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Subspecies-Level Variation in the Phytoextraction of Weathered *p,p′*-DDE by *Cucurbita pepo*

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Previous studies indicate that *Cucurbita pepo* can phytoextract highly weathered persistent organic pollutants (POPs) from soil and translocate large quantities to aerial tissues. To investigate intraspecific variability in uptake potential, a field study was conducted to quantify the phytoextraction of weathered *p,p′*-DDE by 21 cultivar varieties of summer squash from two distinct subspecies, *C. pepo* ssp *texana* and *C. pepo* ssp *pepo*. Significant differences exist between the two subspecies, with average root and stem to soil bioconcentration factors (BCF, dry weight ratio of contaminant concentration in the vegetation to that in the soil) of 7.22 and 5.40 for ssp *pepo* and of 2.37 and 0.454 for spp *texana*, respectively. The amounts of weathered *p,p*-DDE extracted from the soil by ssp *pepo* and ssp *texana* were 0.301 and 0.065%, respectively, with maximum values within each subspecies of 0.780 and 0.182%, respectively. The quantities of 14 inorganic elements were determined in both the soil and tissues (roots, stems, leaves, and fruit) of all 21 cultivar varieties. Phosphorus concentrations in the tissues of ssp *pepo* were 14 (fruit)-73% (stems) greater than those of ssp *texana*. These data support our hypothesis that the unique ability of certain cultivars of *C. pepo* to phytoextract highly weathered POPs from soil is the result of low molecular weight organic acid exudation as a unique phosphorus acquisition mechanism.

Introduction

Persistent organic pollutants (POPs) are problematic given their recalcitrance in natural systems, potential for bioaccumulation/biomagnification, toxicity, and global transport (*1, 2*). Although there is a wealth of information demonstrating that low contaminant availability/mobility is a result of time-dependent sequestration or weathering of POPs (*3, 4*), studies tracking the global distribution of these contaminants are abundant (*5*−*7*). Despite the assumed low availability of these pollutants, recent data indicates that under a variety of circumstances these compounds may enter biota (*8*−*15*). The majority of these studies are unable to definitively link contaminant presence to detrimental effects on organisms or populations, but the global distribution of POPs in both natural media and the biota therein is clearly an issue of significant concern.

Phytoremediation is the in situ application of vegetation to remediate contaminated natural solids and as a technology has received much attention due to its low cost, nonintrusiveness, and success under specific conditions (*16*). Both mechanistically and practically, the field of phytoremediation is divided on the basis of contaminant type. The phytoremediation of inorganic pollutants, largely heavy metals, is a plant-mediated contaminant extraction from soil and sequestration of the pollutant within vegetative tissues (*17*). There are numerous studies utilizing a variety of plant species in the phytoremediation of heavy metals, the most common contaminants cited being Cu, Zn, Pb, Ni, Cd, Se, and As (*17*−*21*). Conversely, the phytoremediation of organic pollutants includes several potential mechanisms. Often, the organic contaminant is degraded outside the plant in the rhizosphere, either by exuded plant enzymes or through the activity of the enhanced microbial community present on and around root tissues (*22*). This type of *ex planta* degradation has been described for PAHs (*23, 24*), chlorinated solvents (*25, 26*), and certain herbicides (*27, 28*). Alternatively, certain hydrophilic organic compounds such as TNT (*29*), atrazine (*30*), and carbon tetrachloride (*31*) may flow with water into the plant tissues, where the contaminants may be degraded or transpired. It has been assumed that organic contaminants with a high $\log K_{ow}$ (octanol water partitition coefficient > 3.5) would be bound too tightly to the soil organic matter for uptake into plants (*22*) and that, taken with the inherent resistance to enzymatic attack, has led to the assumption that POP-contaminated soils would not be amenable to plant-based remediation.

Nearly a decade ago, data indicated that some species of plants, specifically *Cucurbita pepo*, may have a unique ability to remove significant quantities of highly weathered persistent organic pollutants from soil and recent studies support those earlier findings (*32*−*35*). Given that the contaminants become sequestered in vegetative tissues, the removal process is more analogous to the phytoextraction of heavy metals rather than to processes associated with organic pollutants. Initial field studies indicate significant variability in contaminant phytoextraction among different cultivar varieties of *C. pepo*. Although in the same species, White (*34*) showed that while single cultivars of summer squash and pumpkin were effective at accumulating weathered *p,p′*-DDE from soil, two cultivars of winter squash were ineffective. The current study was designed to assess the range in uptake potential of both highly weathered *p,p′*-DDE and inorganic elements by 21 cultivars of summer squash within two subspecies of *Cucurbita pepo*.

Materials and Methods

Field Plot. Twenty-one cultivar varieties of summer squash (*Cucurbita pepo*) were purchased from Johnny's Selected Seeds (Albion, ME), Seedway (Hall, NY), or Gurneys Seed and Nursery Company (Yankton, SD) (Table 1). Taxonomically, two distinct subspecies of domesticated *C. pepo* exist; ssp. *pepo* includes the "true" zucchini and many pumpkins, and ssp. *texana* includes summer squash known as straight-

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TABLE 1. Cultivar Varieties of *Cucurbita pepo* Grown at Lockwood Farm (Hamden, CT) and % of *p,p′*-DDE Phytoextracted in a Single Growing Season

cultivar variety	ssp.	fruit type	seed source	% DDE phytoextracted[a]
"Black zucchini"	*pepo*	zucchini	Gurneys	0.184
"Black magic"	*pepo*	zucchini	Gurneys	0.204
"Black beauty"	*pepo*	zucchini	Seedway	0.078
"Butter scallop"	*texana*	patty pan	Johnnys	0.084
"Costata romanesco"	*pepo*	zucchini	Johnnys	0.284
"Early prolific"	*texana*	straightneck	Gurneys	0.026
"Gurneys pride"	*pepo*	zucchini	Gurneys	0.449
"Goldrush"	*pepo*	zucchini	Johnnys	0.780
"Hybrid butterstick"	*texana*	straightneck	Gurneys	0.145
"Hybrid crescent"	*texana*	crookneck	Gurneys	0.023
"Hybrid jackpot"	*pepo*	zucchini	Gurneys	0.129
"Magda"	*pepo*	zucchini	Johnnys	0.263
"Patty green"	*texana*	patty pan	Johnnys	0.024
"Raven"	*pepo*	zucchini	Johnnys	0.454
"Revenue"	*pepo*	zucchini	Johnnys	0.236
"Seneca"	*texana*	straightneck	Johnnys	0.028
"Starship"	*texana*	patty pan	Johnnys	0.039
"Sunburst"	*texana*	patty pan	Johnnys	0.027
"Sunray"	*texana*	crookneck	Johnnys	0.133
"Yellow crook"	*texana*	crookneck	Johnnys	0.115
"Zephyr"	*texana*	straightneck	Johnnys	0.076

[a] Sum of total *p,p′*-DDE in vegetative tissues expressed as a percentage of contaminant present in 270 kg of soil (estimate of soil volume accessed by roots).

necks, crooknecks, patty pans, and the winter squash (*36*). Experimental field plots were constructed at Lockwood Farm (Hamden, CT) in areas previously shown to contain *p,p′*-DDE at levels ranging from 200 to 1200 ng/g (dry weight). Precise records are not available, but the area received DDT applications for decades. No DDT or DDD has been detected within the field site (detection limit approximately 1 ppb). The soil is 1.4% organic carbon and is designated as a fine sandy loam (56% sand, 36% silt, 8% clay; pH 6.7). The entire plot was covered with 1370 m^2 of polyethylene black plastic, and 30 cm^2 blocks were cut out of the plastic at 2.0-m intervals. Approximately 50 seeds of each cultivar variety were placed between two sheets of moistened germination paper and kept at room temperature for 3 days. In early June 2002, seedlings were planted in mounds of soil within each of the openings in the black plastic; there were adjacent duplicate mounds for each cultivar variety with each mound containing eight seedlings. The mounds were later thinned to 3−5 plants. The vegetation was weeded and watered as necessary. Fruit were harvested and weighed from individual plants as market-size (at least 200 g, wet weight) was achieved. Destructive harvest of the plot began on August 5, 2002.

Soil Extraction. Eight soil cores were collected from the top 15-cm of replicate mounds (four cores per individual mound) prior to planting and were stored at room temperature in 250-mL amber glass bottles sealed with Teflon-lined screw caps. All soils were air-dried and sieved to 0.5 mm for removal of nonsoil debris and to facilitate homogeneous sampling. A representative 3.0-g portion of each sample was placed in an oven at 100 °C for 24 h for moisture determination. Portions (3.0-g) of the soils from each mound were transferred to 40-mL amber vials and were amended with 15 mL of methanol and 1000 ng of transnonachlor as an internal standard. The vials were sealed with Teflon-lined caps and placed in an oven at 70 °C for 5 h. The samples were removed from the oven and cooled for 10 min, and a 1-mL aliquot of the supernatant was passed through a glass microfiber filter (0.2 μm, Laboratory Science Inc., Sparks, NV) for particulate removal prior to analysis.

Vegetative Extraction. For all replicate mounds of vegetation, the total weight of each tissue compartment (roots, stems, leaves, fruit) was determined in the field, and samples of each tissue were brought back to the laboratory for extraction. In preparation for analysis, the vegetation was thoroughly rinsed with tap water to remove soil particles. All vegetation was finely chopped and either extracted immediately or stored in 250-mL amber glass bottles with Teflon-lined caps in a freezer. The method used for extraction of *p,p′*-DDE residues from various portions of the vegetation was that of Pylypiw (*37*). Twenty-five-gram portions of the vegetation were weighed into a one-quart blender container with 25-mL of 2-propanol (Ultra-Resi-Analyzed, J. T. Baker, Phillipsburg, NJ) and 3000 ng of transnonachlor as an internal standard. The sample was blended at low speed for 30 s in an explosion-proof blender (Fisher Scientific, Springfield, NJ). Fifty milliliters of petroleum ether (Ultra-Resi-Analyzed, J. T. Baker, Phillipsburg, NJ) was added to each container, and the sample was blended for 5 min. After settling for 30 s, the sample was decanted into a funnel packed with glass wool, and the extract was collected in a 500-mL glass separatory funnel with a Teflon stopcock. After draining of the solids (20 min), 100 mL of distilled water and 10 mL of saturated sodium sulfate solution were added to each funnel. The funnel was capped, shaken gently for 5 s, and allowed to sit for 20 min to enable phase separation. The water was then drawn off, and the ether layer was rinsed 3 additional times with distilled water. The final extract (30−40 mL) was transferred to a 100-mL graduated cylinder containing 10 g of anhydrous sodium sulfate and was allowed to sit for 3 h prior to GC analysis.

Analysis and Quantitation. The amount of *p,p′*-DDE in the methanol soil extracts and the petroleum ether vegetative extracts was determined on a Hewlett-Packard (Hewlett-Packard Co., Avondale, PA) 5890 gas chromatograph (GC) with a ^{63}Ni electron capture detector (ECD). The column was a 30 m × 0.53 mm 0.5 μm SPB-1 film (Supelco, Inc., Bellefonte, PA). The GC program was as follows: 175 °C initial temperature; ramped at 1 °C/min to 205 °C; then ramped at 15 °C/min to 250 °C; with a hold time of 5 min. The total run time was 38 min. A 2 μL splitless injection was used, and the injection port was maintained at 250 °C. The carrier gas was He, and the makeup gas was 5% CH_4 in Ar at 20 mL/min. The ECD was maintained at 325 °C.

A stock standard of crystalline *p,p′*-DDE was purchased from Chem Service (West Chester, PA). A portion of the stock was dissolved in either petroleum ether (for vegetative extracts) or methanol (for soil extracts) and diluted to prepare a series of calibration standards of *p,p′*-DDE at 10, 25, 50, 100, 150, 250, and 500 ng/mL for each solvent. Each calibration level contained 100 ng/mL transnonachlor. The retention times of transnonachlor and *p,p′*-DDE were 16.5 and 18.4 min, respectively.

All reported concentration values of *p,p′*-DDE are expressed on a dry-weight basis of either soil or vegetation as determined by weight change of the sample after at least 24 h at 100 °C. A total of seven replicate soil samples were extracted from each duplicate mound of vegetation. Tissue compartments (roots, stems, leaves, fruit) from individual replicate mounds were extracted in duplicate; data from individual replicate mounds were composited to give average *p,p′*-DDE concentrations for each cultivar variety. Statistical differences were determined utilizing an unpaired student's *t*-test, and the corresponding *p* values are reported in the text. Recovery of the internal standard was 95% (± 4.0).

Inorganic Elements. Individual tissue samples (roots, stems, leaves, fruit) from each cultivar variety and representative soil samples from the experimental plot were extracted to quantify concentrations of 14 inorganic elements (Ni, Cd, Cu, Mn, Pb, Zn, Al, Ca, Fe, K, Mg, S, P, As). All samples were dried at 95 °C for 24 h. Soils (1.0 g) and vegetation (0.5 g) were digested with 10 mL of concentrated HNO_3 on a hot plate for 30 min. All inorganic element concentrations (except As and Cd) were determined by Inductively Coupled Plasma Emission Spectroscopy (ICP−OES) using the Atom Scan 16 (Thermo-Jarrell Ash, Franklin, MA). The digests were also analyzed for As and Cd with a PE 5100PC graphite furnace atomic absorption spectrometer (GFAAS) (Perkin-Elmer Corp., Wellsley, MA).

Results and Discussion

Soil Analysis. Seven replicate bulk soil samples were extracted for each duplicate mound of soil, and the contaminant concentrations were similar to those reported in previous studies involving this site, ranging from 296 ("Revenue" soil) to 1130 ng/g *p,p′*-DDE ("Black magic" soil).

Vegetation—Uptake and Translocation of *p,p′*-DDE. Measurable quantities of *p,p′*-DDE were detected in the roots, stems, leaves, and fruit of all 21 cultivars of *C. pepo*. With the exception of "Magda" (ssp. *pepo*), the *p,p′*-DDE concentration was highest in the roots and declined through the aerial tissues (stem, leaf, fruit). Within a specific tissue, the concentration of *p,p′*-DDE often varied by well over an order of magnitude among the 21 different cultivars. For example, the *p,p′*-DDE concentration in the roots ranged from 250 to 9240 ng/g and in the stems, 12.7 to 4970 ng/g. Some of the observed variability in *p,p′*-DDE content among the 21 cultivars may be due to differences in the *p,p′*-DDE concentrations in the soil. To eliminate this confounding factor and to allow a direct comparison of the cultivars abilities to bioaccumulate weathered *p,p′*-DDE, bioconcentration factors or BCFs, defined as the ratio of *p,p′*-DDE concentration in the tissue (dry weight) divided by that in the soil, were calculated for each tissue of each cultivar. Root, stem, leaf, and fruit BCFs are shown in Figure 1(a−d). Regardless of variable soil *p,p′*-DDE concentration, considerable differences exist in the contaminant content within cultivar tissues. The minimum and maximum BCFs are 0.575 ("Patty green") and 10.8 ("Goldrush") in the roots, respectively, and 0.040 ("Patty green") and 9.82 ("Goldrush") in the stems, respectively. Interestingly, "Magda" has a stem BCF (9.72) nearly twice that of the root value (5.12). Similarly, the minimum and maximum BCFs range from 0.042 ("Starship") to 1.72 in the leaves ("Raven"), respectively, and from 0.010 ("Yellow crook") to 0.413 ("Gurneys pride") in the fruit, respectively. Use of BCFs assumes that a linear relationship exists between contaminant concentration and release from soil. Although there is evidence in the literature indicating a nonlinear relationship between pollutant concentration and desorption (*38*−*40*), the assumption of linearity in the current study is appropriate given the narrow range of *p,p′*-DDE concentration observed in the soil (<1 order of magnitude), the fact that the *p,p′*-DDE has been weathered for decades, that the labile fraction no longer exists, and that the plot was randomly designed.

Although Lichtenstein (*41*) and Lichtenstein et al. (*42*) described the uptake of chlorinated insecticides now designated as POPs into the roots of various crop species, the contaminants had generally been present in the soil for less than 5 years. The potential uniqueness of *Cucurbita pepo* to accumulate highly weathered POPs through a soil-to-plant route was first noted by Hülster et al. (*32*), who described significant uptake and translocation of weathered PCDD/PCDFs by zucchini and pumpkin. However, the minimal contaminant concentrations in the tissues of a plant in the same family (*Cucurbitaceae*), cucumber (*Cucumis sativa*), were attributed to aerial deposition. In this study, the experimental plot was covered with polyethylene to minimize the soil-air-plant transfer of *p,p′*-DDE. Given that certain cultivars here and certain species in other studies (*33*, *34*) had very low concentrations of contaminant in their tissues, the contribution of aerial deposition is assumed to be negligible for plants containing significant quantities of *p,p′*-DDE. Similar family- and genera-level variability in the uptake and translocation of weathered chlordane and *p,p′*-DDE from soil has been described, again with *Cucurbita pepo* (zucchini or pumpkin) consistently extracting significantly greater quantities of contaminant than other plants (*33*, *35*, *43*). White (*34*) did observe that two cultivars of ssp *pepo* had average root *p,p′*-DDE BCFs of 12.6, whereas two cultivars ssp *texana* had a value of 2.71. Similar variability in contaminant concentration was also observed in the aerial tissues, but the significance of the taxonomic dichotomy was not realized at the time.

Although the roots often have the highest *p,p′*-DDE concentration, the harvested biomass of this tissue compartment is low, ranging from 0.85 to 3.6% of the total dry weight of a given plant. From a practical standpoint, it is likely that the destructive harvest may exclude some portion of the root system, but the majority of the root biomass occurs close to the stem and is collected for analysis. For the 21 cultivars assayed, either the leaf or fruit compartment had the greatest biomass, and in calculating the absolute quantity of *p,p′*-DDE per tissue, the stems or leaves generally were the greatest sink for the contaminant. Across all 21 cultivars, 83.0% of the extracted *p,p′*-DDE was in the shoot system. Given the dimensions of soil excavated at destructive harvest (1.1 m × 1.1 m × 0.23 m) and a measured soil density of 1.04, we have previously estimated a soil compartment mass of 270 kg for *C. pepo*. With the weight of and *p,p′*-DDE concentration in each tissue compartment as well as the concentration of contaminant in the defined mass of soil, one can estimate the relative amount contaminant remediated from the soil. The percent of *p,p′*-DDE phytoextracted by the 21 cultivar varieties of *C. pepo* are shown in Table 1. It is evident that similar subspecies variability exists in the percentage of weathered contaminant extracted from the soil, ranging from 0.023% ("Hybrid crescent") to 0.780% ("Goldrush").

The biomass values and intraplant contaminant distribution reported here are similar to our previous findings for the uptake and translocation of weathered chlordane and *p,p′*-DDE by *C. pepo* (*34*, *44*). In addition, the percent of contaminant phytoextracted also fits within the range previously reported. White (*34*) reported 0.40 and 2.4% *p,p′*-

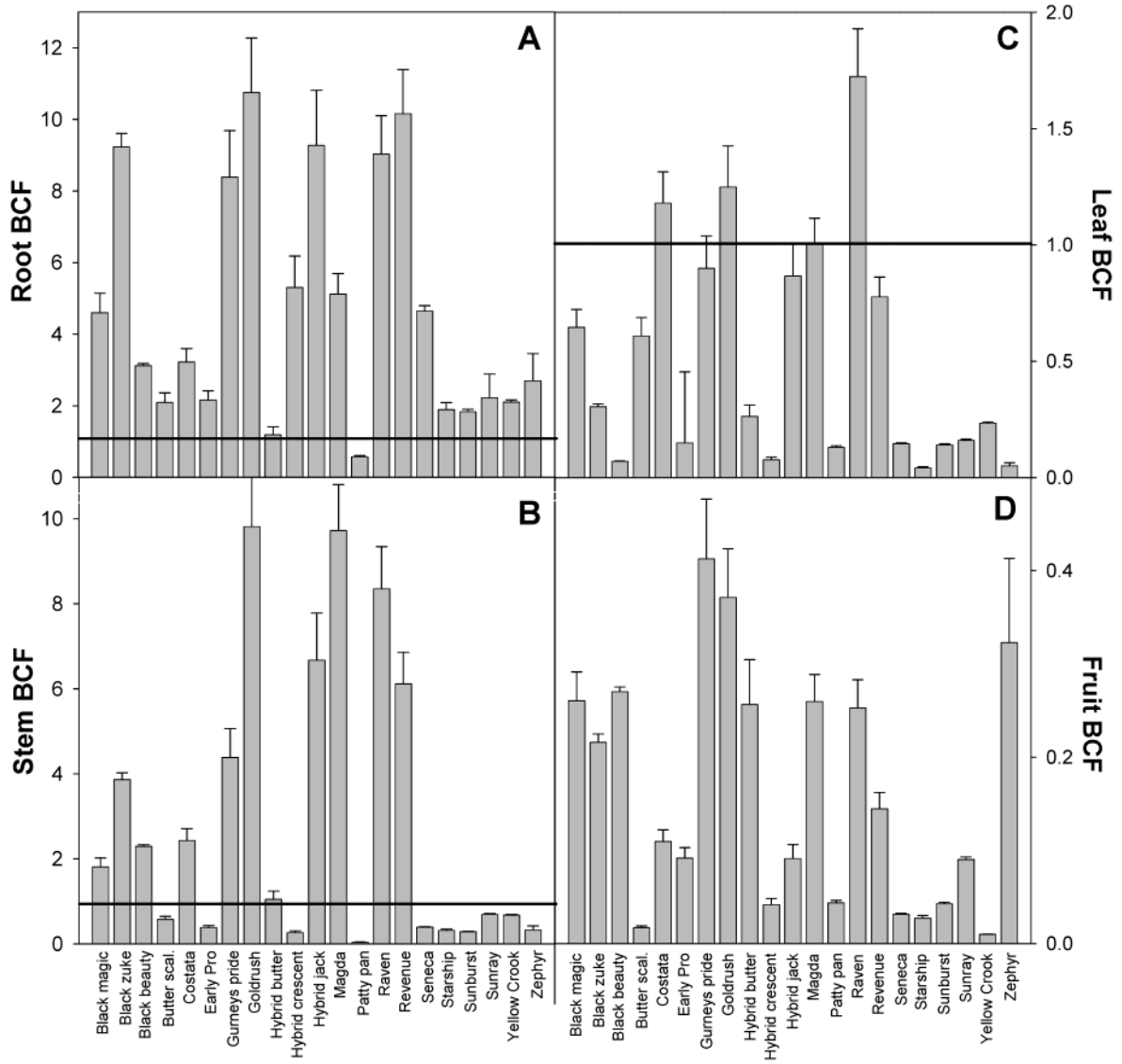

FIGURE 1. Bioconcentration factors (dry-weight ratio of *p,p′*-DDE concentration in the vegetation to that in the soil) for roots (A), stems (B), leaves (C), and fruit (D) of 21 cultivar varieties of *Cucurbita pepo*. Each bar is the average of four values; errors bars represent standard deviation. Bold horizontal line indicates BCF of 1.0.

DDE removal by a zucchini and pumpkin (*C. pepo* ssp *pepo*), whereas winter squash values (*C. pepo* ssp *texana*) were 0.14%. Other genera of plants in the same family, specifically cucumber (*Cucumis sativus*) and melon (*Cucumis melo*), had phytoextraction values of 0.03%. Lee et al. (*44*) also reported similar values for a single cultivar of *C. pepo* ssp *pepo* and weathered chlordane. To our knowledge, these are the only data in the literature reporting findings of percent-level removal of weathered POPs by vegetation. Given that POPs extraction and removal from soil is analogous to heavy metals, it is appropriate to draw some comparisons between the two types of contaminants. For example, Lasat et al. (*45*) describes a 3% removal of radiocesium in a single cropping, with a projected site remediation of 15 years. Alternatively, Brown et al. (*46*) and Lombi et al. (*47*) report Zn and Cd phytoextraction by *Thlaspi caerulescens* at 0.1−2.0% and 1.3−6.3%, respectively. Conversely, Tu et al. (*48*) observed 26% removal of As by brake fern (*Pteris vittata*) in a single growing season. Excluding the extraordinary behavior of *P. vittata*, it is evident that the amount of POPs removed by *C. pepo* ssp *pepo* falls within the range of that observed in heavy metal phytoremediation, and investigations are currently underway to assess the practical implications of these findings.

TABLE 2. Differential Uptake and Translocation of Weathered *p,p′*-DDE by *Cucurbita pepo* ssp *pepo* and *texana*

index	ssp *pepo*	ssp *texana*
average root BCF	7.22 A[a]	2.37 B
average stem BCF	5.40 A	0.454 B
average leaf BCF	0.878 A	0.139 B
average fruit BCF	0.283 A	0.089 B[b]
average stem BCF/root BCF	0.748 A	0.192 B[b]
average % phytoextracted	0.301 A	0.065 B

[a] Across subspecies, values followed by different letters are significantly different ($p = 0.001$). [b] Values significantly different at $p = 0.01$.

In analyzing the BCFs and contaminant extraction data, different patterns of behavior are evident between the two subspecies. Table 2 shows that several indices of contaminant uptake and translocation are significantly greater for ssp *pepo* than for ssp *texana*. Our previous studies focused on a narrow range of cultivars within ssp *pepo* and have consistently reported significant phytoextraction of weathered chlordane (*44*, *49*) and *p,p′*-DDE (*33*, *34*). As described above, White (*34*) did show different uptake potentials of *p,p′*-DDE for a

TABLE 3. BCFs for Inorganic Elements in Vegetative Tissues of Two Subspecies (ssp) of *Cucurbita pepo*

tissue	ssp	Cd	Cu	Mn	Pb	Zn	Al	Ca	Fe	K	Mg	S	P	As
root	*pepo*	4.30 A[a]	1.38	0.275	0.522 A	1.22	0.026	6.92	0.019	35.6	1.32	14.4	14.8 A	0.121 A
	texana	3.66 B	1.33	0.259	0.723 B	1.18	0.026	6.82	0.018	33.0	1.43	15.7	10.8 B	0.174 B
stem	*pepo*	2.84	1.20 A	0.276 A	nd	1.29 A	0.005	9.84 A	0.008	54.1	2.21 A	23.3 A	23.5 A	nd
	texana	2.63	0.852 B	0.238 B	nd	0.869 B	0.005	8.11 B	0.005	47.3	1.78 B	14.5 B	13.6 B	nd
leaf	*pepo*	11.6 A	0.978 A	0.940	nd	2.23 A	0.007 A	36.8	0.014	55.2	2.19	15.8 A	15.7A	nd
	texana	5.33 B	0.866 B	0.957	nd	1.61 B	0.011 B	36.9	0.019	55.3	2.17	13.4 B	13.2 B	nd
fruit	*pepo*	1.32	1.55 A	0.165	nd	1.71 A	0.002	4.86	0.011 A	76.8 A	1.28 A	16.0	31.8 A	nd
	texana	1.05	1.89 B	0.144	nd	1.41 B	0.001	4.46	0.010 B	66.7 B	1.46 B	15.8	27.8 B	nd

[a] Within a tissue type and across subspecies, values followed by different letters are significantly different ($p < 0.05$)

small number of cultivars of ssp *texana* and *pepo*. We have speculated that these differential patterns of POP uptake and translocation are the result of variable patterns of root exudation (*34*, *44*, *50*). Subspecies-level variation in both root exudate quantity and composition has been observed. Wassman and Aulakh (*51*) observed significant variation among rice cultivars in the quantity of exuded carbohydrates and amino and organic acids. Both Wu et al. (*52*) and Fan et al. (*53*) reported similar differences in the exudate profiles among wheat cultivars. Comprehensive mass spectrometry-based investigations are currently underway to qualitatively and quantitatively characterize the root exudates of *C. pepo* cultivars under a range of cultivation and nutrient regimes.

Vegetation—Inorganic Elements. The tissues of all cultivars were digested, and the quantities of 14 inorganic elements were determined. The concentration of each element (mg/kg) was converted to a BCF by expressing each tissue relative to the concentration of the element in the soil. Table 3 shows the root, stem, leaf, and fruit BCF averages for the two subspecies. Although the average biomass of the two subspecies was not significantly different, ssp *pepo* was significantly more effective at concentrating several inorganic elements from soil into its tissues. The high BCF for Cd in the leaves of ssp *pepo* is noteworthy in that it is double that of *texana* leaves and nearly three times that of any of the other tissues within ssp *pepo*. One cultivar of ssp *pepo*, "Costata romanesco", was of particular interest with Cd leaf BCF of 38. In two soils containing 19 and 42 mg/kg Cd, Lombi et al. (*47*) found shoot Cd BCFs of *Thlaspi caerulescens*, a well characterized hyperaccumulator, of 2.76 and 13.8, respectively, and shoot Cd BCFs of maize, a model nonaccumulator, of 0.02 and 0.20, respectively. The soil in the current study has only 0.15 mg/kg Cd, leaving *C. pepo* well below the 100 mg/kg benchmark for designation as a Cd hyperaccumulator (*54*). However, the leaf Cd BCFs reported for *C. pepo* do present the intriguing possibility of simultaneous phytoextraction of heavy metals and POPs, although the mechanisms driving such reactions are unclear. Also of interest are the values for P uptake and translocation for ssp *pepo*, with tissues containing 14—73% more P than *texana*. Figure 2 shows the relationship between P and *p,p′*-DDE BCFs in the roots and stems of both subspecies. Although there is some scatter in the data, the two subspecies of plants appear to group separately, indicating that ssp *pepo* is more efficient than *texana* at phytoextracting both weathered *p,p′*-DDE and P. We have previously hypothesized that the unique phytoextraction abilities of *C. pepo* (specifically ssp *pepo*) are the result of a phosphorus acquisition mechanism involving the root exudation of low molecular weight organic acids (LMWOA) (*44*, *50*). Di- and tricarboxylic acids can chelate and extract inorganic elements from the soil structure, effectively loosening the solid matrix and increasing P availability (*55*, *56*). In white lupin (*Lupinus albus*), P deficiency is directly linked to the formation of proteoid or cluster roots, which subsequently exude large quantities of citric acid over short periods of time, resulting in mobilization

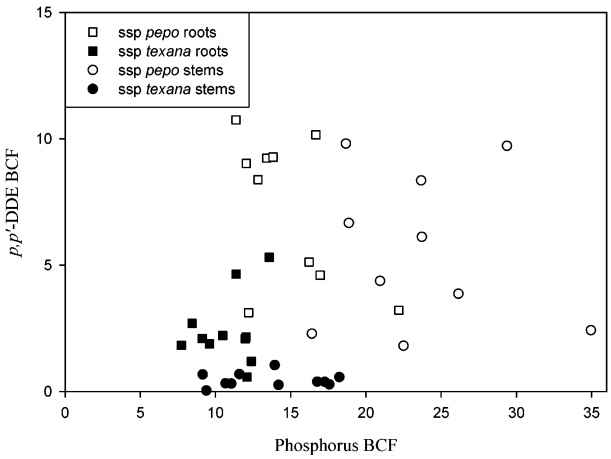

FIGURE 2. Phosphorus and *p,p′*-DDE bioconcentration factors for roots and stems of *C. pepo* ssp *pepo* and ssp. *texana*.

of previous unavailable P (*57*). Neumann and Martinoia (*58*) described cluster root formation among Cucurbitaceae as being functionally linked to the mobilization and acquisition of several required elements through root-induced alterations in the rhizosphere. Under hydroponic conditions, Waters and Blevins (*59*) describe cluster root formation specifically for *Cucurbita pepo* as a response to nutrient deficiency. We have speculated that the these unique methods of nutrient acquisition lead directly to localized destruction of the solid sequestering phase, i.e., the soil structure, and could result in significant increases in the availability of previously sequestered POPs (*50*, *60*). In batch-style abiotic experiments, we have shown that laboratory-prepared solutions of these organic acids increase the aqueous phase concentration of several inorganic elements (from 5—15-fold) as compared to water alone, resulting in up to a 60% increase in desorption of weathered *p,p′*-DDE (*50*) and chlordane (unpublished data). In addition, vegetation grown in *p,p′*-DDE contaminated soil receiving periodic amendments of citric or oxalic acids may have up to 40% more of the weathered pollutant in the tissues as compared to plants grown in soil receiving water alone (*50*, *60*). Preliminary data suggests significant quantities of citric, malic, and acetic acids are in the rhizosphere of one cultivar of *C. pepo* (unpublished data). Intensive rhizosphere-based experiments are currently underway to investigate the relationship between P status and acquisition in soil, root exudation, and contaminant phytoextraction.

Contrary to assumptions regarding the bioavailability of highly weathered hydrophobic pollutants, our findings demonstrate that under specific circumstances these contaminants are accessible from soil. The subspecies dichotomy in POP uptake and translocation observed in this study further demonstrates the importance in selecting appropriate biological receptors for exposure and remediation assessment. As previously reported, *Cucurbita pepo* ssp *pepo* extracts

quantities of weathered contaminants analogous to amounts commonly reported in heavy metal phytoremediation. Last, the data herein indicate that ssp *pepo* is more efficient than *texana* at phytoextracting not only weathered *p,p′*-DDE but also a number of inorganic constituents from soil, including P. Future investigations will seek to characterize and quantify, in situ, the root exudates of plant species of interest, explore the relationship between exudate profile and soil characteristics, assess the potential role of microorganisms such as fungi in POP phytoextraction, and elucidate the mechanism of contaminant translocation within the tissues of *C. pepo* ssp *pepo*.

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