (Terry County, Texas). The physicochemical properties of the soil were determined by A&L Midwest Laboratories (Omaha, NE). The soil was slightly basic ($pH=8.3$) and consisted of 74% sand, 10% silt, and 16% clay with 1.3% organic carbon (OC). A previous analysis of Ottawa sand by Guigard et al. (1996) indicated 98.8% sand, 1.2% silt, 0% clay, and 0% OC. The sand was neutral (pH=7.1; measured using US EPA SW-846 Method 9045D; USEPA, 2004). Sand was used because it is highly homogeneous and represents a case of maximum PPCP bioavailability (Karnjanapiboonwong et al., 2010). Each experimental treatment was conducted using five replicates. In each replicate, a bean seedling was placed in 150 g sand or 120 g soil and grown under continuous light at 25 °C. The sand or soil in each jar was spiked once a week for four weeks with a mixture of EE2 and triclosan to achieve a concentration of 1 μg test compound/g sand (or soil) by adding a stock solution of both compounds at 1000 mg/L in acetonitrile; 150 and 120 μL of stock solution was added to the sand and soil, respectively. The concentrations of test compounds used in this study are consistent with data from our laboratory on PPCP levels (particularly triclosan) in sewage sludge from a wastewater treatment facility (Karnjanapiboonwong et al., 2011).

Control samples with test pharmaceuticals and no plants were also included for each of the uptake experiments and sampled weekly to assess possible degradation of pharmaceuticals in the sand or soil (Carr et al., 2011). In addition, a control in which bean plants were grown in the sand or soil without EE2 and triclosan was also included. Plants were watered daily with Milli-Q water (pH=7.6). For the experiment conducted in sand, there was some concern about the lack of nutrients for plant growth, so plants were also watered once a week with a diluted solution (200 mg/L; pH=7.9) of Miracle-Gro® All-Purpose Plant Food in Milli-Q water. Each week, plants were removed and separated into two parts: the portion above sand/soil level (primarily stems and leaves) and the portion below sand/soil level (primarily roots). Plant tissues, sand, and soil were air dried at 25 °C in a fume hood before extraction and analysis. Both wet and dry weights of plant tissues were recorded. The amount of test compounds in plant tissues and remaining in sand or soil was determined weekly using the extraction and analysis methods described below.

Table 1
Relevant chemical properties of the test compounds.

Compound	Water solubility (mg/L at 20 °C)	log K_{ow}	K_d (mL/g)	Vapor pressure (mm Hg)
17α-ethynylestradiol (EE2)	4.8[a]	3.67[b]	176[c]	2.64×10^{-9}[d]
Triclosan	10[d]	4.76[d]	257[c]	6.45×10^{-7}[d]

[a] Ying and Kookana (2005).
[b] Machatha and Yalkowsky (2005) (estimated value).
[c] Karnjanapiboonwong et al. (2010) (values in sandy loam soil).
[d] National Library of Medicine Toxnet (http://toxnet.nlm.nih.gov) (measured value at neutral pH).

2.3. Sample preparation

2.3.1. Plant tissue

Dried plant tissues were cut into small pieces. In 50-mL glass vials, plant samples were extracted for 2 h with 10 mL of 1:1 (v/v) acetonitrile:water by agitation using an orbital shaker. We tested the extraction efficiency of various ratios of acetonitrile:water and observed that the 1:1 mixture was efficient and (importantly) compatible with the subsequent cleanup step. The plant extract was passed through a C_{18} solid phase extraction (SPE) cartridge (Honeywell Burdick & Jackson, Muskegon, MI) which was first conditioned with 3 mL of acetonitrile followed by 3 mL of water. Samples were passed through cartridges at a flow rate <5 mL min^{-1} and were subsequently eluted with 5×1 mL of acetonitrile. The eluate was evaporated to near dryness under nitrogen and brought to 1 mL with acetonitrile. Samples were then analyzed using HPLC/UV (described below). Preliminary experiments on the recovery of target compounds in plant tissues (1 μg/g), revealed that the efficiency of this extraction and cleanup method was $71.9\pm2.3\%$ for EE2 and $96.5\pm2.7\%$ for triclosan. Method detection limits based on US EPA guidelines (USEPA, 2000) for the HPLC/UV analysis of spiked plant tissues were 0.10 μg/g dry weight for EE2 and 0.12 μg/g dry weight for triclosan.

2.3.2. Sand and soil

Dried sand and soil samples were extracted to determine the amount of EE2 and triclosan remaining. In a 125-mL amber glass jar, sand or soil was added to 50 mL acetonitrile and agitated on an orbital shaker for 2 h. 2 mL of extract was collected from each jar and filtered through a 0.2 μm PTFE syringe filter. The filtrate was then analyzed using HPLC/UV (see below). In preliminary experiments conducted by spiking the sand or soil at 1 μg/g with standard solutions of EE2 and triclosan, the efficiency of the extraction method from sand was 98.3% for EE2 and 99.7% for triclosan and from soil, 99.8% for EE2 and 93.0% for triclosan. We have previously reported on the sorption interactions of EE2 and triclosan to sand and this sandy loam soil (Karnjanapiboonwong et al., 2010). Method detection limits based on US EPA guidelines (USEPA, 2000) for the HPLC/UV analysis of spiked sand were 0.67 ng/g dry weight for EE2 and 0.83 ng/g dry weight for triclosan and for spiked soil were 0.96 ng/g dry weight for EE2 and 1.04 ng/g dry weight for triclosan.

2.4. Instrumental analysis

High performance liquid chromatography (HPLC) with UV detection was used for the determination of EE2 and triclosan. Both compounds were determined under the same instrumental conditions. An Alltech Prevail C_{18} column (25 cm × 4.6 mm i.d., 5 μm) was used for separation. The mobile phase was acetonitrile:water (isocratic; 80:20 v/v) at a flow rate of 0.8 mL min^{-1}. The detection wavelength was 200 nm. While HPLC/UV can be considered ambiguous for forensic measurements of PPCPs in environmental samples, the technique is very reliable for PPCP measurements in samples from controlled laboratory experiments where determination and quantification of known compounds is desired. To determine and account for potential matrix effects and co-eluting compounds, the analytical method was developed using blank plant extracts spiked with the test compounds. We saw little indication of co-eluting compounds or matrix effects for soil or plant extracts and all analyses met pre-determined performance-based quality assurance criteria (Karnjanapiboonwong et al., 2011). Nonetheless, identification of test compounds based solely on UV detection and without second column confirmation, is acknowledged as a potential limitation to the work reported herein.

2.5. Statistical analysis

The concentrations of test compounds in plants were statistically analyzed using SPSS 13.0 software (Chicago, IL, USA). Data comparisons were conducted using analysis of variance (ANOVA). Significant differences were assessed applying the least significant difference of means at the 5% confidence level ($p\leq0.05$).

3. Results

3.1. Plant uptake of EE2 and triclosan

The uptake of EE2 and triclosan in plants (roots and leaves) over time based on dry weight of tissues is shown in Fig. 1.

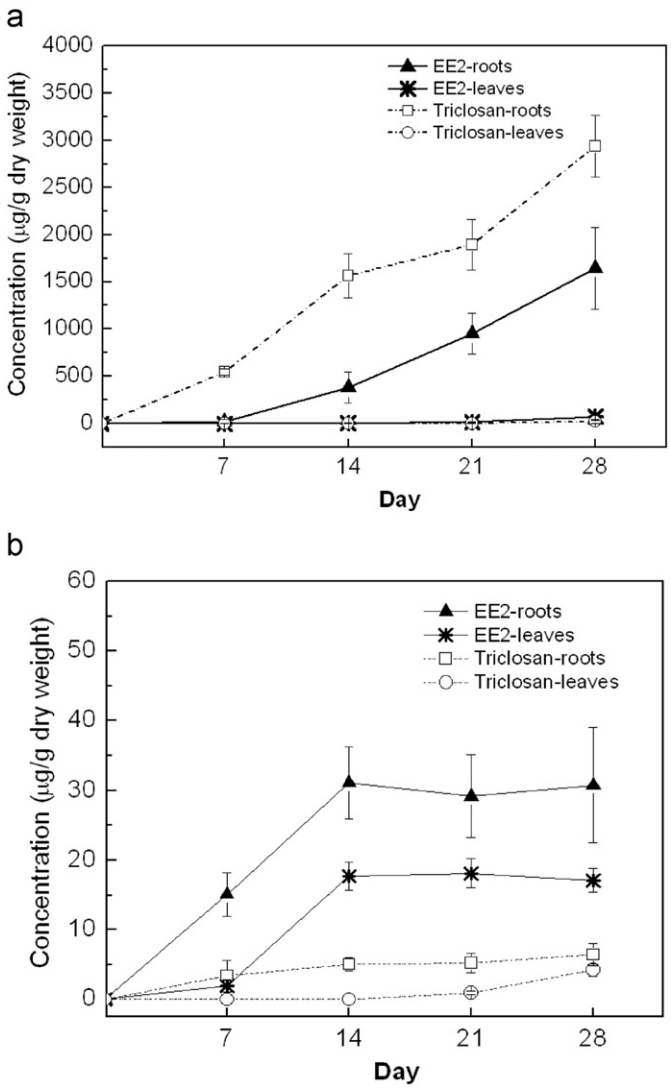

Fig. 1. Mean concentrations of EE2 and triclosan in plant tissues over time in (a) sand and (b) soil treatments based on dry weight (μg/g). Plants were grown in sand or soil spiked (each week) with the test compounds at 1 μg/g. Error bars are one standard deviation of the mean ($n=5$).

Concentrations of EE2 and triclosan in plants grown in sand increased over time in both roots and leaves; accumulation was higher in roots than leaves for both sand and soil treatments. In the sand treatment, EE2 was detected in roots and leaves at up to 1644 ± 433 μg/g dry weight and 74 ± 30 μg/g dry weight, respectively, whereas triclosan was detected in roots and leaves at up to 2940 ± 329 μg/g dry weight and 22 ± 18 μg/g dry weight, respectively. Although the concentrations are quite high, some caution is needed in the interpretation of these data as the dry plant mass for a 4-week pinto bean is quite small.

Among the test compounds detected weekly in each plant part for the sand treatment, concentrations of triclosan in roots were significantly different every week after the first week ($p \leq 0.004$). Concentrations of EE2 in roots were significantly different from EE2 and triclosan in leaves after the second week ($p < 0.002$). In leaves, there were no statistical differences between concentrations of EE2 and triclosan in each week and among weeks ($p > 0.5$), although EE2 was detected at higher concentrations than triclosan each week.

In the soil treatment, concentrations of EE2 and triclosan in both roots and leaves generally increased during the first two weeks and then remained relatively constant until the end of the experiment; the exception was triclosan, which continued to increase in leaves during the experiment. In plants grown in soil, EE2 accumulated in roots and leaves at up to 31 ± 8.3 μg/g dry weight and 17 ± 1.7 μg/g dry weight, respectively, while triclosan was detected in roots and leaves at up to 6.4 ± 1.6 μg/g dry weight and 4.2 ± 0.9 μg/g dry weight, respectively. Among test compounds detected weekly in each plant part for the soil treatment, concentrations of EE2 in roots were significantly different each week ($p \leq 0.04$). Concentrations of EE2 in leaves were significantly different from triclosan in each plant part after the first week ($p < 0.04$). There were also statistical differences between concentrations of triclosan in roots and leaves each week after the first week ($p < 0.05$).

From the data obtained based on dry weight, the ranking of EE2 and triclosan residues in each part of plants grown in sand was: triclosan in roots > EE2 in roots > EE2 in leaves > triclosan in leaves. For the soil treatment, the ranking of residues was: EE2 in roots > EE2 in leaves > triclosan in roots > triclosan in leaves. In addition, the accumulation of EE2 and triclosan in roots and leaves increased over time (Fig. 1), except for EE2 in the soil treatment in which an apparent steady state was reached after two weeks. A longer period of plant uptake may be required to reach steady state for EE2 in sand and triclosan in both sand and soil.

The uptake of EE2 and triclosan in plants over time based on fresh weight of tissues is shown in Fig. 2. In the sand treatment, EE2 accumulated in roots and leaves at up to 141 ± 60 μg/g fresh weight and 15 ± 10 μg/g fresh weight, respectively, while triclosan was detected in roots and leaves at up to 244 ± 60 μg/g fresh weight and 4.7 ± 3.5 μg/g fresh weight, respectively. In the soil treatment, EE2 was detected in roots and leaves at up to 2.8 ± 1.1 μg/g fresh weight and 1.9 ± 0.2 μg/g fresh weight, respectively, whereas triclosan was detected in roots and leaves at up to 0.58 ± 0.22 μg/g fresh weight and 0.47 ± 0.1 μg/g fresh weight, respectively.

We observed some phytotoxicity of test PPCPs, especially to plant roots. Exposed plants had shorter and small roots than controls. Since plants were exposed to both compounds, it is not clear whether the phytotoxicity occurred because of EE2, triclosan, or the combined effects of both compounds.

3.2. Distribution of EE2 and triclosan

The distribution of EE2 and triclosan among roots, leaves, and sand over time based on dry weight is shown in Fig. 3. In sand, both EE2 and triclosan concentrations were greater in roots than leaves. Although the distribution of EE2 in leaves was very low (0–0.7%) during the first three weeks, EE2 in leaves increased over time. The distribution of EE2 in plants was highest in the fourth week (10.8% in roots and 2.5% in leaves). For triclosan, the distribution in plants was primarily to roots after the second week (18.4–19.3%), whereas it was very low in leaves (0.1–0.7%) over the entire study period. Both EE2 and triclosan were accumulated in roots more than in leaves; the distribution of these compounds to leaves was minimal compared to roots. Both compounds degraded in sand during the 4-week study; however, EE2 was more persistent in sand than triclosan.

The distribution of EE2 and triclosan in plants over time for the soil treatment is shown in Fig. 4. The distribution of EE2 to roots decreased over time from 1.5% in the first week to 0.8% in the fourth week whereas it increased in leaves from 0% to 0.3% in the first week and remained constant after the second week at around 2.0%. For triclosan, the distribution to roots increased from 0% to 0.3% in the first week and remained constant after the second week at around 0.2%. The distribution of triclosan to leaves increased over time from less than 0.1% in the first two weeks

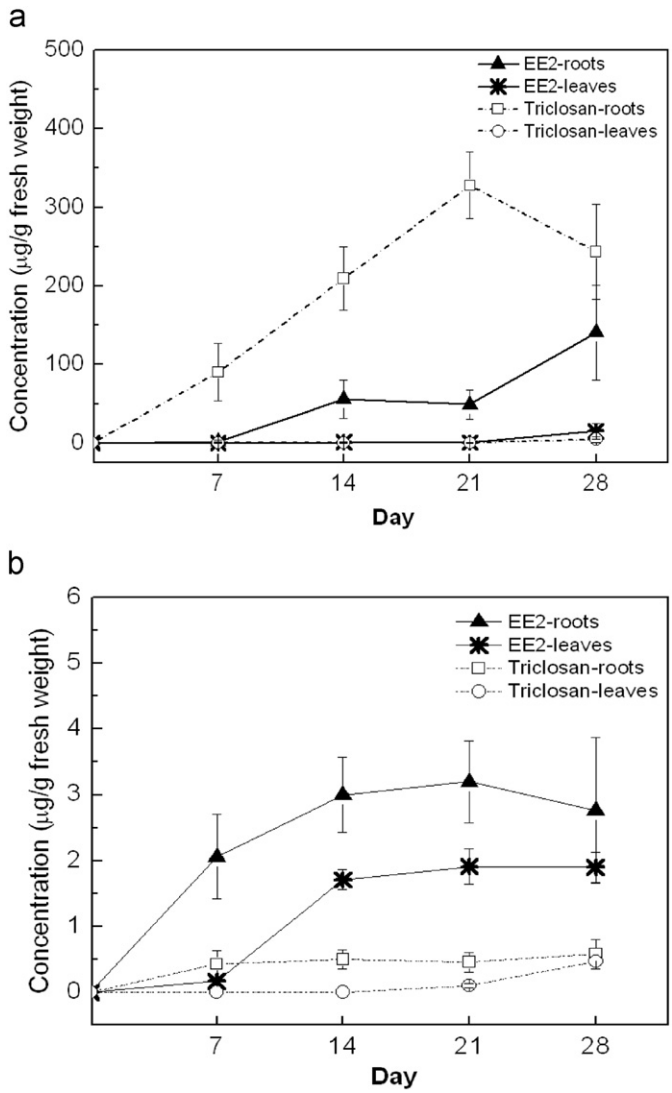

Fig. 2. Mean concentrations of EE2 and triclosan in plant tissues over time in (a) sand and (b) soil treatments based on fresh weight (μg/g). Plants were grown in sand or soil spiked (each week) with the test compounds at 1 μg/g. Error bars are one standard deviation of the mean ($n=5$).

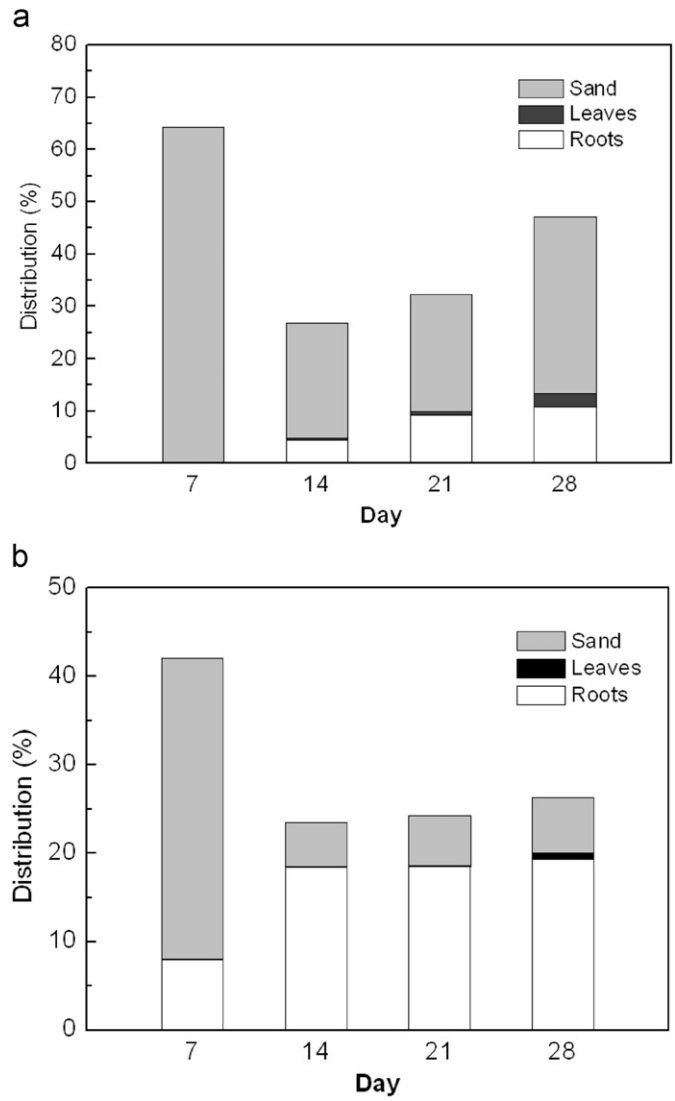

Fig. 3. Distribution of (a) EE2 and (b) triclosan among roots, leaves, and sand over time based on dry weight and the mass of test compounds in the entire compartment.

to 0.5% in the fourth week. Triclosan primarily remained in soil during the study period, however, it did degrade. Since the experimental system was aerobic to accommodate plant root growth, it is unlikely that triclosan was converted to methyl-triclosan. However, we did not test plant or soil extracts for the presence of methyl-triclosan.

Degradation, or the loss of test compounds, was observed in both sand and soil treatments (Figs. 3 and 4). This is consistent with previous work in our laboratory (Carr et al., 2011). The loss of EE2 and triclosan in each treatment over time can be inferred since the sum of chemical distribution among roots, leaves, and sand or soil in each week was not 100%. Degradation of EE2 and triclosan occurred over time in the control treatment containing unplanted sand or soil (Table 2). The loss of EE2 was approximately 13% and 69% in sand and soil, respectively, while the loss of triclosan was approximately 7% and 35% in sand and soil, respectively.

3.3. Bioconcentration factors

Bioconcentration factors (BCF) describing the translocation of test chemicals into bean plants were calculated based on the plant dry mass relative to dry soil concentration:

$$\mathrm{BCF} = \frac{\text{Concentration in dry plant tissue } (\mu g/g)}{\text{Concentration in dry sand or soil } (\mu g/g)}$$

BCFs for leaves and roots in sand and soil treatments are shown in Table 3. BCFs for both EE2 and triclosan in roots were higher than those in leaves in both soil and sand treatments during the study period. Moreover, BCFs for both compounds in plants grown in sand were higher than those grown in soil every week with the exception of EE2 in roots during the first week. In sand, BCFs of EE2 and triclosan in roots reached as high as 1424 and 11,582, respectively, whereas BCFs were as high as 55 for EE2 and 85 for triclosan in leaves.

In the soil treatment, the root BCF for EE2 decreased from 154 in the first week to 32 in the fourth week, while it fluctuated in leaves from 18–20 during the study period. For triclosan, the BCF in the soil treatment increased slightly over time up to 12 in roots and 8 in leaves. For both test compounds, roots were the primary site of bioconcentration in plants during the 4-week study period. In addition, both compounds were more bioavailable for plant uptake in sand. This was consistent with sorption data from our laboratory on the test compounds (Karnjanapiboonwong et al., 2010).

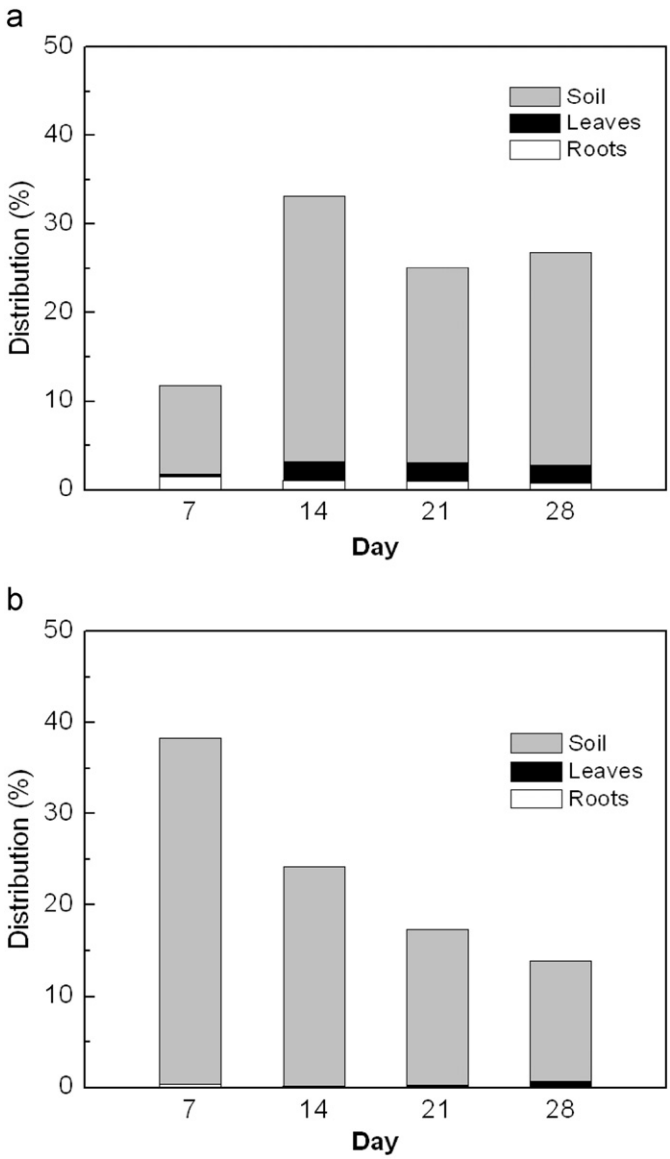

Fig. 4. Distribution of (a) EE2 and (b) triclosan among roots, leaves, and soil over time based on dry weight and the mass of test compounds in the entire compartment.

Table 2
Concentrations of EE2 and triclosan remaining in sand and soil in the control treatment based on dry weight (μg/g).

Treatment[a]	Compound	Week			
		1	2	3	4
Sand	EE2	0.82 ± 0.03	1.60 ± 0.09	2.61 ± 0.06	3.32 ± 0.12
	Triclosan	0.85 ± 0.03	1.86 ± 0.01	2.76 ± 0.11	3.70 ± 0.05
Soil	EE2	0.24 ± 0.06	0.62 ± 0.11	0.76 ± 0.05	1.20 ± 0.15
	Triclosan	0.65 ± 0.04	0.71 ± 0.05	1.02 ± 0.06	1.06 ± 0.08

[a] Each sample was spiked with test compounds at 1 μg/g every week. Data represent mean ± standard deviation (n=5). The loss of test compounds over time was from biodegradation (Carr et al., 2011).

4. Discussion

Uptake and distribution of organic chemicals in plants is dependent on the physical–chemical properties of the chemical and characteristics of the soil (Ryan et al., 1988; Bacci et al., 1990; Trapp et al., 1990; Paterson et al., 1994); these properties also govern plant uptake of EE2 and triclosan in sand and soil. EE2 and triclosan were detected in plants grown in sand at higher concentrations than in plants grown in soil; both EE2 and triclosan were less bioavailable for uptake from soil compared to sand. The organic carbon (OC) content of the soil (1.3%), although relatively low, was much higher than OC content of the sand (essentially 0%). Sorption of EE2 and triclosan was greater in soil, resulting in less chemical in pore water available for plant uptake. Interestingly, the matrix (sand vs. soil) influenced which test compound accumulated to the greatest extent: sand favored triclosan in roots, while soil favored EE2 in roots. By the end of the 4-week study, there was a significant mass of root tissue in the sample containers. This ratio (sand or soil mass:root mass) may have also affected the PPCP distribution we observed.

Table 3
Bioconcentration factors for test compounds in plants based on dry weight.

Compound	Treatment		Week			
			1	2	3	4
EE2	Sand	Roots	31	874	1,424	1218
		Leaves	0	14	23	55
	Soil	Roots	154	52	44	32
		Leaves	20	30	28	18
Triclosan	Sand	Roots	1613	16,364	11,151	11,582
		Leaves	5	17	10	85
	Soil	Roots	9.0	10	10	12
		Leaves	0	0	1.8	8

Plants accumulated EE2 and triclosan in roots more than in leaves. Organic contaminants are taken up by plant roots via partitioning between soil and plant cells (passive transport) as well as against the electro-chemical potential gradient (active transport). Contaminants then move into the plant transpiration stream to other parts of the plant (Briggs et al., 1982; Chiou et al., 2001; Li et al., 2005; Su and Zhu, 2007). Roots have a higher potential to accumulate lipophilic contaminants as lipophilicity tends to favor partitioning to roots and hinder movement out of roots and into the plant transpiration stream (Briggs et al., 1982).

Plants accumulated EE2 at higher concentrations than triclosan. This result may be partially explained by the sorption of these compounds in soil. Both $\log K_{ow}$ and K_d of triclosan are higher than those of EE2, indicating that triclosan has a higher tendency to sorb to the soil resulting in less of the chemical available for plant uptake (Karnjanapiboonwong et al., 2010). The lower lipophilicity for EE2 translated into greater bioavailability in soil and higher translocation to leaf tissue.

There was loss of both EE2 and triclosan from the control (non-vegetated) treatments. This was believed to be due primarily to biodegradation of the test compounds and not volatilization; the vapor pressures of the test compounds are relatively low. Several studies reported that EE2 and triclosan can be biologically degraded in soil (Colucci and Topp, 2001; Ying and Kookana, 2005; Ying et al., 2007; Carr et al., 2011). We observed greater transformation of both test compounds in soil than in sand, likely the result of higher microbial biomass and/or activity. This is also supportive of aerobic biodegradation as the primary loss mechanism from controls. Photodegradation of the test compounds on the soil surface may have also occurred; however, this transformation mechanism is more likely for triclosan than EE2. It is believed that EE2 does not photodegrade at visible wavelengths (Liu et al., 2003) while triclosan has been found to photodegrade (Aranami and Readman, 2007).

The possible cause of loss of EE2 and triclosan in planted treatments, other than degradation in sand or soil, was transformation of these compounds in plants (Farkas et al., 2008). However, possible metabolites of EE2 and triclosan in plant tissues were not quantified in this study. In addition, the loss of target compounds in plant tissues may occur during sample preparation and extraction since the efficiency of the extraction method applied in the test was not 100%, especially for triclosan (71.9% extraction efficiency).

To date, data on bioaccumulation and phytotoxicity of EE2 and triclosan in plants are still very limited. Results of the available studies on uptake of these compounds in plants have also varied among plant species and experimental designs. However, our results are supported by other studies on the accumulation and toxicity of triclosan in plants (Liu et al., 2009; Stevens et al., 2009; Herklotz et al., 2010; Wu et al., 2010). In greenhouse studies, triclosan was readily taken up from soil by soybean (*Glycine max*) from both biosolids application and wastewater irrigation (Wu et al., 2010); accumulation in roots and translocation to shoots (including beans) was observed. Stevens et al. (2009) found that, in wetland plants, triclosan was accumulated in roots more than shoots, and can cause changes to root morphology. In addition, Liu et al. (2009) and Herklotz et al. (2010) observed that triclosan inhibited growth and/or killed plants following germination. However, since our study was conducted with both EE2 and triclosan, we could not determine whether the effects we observed were due to EE2, triclosan, or a combination of both compounds.

Our results also indicate that plants can serve as a transfer pathway of EE2 and triclosan to higher organisms feeding on plants, especially roots. There is no maximum residue level for triclosan and EE2 regulated in edible crops since these compounds are not regulated in plant-based products and there are no direct food uses associated with these compounds. Triclosan and EE2 both show acute toxicity with high-concentration exposure ($LD_{50} > 2$ g/kg) and chronic toxicity with low-concentration (mg/kg/day) exposure to mammals such as rats and rabbits (Zimmermann et al., 1996; Barbolt, 2002; USEPA, 2008). We did not determine EE2 and triclosan residues in the pinto bean plant tissue typically consumed by humans (seeds). Therefore, it is difficult to make any conclusions about potential human exposure. Based on our data, acute toxicity of these compounds from edible crops is not likely to occur in wildlife; however, chronic toxicity may occur via long-term consumption of these compounds in food. Bioconcentration data from our study could be used to estimate the potential intake of EE2 or triclosan from crops irrigated with gray water to assess risk.

5. Conclusions

The results of this study provide information on uptake of PPCPs from soil into plants as a potential exposure route of these chemicals to animals including humans. In addition, our results suggest that estrogens and antimicrobial compounds in agricultural runoff have the potential to be taken up by vegetation. Roots were the primary plant part in which EE2 and triclosan accumulated, and the accumulation of both test compounds was higher in plants grown in the low OC substrate. Although PPCPs were also taken up by bean plants from soil, the OC content of the soil played an important role in reducing the bioavailability of these compounds to plants. Biodegradation of the test compounds also reduced their uptake into plants. The potential for accumulation of PPCPs in plants irrigated with gray water or treated wastewater exists provided the compounds have characteristics amenable to uptake and translocation. Such exposure routes should be considered when assessing risks associated with the use of recycled wastewater and/or the land application of biosolids.

Acknowledgments

This study was supported in part by the US EPA through the Water Law and Policy Center at Texas Tech University. The Texas Water Resources Institute (TWRI) is acknowledged for supporting A.K. and the research. D.A.C. was supported by a Water Conservation Research Fellowship at Texas Tech University.

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