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Uptake by Cucurbitaceae of Soil-Borne Contaminants Depends upon Plant Genotype and Pollutant Properties

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Three Cucurbitaceae, *Cucurbita pepo* L. subsp. *pepo* (cv. Black Beauty, true zucchini), *Cucurbita pepo* L. intersubspecific cross (cv. Zephyr, summer squash), and *Cucumis sativis* (cv. Marketmore, cucumber), were grown in rhizotrons containing soil contaminated with three classes of highly weathered, hydrophobic organic contaminants: (1) technical chlordane, (2) dichlorodiphenylethanes (DDT and DDD) and -ethene (DDE), (3) polyaromatic hydrocarbons (PAHs), and heavy metal residues. Movement of the contaminants through the soil/plant system was studied by comparing contaminant concentration in the bulk soil, the rhizosphere soil pore water, the xylem sap, and aerial tissue. This permitted, for the first time, calculation of bioconcentration factors (BCFs) based on concentration in the xylem sap versus that in the rhizosphere soil pore water. The bioconcentration factors so determined for the sum of five chlordane residues (two enantiomers of *trans*-chlordane, TC; two enantiomers of *cis*-chlordane, CC; and achiral *trans*-nonachlor, TN) were 36, 40, and 23 for Black Beauty, Zephyr, and Marketmore, respectively. In addition, the xylem sap of each cultivar had a consistent enantioselective profile for some of the chiral chlordane components. For the sum of dichlorodiphenylethanes and -ethene, comparable BCF values were 19, 4, and 0.8, respectively. In the case of PAHs, different BCF patterns among the cultivars were noted for three- versus four-ring compounds. Similarly, movement of heavy metals was cultivar-dependent, with cadmium BCF values 9.5, 3.5, and 0.6 for Black Beauty, Zephyr, and Marketmore, respectively; the analogous BCFs for zinc were 9, 11, and 2. Thus, passage from ex planta to in planta regions of the soil/plant system is dependent not only on properties of the plant, but also on those of the pollutant. Such data will provide insight into transport mechanisms of highly hydrophobic organic contaminants, as well as heavy metal contaminants, in the soil/plant system.

Introduction

Sequestration of xenobiotics upon aging within the soil matrix is well-documented for organic and elemental substances (*1*−*5*). For organic contaminants, sequestration has been noted primarily for hydrophobic compounds whose physicochemical properties, such as log K_{ow} > 3.5, favor partitioning to the soil matrix (*6*). Empirical evidence for sequestration comes from two types of observations: those of biotic systems, for example, reduced bioavailability of xenobiotics to organisms such as microbes and earthworms as a function of the xenobiotic's aging in the soil compartment (*7*), as well as those of abiotic systems, namely, the more vigorous extraction methods required to recover contaminants from the soil matrix as weathering proceeds (*8*).

Reduced bioavailability upon aging of hydrophobic, soil-bound, organic contaminants to microbes and fauna was assumed to pertain to flora as well (*9*). However, beginning in the 1990s, reports of an apparently distinct difference between contaminant uptake by plants of the Cucurbitaceae family versus most other terrestrial plant families began to appear in the literature. For example, researchers in Germany reported that aerial plant tissues of *Cucurbita pepo* were more contaminated with soil-borne dioxins and furans than other common crops (*10*).

For more than a decade, our laboratory has conducted extensive research in field and greenhouse trials focused on uptake by Cucurbitaceae and other terrestrial plants of highly weathered, soil-borne, organic "opportunistic analytes". As noted by Schröder et al. (*11*), terrestrial plants neither need nor want such substances, which have been introduced to the biosphere relatively recently. Nevertheless, if plants take them up, we hypothesize that such analytes are accumulated in planta using endogenous uptake systems which evolved in response to the plant's physiological requirements.

The move from field studies to the rhizotron-based trials described in this paper was motivated by two considerations: first, the need to minimize experimental variations—including, but not limited to, soil compartment size and contaminant concentration in soil—which are inherent in field studies (*12*, and references therein). Such experimental variations might confound observations and associated conclusions. Second was the desire to obtain in situ access to portions of the soil/plant system, such as the rhizosphere, rhizosphere soil pore water, and xylem sap. The rhizosphere is defined as that volume of soil influenced by root exudates (*13*). Operationally, for most plants and soil types, it encompasses that portion of the soil 1 to 2 mm laterally displaced from the root surface; within this region, the impact of plant roots on contaminant bioavailability is maximized in the soil pore water and the soil gas.

Three Cucurbitaceae were selected for these rhizotron experiments: *Cucurbita pepo* subsp. *pepo* (cv. Black Beauty), *Cucurbita pepo* intersubspecific cross (*pepo* × *texana*, cv. Zephyr) (Harry Paris personal communication; *14*, *15*), and *Cucumis sativis* (cv. Marketmore). Among terrestrial plants, these Cucurbitaceae represent extremes of contaminant uptake on the basis of our previous field experiments: Black Beauty is considered an exceptional accumulator, whereas Zephyr and Marketmore are termed nonaccumulators (*12*, *16*). Limiting this study to a plant family whose genera and species display a range of accumulator characteristics lays the foundation for the eventual identification of the genetic basis of the observed variations.

The objectives of this series of rhizotron experiments were (1) to track and compare movement of target analytes, for example, heavy metals and organic pollutants, through the soil/plant system, from the bulk soil to the rhizosphere pore water to the xylem sap to the aerial plant tissue, of different Cucurbitaceae; (2) to compare movement of target analytes from the bulk soil to the rhizosphere soil pore water in

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TABLE 1. Concentration[a] of Selected Heavy Metals in Bulk Soil (dry weight basis), Rhizosphere Soil Pore Water and Xylem Sap[b] for Three Cultivars of Cucurbitaceae

	Cd			Pb			Zn		
cultivar	bulk soil	pore water	sap[c]	bulk soil	pore water	sap	bulk soil	pore water	sap
Black Beauty	1.67 ± 0.03	2.64 ± 2.06 $n = 30$	25.1 ± 12.8 (A)	203 ± 1.0	22.3 ± 11.2 $n = 30$	38.0 ± 12.2 (A)	67.1 ± 0.77	0.11 ± 0.05 $n = 9$	1.0 ± 0.3 (A)
BCF		9.5			1.7			9.1	
Zephyr	1.67 ± 0.03	2.37 ± 2.20 $n = 18$	8.3 ± 2.6 (B)	203 ± 1.0	24.8 ± 9.3 $n = 21$	48.6 ± 23.4 (A)	67.1 ± 0.77	0.11 ± 0.06 $n = 7$	1.2 ± 0.5 (A)
BCF		3.5			2.0			10.9	
Marketmore	1.67 ± 0.03	2.90 ± 2.57 $n = 30$	1.8 ± 1.0 (C)	203 ± 1.0	18.6 ± 9.7 $n = 32$	24.3 ± 9.2 (A)	67.1 ± 0.77	0.17 ± 0.09 $n = 9$	0.4 ± 0.1 (B)
BCF		0.62			1.3			2.4	
Control		4.35 ± 3.53 $n = 74$			11.1 ± 6.8 $n = 67$			0.15 ± 0.08 $n = 30$	

[a] Bulk soil units are milligrams/killigram; pore water for Cd and Pb, in nanograms/milliliter and for Zn, in micrograms/milliliter; sap for Cd and Pb, in nanograms/milliliter and for Zn, in micrograms/milliliter. [b] For each element, letters in parentheses following xylem sap concentration show statistical pairwise comparison (Tukey test) of the log-transformed xylem sap flux data. [c] For each cultivar, sap was collected from each of six plants over three consecutive periods (see text) for a total of 18 separate sap samples.

vegetated systems with the nonvegetated system; and (3) to determine if a correlation exists between root exudates and analyte movement determined in item no. 1. Movement of selected analytes in the ex planta portion of the soil/plant system, as well as collection and analysis of root exudates, are presented and discussed in another publication (*17*). The present manuscript focuses on data pertaining primarily to item no. 1.

Materials and Methods

Rhizotrons (*18*) were constructed as previously described in detail (*19*) and filled with 3.5 kg of contaminated sandy loam soil containing 6.5% organic matter (*20*). For each cultivar, six rhizotrons were planted with the aforementioned cucurbits: Black Beauty, Marketmore (Seedway, Hall, NY), and Zephyr (Johnny's Selected Seeds, Albion, ME). After germination, each was thinned to one plant per rhizotron. Six additional rhizotrons were left unplanted to serve as controls, making a total of 24 rhizotrons, which were fully randomized on a greenhouse bench for this trial. All rhizotrons were watered automatically twice daily throughout the 10-week growing period in the greenhouse.

"Mini rhizotrons" (MR) to encompass the rhizosphere were constructed from 10-mL glass serological pipets (Fisher Scientific, Pittsburgh, PA), scored and cut at the 4 mL mark, to result in a tube of 13-cm final length. The MRs were filled with approximately six grams of contaminated soil and installed on the soil surface in a rhizotron after root growth was clearly visible. For a vegetated rhizotron, the growing end of a viable root was carefully teased from the soil and directed to grow into the MR. When a root was about to exit the far end of the MR, the root was severed from the plant at the point where it entered the MR, the MR was removed and centrifuged, and rhizosphere soil pore water was collected and frozen until analysis. For each MR containing plant roots, an MR packed with contaminated soil was placed in one of the six nonvegetated rhizotrons for a comparable time period to serve as control.

To collect xylem sap, the Plexiglas window on the long rhizotron dimension was removed, and the bottom end of the rhizotron was propped up at an angle of ~30° to the horizontal. The stem of the plant was cleaned of soil particles and severed 2−3 cm above the soil surface, and the sap was allowed to flow for 5 s, after which the rooted and now severed stem end was dabbed clean and placed into a clean 16-mL amber glass vial for sap collection. Water delivered via a peristaltic pump kept the soil moist throughout sap collection, although no attempt was made to achieve typical field moisture conditions. Collection was divided into two consecutive 4-h periods, followed by a third, 12-h collection. Samples were chilled on ice throughout collection and then frozen until analysis. For each plant, the sap volume and collection time were summed over the three collection periods. The final analyte sap concentration for the plant in a given rhizotron is calculated from the sum of the analyte quantity, determined separately for each collection period, divided by the total volume. The sap concentrations listed in Tables 1, 2, and 3 are the averages across the six plants of each cultivar.

Elemental analysis of soils on an Atom Scan 16 inductively coupled plasma optical emission spectrometer (Thermo-Jarrel Ash, Franklin, MA) has been described previously (*21*). Pore water and xylem sap samples were analyzed without digestion for Cd and Pb on a PE 51 OOPC graphite furnace atomic absorption spectrometer (GFAAS) (Perkin-Elmer Corp., Wellsley, MA) and for Zn by ICP OES. Limits of detection (LOD) are provided in Table 1 of the Supporting Information.

For organic contaminants, soil and plant tissue samples were extracted in duplicate, using methods published previously (*22*). Soils were spiked with 100 μL of an internal standard (IS) solution containing 4 μg/mL of $^{13}C_{10}$-TC and $^{13}C_{12}$-*p,p′*-DDE (2,2-bis(4-chlorophenyl)-1,1-dichloroethene), as well as 2 μg/mL each of $^{13}C_{10}$-TN, d_{10}-fluoranthene, d_{10}-phenanthrene, and d_{10}-pyrene (Cambridge Isotope Laboratories, Andover, MA) in toluene prior to extraction. Plant tissue samples were spiked with 50 μL of the above IS prior to extraction.

For analysis of organic contaminants in rhizosphere pore water or sap samples, the above IS was diluted into methanol such that all labeled achiral compounds and each enantiomer were at 20 ng/mL in the methanolic solution. For each pore water or sap sample, 950 μL was transferred to a GC autosampler vial which contained 50 μL of the methanolic IS. Solid-phase microextraction (SPME) was then conducted on these liquid samples using a 65-μm PDMS-DVB fiber (Supelco, Bellefonte, PA), precleaned by thermal desorption and inserted through the septum on the vial's cap directly into the sample solution. Vial and SPME apparatus together were placed on a Reacti-therm heating mantle (Pierce Chemical Co., Rockford, IL) at 57 °C for 140 min. The analytes of interest were desorbed from the fiber in the injection port of the GC/MS system.

Target organic analytes were quantified on a Saturn 2000 ion trap GC/MS system (Varian, Sugar Land, TX) with minor modifications to the GC oven program published previously

TABLE 2. Concentrations,[a] EFs, BCFs of Chlordane Components in Soil/Plant/Air Compartments[b]

cultivar	(+)-TC	(−)-TC	(−)-CC	(+)-CC	MC-5a	MC-5b	TN	SUM 5	(−)-exo-HepX	(+)-exo-HepX
Bulk Soil										
$n = 1$ or 2	1020	1130	1150	1290	210	203	1080 ± 100	5680	65.3 ± 5.9	90.6 ± 7.6
EF	0.47		0.53		0.51				0.58	
Pore Water										
Black Beauty $n = 31$	0.188 ± 0.095	0.206 ± 0.102	0.234 ± 0.101	0.283 ± 0.114	0.062 ± 0.027	0.069 ± 0.029	0.190 ± 0.095	1.10 ± 0.501	0.198 ± 0.080	0.266 ± 0.096
EF	0.48		0.55		0.48				0.57	
Zephyr $n = 24$	0.170 ± 0.085	0.201 ± 0.107	0.224 ± 0.111	0.263 ± 0.111	0.060 ± 0.030	0.064 ± 0.030	0.183 ± 0.094	1.04 ± 0.50	0.169 ± 0.070	0.229 ± 0.095
EF	0.46		0.54		0.48				0.57	
Marketmore $n = 35$	0.151 ± 0.061	0.174 ± 0.067	0.193 ± 0.070	0.235 ± 0.077	0.048 ± 0.024	0.055 ± 0.023	0.164 ± 0.060	0.917 ± 0.327	0.150 ± 0.046	0.201 ± 0.064
EF	0.47		0.55		0.47				0.57	
control $n = 81$	0.137 ± 0.088	0.155 ± 0.098	0.178 ± 0.105	0.215 ± 0.114	0.050 ± 0.032	0.054 ± 0.027	0.142 ± 0.094	0.826 ± 0.496	0.149 ± 0.048	0.198 ± 0.058
EF	0.47		0.55		0.48				0.57	
Xylem Sap[c]										
Black Beauty $n = 18$	5.27 ± 1.62 (A)	5.73 ± 1.62 (A)	8.79 ± 2.40 (A)	9.83 ± 2.90 (A)	1.59 ± 0.39 (A)	3.88 ± 0.99 (A)	2.59 ± 0.72 (A)	32.21 ± 9.21 (A)	0.55 ± 0.37 (A)	4.21 ± 1.27 (A)
BCF	35.1	34.3	45.7	42.5	28.3	68.4	16.3	35.8	3.60	20.5
EF	0.48		0.53		0.29				0.65	
Zephyr $n = 18$	6.23 ± 0.74 (A)	7.67 ± 1.04 (A)	9.84 ± 1.57 (A)	11.10 ± 1.96 (A)	1.20 ± 0.20 (B)	0.84 ± 0.15 (B)	3.27 ± 0.50 (A)	38.10 ± 5.68 (A)	0.80 ± 0.23 (A)	7.96 ± 2.30 (A)
BCF	38.3	42.3	48.2	44.7	19.2	14.0	19.5	39.5	5.42	39.3
EF	0.45		0.53		0.59				0.58	
Marketmore $n = 18$	3.12 ± 0.53 (B)	3.66 ± 0.73 (B)	5.07 ± 0.92 (A)	4.55 ± 0.74 (B)	0.27 ± 0.06 (C)	0.26 ± 0.06 (C)	3.44 ± 0.55 (A)	19.86 ± 3.39 (B)	0.30 ± 0.14 (B)	2.01 ± 0.71 (B)
BCF	22.2	23.1	27.8	20.9	5.06	4.71	23.2	23.4	2.09	10.3
EF	0.46		0.47		0.51				0.48	
Aerial										
Black Beauty $n = 6$	1017 ± 386	1020 ± 358	1470 ± 541	1470 ± 537	132 ± 26.4	306 ± 82.9	445 ± 146	5420 ± 1960	431 ± 74.8	806 ± 133
EF	0.50		0.50		0.30				0.65	
Zephyr $n = 6$	343 ± 52.0	380 ± 51.1	532 ± 64.4	491 ± 64.9	52.4 ± 15.2	43.4 ± 10.5	204 ± 31.8	1950 ± 254	218 ± 78.4	376 ± 119
EF	0.47		0.48		0.55				0.63	
Marketmore $n = 6$	228 ± 49.2	347 ± 71.1	624 ± 127	542 ± 113	33.4 ± 12.7	31.3 ± 8.44	515 ± 103	2260 ± 450	163 ± 58.3	144 ± 58.1
EF	0.40		0.46		0.52				0.47	
Air										
$n = 10$	0.790 ± 0.330	0.812 ± 0.326	0.632 ± 0.240	0.662 ± 0.251	0.052 ± 0.024	0.051 ± 0.022	0.680 ± 0.260	3.68 ± 1.44	0.102 ± 0.032	0.133 ± 0.044
EF	0.49		0.51		0.51				0.57	

[a] Bulk soil and aerial tissues on dry weight basis in nanograms/gram; pore water and xylem sap in nanograms/milliliter; air in nanograms/meter^3. [b] For a given component, letters in parentheses in the "xylem sap" section show statistical pairwise comparisons among cultivars (Tukey test) of the log-transformed flux data. [c] For each cultivar, sap was collected from each of six plants over three consecutive periods (see text) for a total of 18 separate sap samples.

TABLE 3. Concentrations[a] and BCFs of Dichlorodiphenyl Compounds and PAHs in Soil/Plant/Air Compartments[b]

cultivar	SUM DDs	phenanthrene	anthracene	fluoranthene	pyrene
		Bulk Soil			
$n = 1$ or 2	3411	1661 ± 304	178 ± 16	3970 ± 416	3135 ± 186
		Pore Water			
Black Beauty, $n = 31$	1.10 ± 0.65	0.14 ± 0.12	0.02 ± 0.02	0.12 ± 0.06	0.09 ± 0.05
Zephyr, $n = 24$	1.23 ± 0.90	0.28 ± 0.43	0.07 ± 0.20	0.14 ± 0.08	0.11 ± 0.07
Marketmore, $n = 35$	1.06 ± 0.45	0.37 ± 0.41	0.12 ± 0.19	0.15 ± 0.08	0.11 ± 0.06
control, $n = 81$	1.04 ± 0.67	0.25 ± 0.32	0.06 ± 0.11	0.19 ± 0.33	0.15 ± 0.27
		Xylem Sap[c]			
Black Beauty, $n = 18$	21.3 ± 4.30, (A)	1.63 ± 0.67, (A)	0.53 ± 0.26, (A)	0.62 ± 0.23, (A)	0.27 ± 0.07, (A)
BCF	19.4	11.6	26.5	5.17	3.0
Zephyr, $n = 18$	4.73 ± 0.59, (B)	1.63 ± 1.22, (A)	0.69 ± 0.52, (A)	0.16 ± 0.06, (B)	0.08 ± 0.04, (B)
BCF	3.84	5.8	9.8	1.1	0.72
Marketmore, $n = 18$	0.82 ± 0.22, (B)	1.04 ± 0.45, (A)	0.42 ± 0.20, (A)	0.55 ± 0.13, (A)	0.44 ± 0.08, (A)
BCF	0.77	2.8	3.5	3.6	4.0
		Aerial			
Black Beauty, $n = 12$	2990 ± 910	192 ± 35.2	12.7 ± 2.10	136 ± 28.6	111 ± 23.1
Zephyr, $n = 12$	436 ± 136	189 ± 44.7	14.6 ± 2.34	144 ± 48.6	115 ± 36.1
Marketmore, $n = 12$	257 ± 79.5	193 ± 68.6	15.0 ± 4.18	161 ± 60.5	131 ± 41.7
		Air			
$n = 10$	0.67 ± 0.25	7.57 ± 2.22	0.000	1.49 ± 0.80	1.31 ± 0.46

[a] Bulk soil and aerial tissues on dry weight basis in nanograms/gram; pore water and xylem sap, in nanograms/milliliter; air, in nanograms/meter^3. [b] For a given compound, letters in parentheses in the "xylem sap" section show statistical pairwise comparisons among cultivars (Tukey test) of the log-transformed flux data. [c] For each cultivar, sap was collected from each of six plants over three consecutive periods (see text) for a total of 18 separate sap samples.

(*19*). To accommodate the additional analytes included in this study, mass spectrometer scan ranges were set to 6 min filament delay; 6−13 min, m/z 120−140; 13−31 min, m/z 145−170; 31−46 min, m/z 170−195; 46−53 min, m/z 265−345; 53−85.5 min, m/z 195−425; 85.5−92 min, m/z 220−340; 92−116 min, m/z 220−250.

For quantitation of soils and plant tissue, calibration standards containing *p,p′*-DDE, TC, CC, and TN (ChemService, West Chester, PA), *p,p′*-DDD (2,2-bis(4-chlorophenyl)-1,1-dichloroethane); *p,p′*-DDT (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane) (EPA, Research Triangle Park, NC); and a mixture of PAHs (Supelco, Bellefonte, PA, Catalogue No. 48905-U) were prepared in isooctane at 10, 25, 50, 100, 250, 500, and 1000 μg/L. Each solution contained TC and CC at the cited concentration and TN, *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, and the PAHs at one-half the cited amount. The following labeled ISs were also added such that the solution contained 50 μg/L of each component: $^{13}C_{10}$-TN, $^{13}C_{10}$-TC, $^{13}C_{12}$-*p,p′*-DDE, and the aforementioned deuterated PAHs. A set of the seven calibration standards was injected at the beginning and end of each run of approximately 20 samples, each of which was injected once. All soil and plant concentrations were reported on a dry weight basis. For quantitation of soil pore waters and xylem saps, calibration curves were constructed by spiking known quantities of standards containing native and labeled components into DI water. These spiked solutions were then exposed to SPME fibers as described above and desorbed into the GC inlet.

Data reduction and stringent data quality criteria have been described previously (*22*, *23*). The limits of quantitation (LOQ) for each matrix at a S/N of 2.5 were calculated in nanograms/gram as follows: pore water, 0.05; xylem sap, 0.1; soil, 1.0; vegetation, 5.0. So as not to bias the data, components observed at less than the limit of quantitation (LOQ) were replaced by an area equivalent to 1/2 the minimum quantifiable area for that component in any sample (*24*).

Results and Discussion

Movement of Heavy Metals through the Soil/Plant System. Rhizotrons were filled with a contaminated soil that has been previously described in detail (*22*). Three heavy metals, Cd, Pb, and Zn, which could be traced at concentrations greater than the LOQ from their origin in the bulk soil into the plant tissues will be discussed.

Data from duplicate analysis of a soil sample composited from each of the 24 rhizotrons are shown in Table 1 in the columns headed "bulk soil" and are in good agreement with analyses of various samples of this same soil collected for use in earlier experiments (unpublished data). Furthermore, xylem sap concentrations of Cd and Zn for Black Beauty and Marketmore in the present trial are in agreement with published data from an earlier rhizotron trial (*25*).

Collection of both rhizosphere soil pore water from the mini rhizotrons and xylem sap from the severed plant stems permits us to track these three heavy metals through the soil/plant system for comparisons between compartments which have not been reported previously. BCF values tabulated in Table 1 for Cd, Pb, and Zn were calculated from eq 1:

$$\mathrm{BCF} = C_{\mathrm{sap}}/C_{\mathrm{porewater}} \quad (1)$$

For organic contaminants, this concentration ratio has been referred to as the "transpiration stream concentration factor (TSCF)" (*26*). Although xylem sap has been collected and analyzed for metals including Zn (*25*, *27*) and Cd (*25*, *28*) using methods basically the same as those described above, and rhizosphere pore water has been frequently analyzed for metals (*29*), albeit via a collection technique differing substantially from the MR method described above, these data represent the first direct comparison of heavy metal concentrations in these two aqueous compartments of the soil/plant system.

If xylem sap flow differs from one cultivar to the next, this might account for the variation in the tabulated BCF values. Sap flow (mL/h) versus root biomass (dry weight in grams) data were plotted (Figure 1, Supporting Information) and showed that sap flow varies in direct proportion to root biomass across the three cultivars. To support this statement, data were converted to the osmotically driven xylem sap flow, J_v, expressed in milliliters of sap per hour per gram of

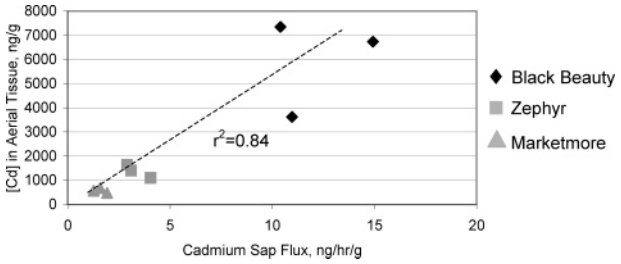

FIGURE 1. Correlation of cadmium concentration in aerial tissue with cadmium xylem sap flux.

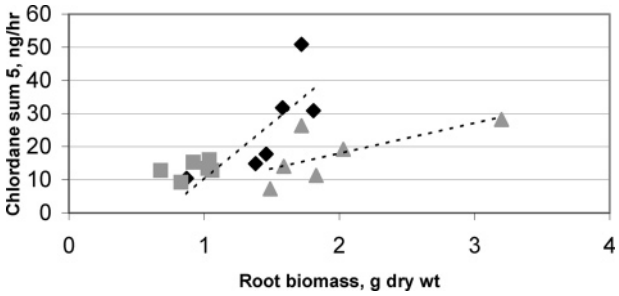

FIGURE 2. Chlordane flow in xylem sap (see text for definition of "sum 5") as function of root biomass. Linear regression lines shown. See Figure 1 for symbol key.

dry root biomass (*30*) and log-transformed (Table 2, Supporting Information). A one-way ANOVA of the log-transformed data indicated no significant difference in sap flow among the cultivars ($P > 0.05$). Thus, for the conditions and cultivars under which sap was collected, flow varies with root biomass, rather than other cultivar-dependent parameters.

Having established that sap flow among the three cultivars is not significantly different, we examined in planta movement of Cd, Pb, and Zn. The solute flux, J_s (*30*), of each element, defined in eq 2, was calculated,

$$J_s = \text{flow rate of element (mass per hour)} / \text{dry root biomass (g)} \quad (2)$$

and the elemental flux values were summarized (Table 3, Supporting Information). The elemental flux values for each cultivar were log-transformed, and one-way ANOVA was used to compare separately Cd, Pb, and Zn movement among the cultivars. For a given heavy metal, pairwise multiple comparisons using the Tukey test permitted cultivar differences to be identified (letters in parentheses following the sap concentrations, Table 1). In the case of lead, there is no statistical difference among the three cultivars for in planta flux ($P > 0.05$). However, for cadmium, there is a statistically significant difference ($P < 0.05$) in its xylem flux among the three cultivars, with Black Beauty > Zephyr > Marketmore. Finally, Black Beauty, and Zephyr sap fluxes for zinc are similar and significantly larger than in Marketmore. It should be noted from BCF values in Table 1, however, that although Black Beauty bioconcentrates ~15× more cadmium than Marketmore in its xylem sap, for zinc, it bioconcentrates only ~4× more.

From these data, we conclude that heavy metal movement is dependent on cultivar as well as on element. Although it has been assumed that for elements in the same group (Group IIB for Zn and Cd) in the periodic table, in planta movement was similar, our data are consistent with variations observed by Lombi et al. for transport of Cd and Zn in species of *Thlaspi* (*31*). Across the three Cucurbitaceae cultivars, Black Beauty appears to provide optimal in planta movement of all three heavy metals, followed closely by another *C. pepo*, Zephyr. It is of particular interest to note that in field trials conducted in Poland, it has been reported that *C. pepo* (field pumpkin) was the best accumulator of Cd, Pb, and Zn among the nine crops examined (*32*), data in agreement with the xylem sap data presented here.

Concentrations of the three heavy metals in the aerial tissues, the final compartment in the soil/plant continuum to be examined, are provided in Table 4, Supporting Information. What is notable through comparison of the Cd and Zn xylem sap flux data with the aerial tissue concentration data is that the flux and aerial tissue concentration are in the same order: Black Beauty > Zephyr > Marketmore. Furthermore, the statistical pairwise comparisons across the three cultivars are the same for Cd and Zn for the sap flux and the aerial tissue concentration. Figure 1 presents graphically the correlation between flux and aerial tissue concentration for cadmium. Mathematically, the correlation between the two variables is highly significant ($P < 0.001$). Comparison of Pb xylem sap flux and aerial tissue concentration data shows similar trends, although the agreement is not so consistent as for Cd and Zn. Thus, the data provide explicit support for the assumption that Cd, Zn, and Pb contamination in the aerial tissues derives from the movement of metals in the xylem sap.

Movement of Weathered, Hydrophobic Organic Compounds through the Soil/Plant System. We now turn our attention to the three classes of weathered organic contaminants detected above typical background levels in the soil used in these trials. Tables 2 and 3 summarize the pollutant concentrations in various compartments, the BCFs, and the (+) enantiomeric fractions, defined in eq 3, for several chiral chlordane components (*33*),

$$\mathrm{EF}_+ = \frac{C_+}{(C_+ + C_-)} \quad (3)$$

where C is the concentration of the indicated enantiomer. If elution order of the (+) and (−) enantiomers cannot be specified, EF is computed on the basis of the first eluting enantiomer; thus, for MC-5 enantiomers, EF is specified for the MC-5a enantiomer.

The discussion is best continued by examining separately the movement through the soil/plant system of each class of compounds.

Technical Chlordane Residues. We have established the persistence in this particular soil of the technical chlordane components specified at the top of Table 2, and the "SUM 5" ([+TC] + [−TC] + [−CC] + [+CC] + TN) as a reasonable surrogate for total weathered technical chlordane (*22*). With these data, we may calculate for the first time BCF values (xylem sap section, Table 2) on the basis of sap versus rhizosphere pore water concentrations for the specified enantiomers and achiral residues. Although TSCFs for 10 pesticides have been determined previously using soybeans (*34*), the experiments were conducted in hydroponic solution with pesticides, most with log $K_{ow} < 3$. This contrasts with the soil-sequestered and highly hydrophobic pesticides in the present study.

Since all sap collections were timed, Figure 2 was prepared from the SUM 5 chlordane flow rates; the graphed data imply differences in the chlordane fluxes among cultivars. From the discussion in the preceding section, we know that variation in the chlordane BCFs (Table 2) and the hourly chlordane flows (Figure 2) are not attributable to cultivar differences in sap flow rates. The trend in SUM 5 BCFs and in the hourly chlordane flows, that is, Black Beauty ~ Zephyr > Marketmore, may be examined more closely by converting

the SUM 5 sap flow to flux (mass per hour per gram dry root biomass) (see Table 5, Supporting Information). One-way ANOVA on the log-transformed fluxes shows that there is a statistically significant difference in the SUM 5 fluxes between Black Beauty and Marketmore, between Zephyr and Marketmore, but not between Black Beauty and Zephyr (letters in parentheses in the xylem sap section, Table 2). Both subspecies of *C. pepo*, Black Beauty and Zephyr, exceed *C. sativis* in the movement of chlordanes in planta.

Resolution of enantiomeric pairs for chiral chlordane components permitted us to examine "fine structure" in the movement of the chlordane residues, which might be obscured in the SUM 5 data. For each component, the flux (mass per hour per gram dry root biomass) was log-transformed, and the flux values among cultivars was compared using one-way ANOVA (Table 5, Supporting Information). Within each component column, Tukey pairwise comparisons are indicated in Table 2 next to the xylem sap concentration values. For most of the chlordane components, the pairwise comparisons show that movement in Black Beauty and Zephyr is similar and different from Marketmore. The notable exceptions are the achiral TN component and the MC-5 components. In the case of achiral TN, in planta movement of the component across all three cultivars is similar. In the case of the MC-5 enantiomers, in planta movement of each enantiomer is significantly different from one cultivar to the next. In particular the MC-5 concentration in the sap of Black Beauty is remarkably nonracemic ($EF_a = 0.29$), with preferential accumulation of the different enantiomer (MC-5b) than is accumulated in both Zephyr and Marketmore (MC-5a). From the structures of TC, CC, MC-5, and TN (*35*), we note that for the fully symmetrical TN, no in planta distinction is apparent among the three cultivars. However, for the MC-5 structure, which has fewer symmetry elements than TC or CC, the enantioselectivity is most extreme, with a remarkable BCF of 68 for the MC-5b enantiomer in Black Beauty.

Finally, in Table 2, data for chlordane concentrations found in the aerial tissue are provided. In our previous reports, we have shown that Cucurbitaceae are impacted by air-to-plant contaminant uptake as well as soil-to-plant uptake. A straightforward correlation between xylem sap flux and aerial tissue contamination, as demonstrated above for cadmium, is, therefore, more elusive; however, the EF values may elucidate contributions to aerial tissue concentration from sap versus air pathways. Equation 4 may be rearranged to solve for *P*, the proportional contribution of the sap to the final EF in the aerial tissue (*33*).

$$EF_{aerial} = EF_{sap}P + EF_{air}(1 - P) \quad (4)$$

$$P = (EF_{aerial} - EF_{air})/(EF_{sap} - EF_{air}) \quad (5)$$

Using eq 5, 12 *P* values were calculated, namely, one for each optically active chlordane component in each cultivar. Only values of $P \leq 1$ are reasonable, according to eq 4. Of the 12 possible *P* values, two *P* values distinctly met this criterion: $P = 0.95$ for MC-5 in Black Beauty and $P = 1.0$ for exo-HEPX, also in Black Beauty. A review of Table 2 shows that all EF values of the chlordane components in the soil are reflected in the air EF values; however, the enantiomeric profile of the MC-5 and exo-HEPX components in the xylem sap of Black Beauty change by −43% and +12%, respectively, from their values in either soil or air. Most notably, for both MC-5 and exo-HEPX, the EF in the sap is preserved in the aerial tissue. A review of the data presented here supports the conclusion that *C. pepo* spp. *pepo* is not only exceptional among terrestrial plants examined to date, but also among members of the Cucurbitaceae family, as well, with regard to quantitative and qualitative in planta movement of hydrophobic organic contaminants.

Dichlorodiphenyl Compounds. In previous field studies with a variety of Cucurbitaceae grown in DDE contaminated soil (*12*), *C. pepo* Zephyr was operationally classified as a "nonaccumulator" and *C. pepo* Black Beauty was adjudged an "accumulator". For analytical reasons, we focus on the "SUM DDs" (DDT + *o*,*p*′-DDE + *p*,*p*′-DDE + DDD). From sap concentrations and BCF values (Table 3), which are generally consistent with field studies, we see that in planta movement of the DDs is in the order Black Beauty > Zephyr > Marketmore. From the ANOVA of the log-transformed SUM DDs flux values (Table 6, Supporting Information), it is evident that the flux in Black Beauty is significantly larger than that in either Zephyr or Marketmore. What is most interesting, however, is the comparison of data in Table 3 with that in Table 2. From this comparison, we learn (1) on the basis of BCF values for both chlordane (BCF range, 3.6−68.4) and DD (BCF 19.4) components, Black Beauty is an accumulator; (2) Marketmore can be termed a nonaccumulator of DDs (BCF < 1) and the poorest accumulator of chlordanes (BCF range 2.1−27.8); (3) Zephyr is a modest accumulator of the DDs (BCF 3.8), although for many chlordane components its BCF (BCF range 5.4−48.2) is equal to or larger than the BCF of Black Beauty. We have pointed out in the previous section that the data in Table 2 support enantioselective dependence of in planta movement of the chlordane components. From these additional observations, it is evident that the data in Tables 2 and 3 also support compound-specific in planta movement. In other words, a cultivar (e.g., Zephyr) may move chlordanes well, but DDs poorly, in its xylem sap. The conclusion must be reached that generic models of pollutant uptake in the soil/plant system may be unrealistically simplistic (*36*).

Without the optical handle that some chlordane components provide, assessing the impact of the sap on the final aerial tissue concentration of the dichlorodiphenyl compounds is problematic with the available data.

Polyaromatic Hydrocarbons. Four PAHs were present in the contaminated soil at levels which were readily quantifiable (Table 3). Determination of PAH concentration in the rhizosphere soil pore water and the xylem sap (Table 3) reveals additional patterns of in planta movement among the three cultivars. Two of the PAH compounds, phenanthrene and anthracene, which are three ring (C_{14}) structures, have BCF (Table 3) and flux values (Table 6, Supporting Information) in the same order as the pattern noted previously across the three cultivars for chlordanes and DDs, namely, Black Beauty > Zephyr > Marketmore. The sap flow as a function of root biomass for phenanthrene is shown in Figure 3A of the Supporting Information. ANOVA of the log-transformed flux values is not significantly different for the three cultivars.

The second two PAHs in this study, fluoranthene and pyrene, are four-ringed, C_{16} compounds. In this case, both the BCF values (Table 3) and the sap flow (Figure 3B, Supporting Information) fall into a pattern not previously observed: Black Beauty and Marketmore are both better at moving these two PAHs than Zephyr, and in fact, the fluxes for the two former cultivars are similar and significantly higher than that of the latter (Table 6, Supporting Information). This is the first report for which Marketmore uptakes and moves in planta a weathered POP at levels equal to that of Black Beauty.

As for the dichlorodiphenyl compounds, the data for the PAHs do not permit conclusions regarding the impact of xylem sap concentration on aerial tissue concentrations.

Comparison of Empirical and Theoretical BCF Values. BCF values derived empirically from these rhizotron experiments for the hydrophobic organic chemicals studied may

be compared with those predicted from uptake models. Specifically, Briggs has derived eq 6,

$$\mathrm{TSCF} = C_{\mathrm{xylemsap}}/C_{\mathrm{soilwater}} = 0.784 \times \exp\left\{\frac{-(\log K_{\mathrm{ow}} - 1.78)^2}{2.44}\right\} \quad (6)$$

to describe movement of organic chemicals from the soil solution into herbaceous plants (*26*). Using chlordane as typical of hydrophobic contaminants and the published values of K_{ow} for chlordane (*37*) in eq 6, TSCF is determined to be 2.6×10^{-4}, orders of magnitude smaller than the empirical values in Table 2. It must be deduced that the plant used for the development of the uptake model behaves very differently from the Cucurbitaceae cultivars examined in the present experiments.

Among terrestrial plants examined to date, *C. pepo* appears to maximize uptake of soil-sequestered, organic, and elemental pollutants. This has now been shown for contaminated soils of different type and organic matter content (8.1% and <2%, ref *10*; 1.2%, ref *32*; 6.5%, present experiments). One intriguing observation which has been published regarding cadmium is the evidence that chloro complexes can be absorbed by the plant root, in addition to uncomplexed, free metal ion species. Neutral $CdCl_2$ is one such chloro complex (*38*). Although the data do not permit conclusive support for an uptake mechanism, it is interesting to speculate on a role for transmembrane channel proteins, for example, aquaporins and glyceroaquaporins, in this process. To date, these channels are known to be specific for passage of neutral species into the plant interior. $CdCl_2$ fits the criterion of neutrality.

For organic contaminants, data from the present set of experiments point to enantioselective and compound-selective in planta movement. For aquaporin transmembrane channel proteins, the central channel is known to be sided (*39*), having an hourglass shape. When the three-dimensionality of the channel is considered, stereoselective preference of certain neutral organic species may result. Mathematically we note a statistically significant correlation between Cd xylem sap flux and xylem sap flux of the chiral MC-5 enantiomers, but no correlation between Cd xylem sap flux and xylem sap flux of the achiral TN component (Figure 4, Supporting Information). It remains to be determined if this intriguing mathematical correlation relates to a mechanistic one, as well.

Supporting Information Available

Tables supplying limits of detection, plant biomass, in planta fluxes of chlordanes, DDs, and PAHs, and graphs of correlation of chlordane and Cd fluxes. This material is available free of charge via the Internet at http://pubs.acs.org.

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