

Multi-species interactions impact the accumulation of weathered 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE) from soil

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Interactions between earthworms and plants affect both the phytoextraction and bioaccumulation of p,p'-DDE in soil.

Abstract

The impact of interactions between the earthworms *Eisenia foetida* and *Lumbricus terrestris* and the plants *Cucurbita pepo* and *Cucurbita maxima* on the uptake of weathered *p,p'*-DDE from soil was determined. Although some combinations of earthworm and plant species caused significant changes in the *p,p'*-DDE burden in both organisms, the effects were species specific. Contaminant bioconcentration in *C. pepo* was increased slightly by *E. foetida* and by 3-fold when the plant was grown with *L. terrestris*. *E. foetida* had no effect on the contaminant BCF by *C. maxima*, but *L. terrestris* caused a 2-fold reduction in *p,p'*-DDE uptake by the plant. Contaminant levels in *E. foetida* and *L. terrestris* were unaffected by *C. pepo*. When grown with *C. maxima*, the concentration of *p,p'*-DDE decreased by approximately 4-fold and 7-fold in *E. foetida* and *L. terrestris*, respectively. The data suggest that the prediction of contaminant bioavailability should consider interactions among species.

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1. Introduction

Soil pollutants can threaten both human and environmental health. However, the risk of a toxin depends on the physical, chemical, and biological properties of a contaminated site. A growing body of evidence indicates that the mere presence of a compound in soil is not a reliable indicator that organisms

will be adversely affected by it (Kelsey et al., 1997; Alexander, 2000). Under certain conditions, pollutant biological availability can be reduced as the compound remains in soil and becomes entrapped within soil solids. This time-dependent loss in bioavailability is commonly referred to as sequestration (Alexander, 2000). A number of compounds, including atrazine (Kelsey and Alexander, 1997; Kelsey et al., 1997), simazine (Scribner et al., 1992), 1-2 dibromoethane (Steinberg et al., 1987), dichlorodiphenyltrichloroethane (DDT) (Nash and Woolson, 1967; Robertson and Alexander, 1998), dieldrin (Nash and Woolson, 1967), and many polycyclic aromatic hydrocarbons

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(PAHs), including anthracene, phenanthrene, benzo[*a*]pyrene, and pyrene (Erickson et al., 1993; Hatzinger and Alexander, 1995; Alexander and Alexander, 1999), have been shown to undergo sequestration in both laboratory and field studies. The prediction of the risk of toxic substances, therefore, should not be based solely on the total chemical concentration of the pollutant in soil (Alexander, 1995, 2000). Furthermore, although a limited amount of data have been published in this area, organisms likely differ in their ability to access the same sequestered compound. For example, the bioavailability of atrazine and phenanthrene to bacteria was far greater than that to earthworms (Kelsey et al., 1997). In other studies, bioconcentration of weathered 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE) and chlordane by *Cucurbita pepo* (includes zucchini and pumpkin) was considerably higher than that by other plants (White, 2001, 2002; Mattina et al., 2000). Similarly, the bioaccumulation of *p,p'*-DDE from soil by earthworms was nearly an order of magnitude higher for *Eisenia foetida* than for *Lumbricus terrestris* or *Aporrectodea caliginosa* (Kelsey et al., 2005). The utilization of sorbed PAHs was also shown to differ between two species of bacteria (Guerin and Boyd, 1992; Tang et al., 1998). Although these reports provide important insights into the variations in uptake among isolated organisms, like most studies designed to assess the uptake of soil toxins, they ignore multi-species interactions.

Little work has been conducted on the bioaccumulation of soil pollutants by multiple receptors exposed simultaneously. Burrows and Edwards (2004) examined the effect of the pesticide carbendazim on organisms in soil microcosms and evaluated the activity of a number of biological receptors. They noted that uptake by plants has the potential to mitigate the effects of pollutants on other organisms. However, in their system, they did not observe any reduction in the toxicity of carbendazim to soil invertebrates as a result of plant growth (Burrows and Edwards, 2004). Microcosms were also used to study the ecotoxicity of the antibiotic doxycycline to plants, earthworms, and microorganisms, but the effect of species interactions on uptake was not examined directly (Fernandez et al., 2004). In another microcosm experiment, the presence of the earthworm *L. terrestris* increased the uptake of trace metals from soil by ryegrass (Abdul Rida, 1996). To our knowledge, though, no direct measurement of the effect of the interactions between plants and earthworms on the bioaccumulation of organic pollutants by both organisms has been conducted. Data concerning the biological factors and interactions that affect the bioavailability of toxic substances in soil are critical in that altered exposure could yield different estimations of risk from contaminated sites. In addition to the potential adverse effects associated with the uptake of

soil toxins, factors affecting biological availability also influence the bioremediation of polluted soil. Effective and efficient remediation of contaminated sites depends on the ability of organisms to access pollutants. Phytoremediation is an important in situ method that uses the inherent physiological processes of plants to clean up contaminated soil; a number of comprehensive reviews of this technology are available (Cunningham et al., 1996; Salt et al., 1998; Meagher, 2000; Schnoor, 2002). Plants have been used to remediate soils contaminated with both metals (Lasat, 2002) and a range of organic compounds such as industrial solvents (Anderson and Walton, 1995), polycyclic aromatic hydrocarbons (Aprill and Sims, 1990), and pesticides (Burken and Schnoor, 1996; White, 2002). A high degree of specificity exists with regard to a plant's ability to take up a contaminant from soil. For example, profound differences in the ability to bioconcentrate highly weathered and sequestered *p,p'*-DDE have been observed among different plant species. Under both field and laboratory conditions, *C. pepo* has been shown to extract and translocate much higher quantities of the pollutant than *Cucurbita maxima* and other species (White, 2001, 2002). Pier et al. (2002) examined native vegetation growing in PCB-contaminated soils on 61 military installations in Canada, and the PCB content of the 1043 sampled species varied by 3 orders of magnitude. In addition, a number of environmental factors influence the ability of these plants to take up the sequestered compound, and remediation rates can vary widely. For example, crowding and nutrient stress significantly influenced *p,p'*-DDE phytoextraction by *C. pepo* and *Cucumis sativus* (cucumber) (Wang et al., in press). Other soil organisms may affect uptake by plants as well. Earthworms could have an important effect on the efficiency of phytoremediation as they have been shown to enhance the availability of both nutrients and toxins to plants (Lee, 1985; Stephens et al., 1994; Ma et al., 2002, 2003). Moreover, earthworms can improve soil fertility by stimulating microbial activity and improving soil structure (Edwards and Bohlen, 1996; Lavelle et al., 1998) and may promote the growth of roots (Springett and Syers, 1979; Edwards and Lofty, 1980; Tomati et al., 1988; Cannellas et al., 2002) as well as above-ground tissues (Stephens et al., 1994). The effect of earthworms on soil fertility and plant growth varies with species, though; both enhancement and inhibition have been noted (Derouard et al., 1997; Lavelle et al., 1998).

In the current study, we examined the effect of interactions between plants (*C. pepo* or *C. maxima*) and earthworms (*E. foetida* or *L. terrestris*) on the uptake of weathered *p,p'*-DDE from soil. Our objective was to assess the uptake of weathered *p,p'*-DDE by two receptors, plants and earthworms, grown simultaneously in the same contaminated soil. Of interest was the

influence of the presence of earthworms on the phytoextraction efficiency of the *p,p'*-DDE, as well as the effect that plant growth had on the bioaccumulation of the potentially toxic compound by earthworms.

2. Materials and methods

2.1. Soil

Soil (56% sand, 36% silt, 8.0% clay, 1.4% organic carbon, and pH 6.7) was collected from the Connecticut Agricultural Experiment Station's Lockwood Farm (Hamden, CT, USA) and then stored in covered plastic bins at 22 ± 2 °C. As a result of historical applications of DDT, this soil contains *p,p'*-DDE residues. The soil was air dried for 24 h and hand sieved to 2.0 mm to facilitate homogeneous sampling and to remove non-soil debris. Three-gram samples were dried in an oven at 100 °C for 24 h for moisture determination. Additional 3-g samples of the soils were transferred to 40-mL amber vials and were amended with 15 mL of *n*-hexanes and 1 µg of transnonachlor (unless otherwise noted, Chem-Service, West Chester, PA, USA) (in 100 µL hexanes) as an internal standard. The vials were sealed with caps and placed in an oven at 70 °C for 5 h. After the samples were removed from the oven, they were cooled for 10 min. After cooling, a 1-mL aliquot of the supernatant was filtered with a glass microfiber filter (0.2 µm, Laboratory Science Inc., Sparks, NV) for particulate removal prior to analysis (Section 2.5). Analysis of three replicate samples indicated that the soil used contained 178 ± 14.0 ng DDE/g of soil. This extraction method has been previously validated through comparison with microwave assisted extraction (MAE) of *p,p'*-DDE-contaminated soil (White, 2002).

2.2. Preparation of pots

Five hundred grams of the dried, sieved soil were added to 38 square plastic pots (Hummert International, Earth City, MO, USA). The depth of the pots was 12.5 cm and the width of the pots was 10.5 cm. As is described in detail below, eight separate treatments were prepared: four contained a single species (*E. foetida*, *L. terrestris*, *C. pepo*, or *C. maxima*) and four contained two species (*C. pepo* with either *E. foetida* or *L. terrestris*; *C. maxima* with either *E. foetida* or *L. terrestris*). All treatments consisted of five replicate pots (except those that contained earthworms only, which consisted of four pots per treatment).

2.3. Earthworms

Two species of earthworms were used: *E. foetida* and *L. terrestris* (obtained from Carolina Biological Supply,

Burlington, NC, USA). Approximately 3 g of earthworm biomass was added to replicate soil samples (either 3 individual *L. terrestris* or 30 individual *E. foetida*). In all cases, mature earthworms were obtained from uncontaminated soil and washed with tap water. The experimental soil was brought to 20% moisture (by weight) prior to the addition of the earthworms. The organisms were added to the tops of the soil (in the pots) and they burrowed (into the soil) beneath the surface. After 14 days, the worms were removed from the soil, washed with tap water, and transferred to Petri plates for depuration (the plates were maintained in the dark at 22 ± 2 °C). After an additional 24 h (*E. foetida*) or 48 h (*L. terrestris*), earthworms from replicate plates were pooled and washed with tap water. The worms were divided into approximately equal portions by mass and transferred to 30-mL vials. The number of replicate samples for each treatment was as follows: *E. foetida* with no plants, 6; *E. foetida* with *C. pepo*, 8; *E. foetida* with *C. maxima*, 10; *L. terrestris* with no plants, 6; *L. terrestris* with *C. pepo*, 11; *L. terrestris* with *C. maxima*, 10. Ten milliliters of hexanes and 1 µg of transnonachlor (in 100 µL hexanes) as an internal standard were then added to each vial. The vials were sealed with Teflon-lined closures, placed upright in a test tube rack in a 70-°C oven, and agitated at 110 rpm for 5 h. The samples were removed from the oven, allowed to cool for 10 min, and a 1-mL aliquot of the supernatant was removed. The 1-mL sample was then passed through a glass microfiber filter (0.2 µm, Laboratory Science Inc., Sparks, NV, USA) for particulate removal prior to analysis (Section 2.5).

2.4. Plants

Two species of plants were used: *C. pepo* (Baby bear) and *C. maxima* (Prizewinner) (Johnny's Selected Seeds, Winslow, ME, USA). Seeds were sown in seed germination medium (Frank's Nursery and Crafts, Inc., Troy, MI, USA) and maintained in the dark at 22 ± 2 °C for 1 week. Seedlings were then transferred to the 500-g soil samples and grown under four 40-W plant and aquarium grow lamps (Philips Lighting Co., Somerset, NJ, USA) at 22 ± 2 °C. The pots were exposed to 10 h of light during each 24-h period. A single plant was grown in each pot. At the time of transfer of the seedling to the contaminated soil, a 4-mL aliquot of NH_4NO_3 (100 mg/L aqueous solution) was added with each seedling. Plants were watered daily and the pots were maintained at approximately 20% moisture (by weight). All of the plants were grown for a total of 28 d. Half of the pots were amended with earthworms (either *E. foetida* or *L. terrestris*) after 14 d of plant growth (i.e., in the treatments containing two species, the plants and the worms grew together for 14 d). Worms were added to the top of the soil and burrowed beneath the surface.

The remaining plant samples were left unamended with worms. After 28 d of growth, plants were destructively harvested and divided into roots, stems, and leaves. Earthworms were removed from the soil and analyzed as described above. All vegetation was washed with water to remove attached soil particles. Roots, stems, and leaves from all pots for each treatment were pooled, and three replicate sub-samples of approximately equal mass were taken from each composite. The exceptions were the roots in the no-worm, *L. terrestris* + *C. maxima*, and *L. terrestris* + *C. pepo*, treatments, from which only two replicates were taken due to limited biomass.

The extraction method of Pylypiw (1993) was used to recover *p,p'*-DDE from the vegetation. Replicate samples of roots, stems, or leaves, 30 mL of 2-propanol, and 3 µg of transnonachlor (in hexanes) as an internal standard were added to 2-qt blender jars. After blending at high speed for 30 s, 60 mL of petroleum ether was added, and the sample was blended at high speed for an additional 4 min. The extract was then decanted through a funnel packed with glass wool and was collected in a 500-mL glass separatory funnel with Teflon stopcock. The extracts were allowed to settle for 20 min, and then 100 mL of deionized water and 10 mL of saturated sodium sulfate solution were added to each funnel. The funnels were swirled for 5 s, and then allowed to settle for 20 min so phase separation could be achieved. The water layer was then removed from the funnel. The rinsing procedure was then repeated twice (100 mL of deionized water and 10 mL of saturated sodium sulfate solution, swirling, and 20 min of settling). A final rinse with 30 mL of distilled water and 5 mL of saturated sodium sulfate solution was then carried out. The remaining petroleum ether was collected in a graduated cylinder containing 10 g of anhydrous sodium sulfate and allowed to settle for 2 h. A 5-mL sample was removed and stored in a freezer at -18°C . Prior to analysis, a 1-mL subsample was passed through a glass microfiber filter (0.2 µm, Laboratory Science Inc., Sparks, NV, USA) (Section 2.5).

2.5. Chemical analysis

The *p,p'*-DDE content in each extract (soil, worm, or plant) was determined on a Agilent (Avondale, PA, USA) 6890 gas chromatograph (GC) with a ^{63}Ni micro-electron capture detector (ECD). The column (30 m \times 0.53 mm \times 0.5 µm) contained an SPB-1 film (Supelco, Bellefonte, PA, USA) and the GC program was 175 $^{\circ}\text{C}$ initial temperature ramped at 3.5 $^{\circ}\text{C}/\text{min}$ to 225 $^{\circ}\text{C}$, then ramped at 25 $^{\circ}\text{C}/\text{min}$ to 250 $^{\circ}\text{C}$ with a hold time of 4.71 min. The total run time was 20 min. A 2-µL splitless injection was used, and the injection port was maintained at 250 $^{\circ}\text{C}$. The carrier gas was He, and the make-up gas was 5% CH_4 in Ar at 20 mL/min. The electron capture detector was maintained at 325 $^{\circ}\text{C}$.

The retention times of transnonachlor and *p,p'*-DDE were 9.9 and 10.3 min, respectively.

Stocks of crystalline *p,p'*-DDE and transnonachlor were acquired from the EPA National Pesticide Standard Repository (Fort Meade, MD, USA) and portions were transferred to hexanes (soils and earthworms) or petroleum ether (vegetation). The *p,p'*-DDE solution was diluted to prepare calibration standards of 10, 25, 50, 100, 150, 250, and 500 ng/mL and each calibration level was amended with 100 ng/mL transnonachlor as an internal standard.

2.6. Statistical analysis

All reported *p,p'*-DDE concentration values are means of replicate samples expressed on a dry-weight basis for either biomass or soil. After exposure to the contaminated soil, the plants and earthworms from each treatment were pooled and divided into replicates of nearly equal mass. Statistical differences were determined by either a Student's *T*-test or an analysis of variance (ANOVA) followed by a multiple comparison test. Specific analyses are cited in Section 3.

3. Results

3.1. Bioconcentration in plant tissues

In all pots, both plants and earthworms survived the experimental period and appeared in good health at harvest. Tissue concentrations of *p,p'*-DDE in the two plants grown in isolation (i.e., without the addition of worms to their pots) were statistically different ($p < 0.05$, Student's *T*-test). As is evident in Table 1, the bioconcentration of *p,p'*-DDE into the roots of *C. maxima* was substantially higher than that of *C. pepo*, but the concentration of the compound in the stems (the result of translocation) was approximately an order of magnitude higher in *C. pepo* than in *C. maxima*.

As measured by either tissue concentration or bioconcentration factor (BCF; dry-weight ratio of contaminant concentration in the plant tissue to that in the soil), extraction of *p,p'*-DDE by both plants was affected by the presence of earthworms (Table 1). For *C. pepo*, significant increases in root concentration of the compound were observed when either species of earthworm was added (Table 1). Grown with *E. foetida*, the root BCF for *p,p'*-DDE was approximately 30% higher than that in plants grown alone, and the concentration of *p,p'*-DDE in the roots of plants grown with *L. terrestris* was over 3-fold higher than that in the isolated plants. Translocation to *C. pepo* stems and leaves was unaffected by the earthworms. For *C. maxima*, root concentration of the compound was unaffected by the presence of *E. foetida* but declined in

Table 1

The effect of the presence of earthworms on the bioconcentration of *p,p'*-DDE by the plants *Cucurbita pepo* and *Cucurbita maxima* from contaminated soil

Species	Tissue	Treatment	Tissue conc. (ng/g)	BCF ^a
<i>C. pepo</i>	Roots	Plant only	2626.4 ± 254.5A ^b	14.7 ± 1.4A
		Plant + <i>E. foetida</i>	3492.2 ± 269.3B	19.6 ± 1.5B
		Plant + <i>L. terrestris</i>	9127.9 ± 151.8C	51.2 ± 0.8C
	Stems	Plant only	741.3 ± 247.0A	4.2 ± 1.4A
		Plant + <i>E. foetida</i>	858.5 ± 255.2A	4.8 ± 1.4A
		Plant + <i>L. terrestris</i>	953.7 ± 121.8A	5.3 ± 0.7A
	Leaves	Plant only	69.9 ± 4.51A	0.39 ± 0.02A
		Plant + <i>E. foetida</i>	62.1 ± 16.5A	0.35 ± 0.01A
		Plant + <i>L. terrestris</i>	129.8 ± 33.1A	0.73 ± 0.18A
<i>C. maxima</i>	Roots	Plant only	4594.2 ± 440.5A	25.7 ± 2.5A
		Plant + <i>E. foetida</i>	4884.7 ± 1205.6A	27.4 ± 6.8A
		Plant + <i>L. terrestris</i>	2573.9 ± 209.6A ^c	14.4 ± 1.1A ^c
	Stems	Plant only	77.8 ± 16.1A	0.44 ± 0.09A
		Plant + <i>E. foetida</i>	77.5 ± 3.7A	0.43 ± 0.02A
		Plant + <i>L. terrestris</i>	24.4 ± 11.6B	0.14 ± 0.06B
	Leaves	Plant only	78.7 ± 55.7A	0.44 ± 0.31A
		Plant + <i>E. foetida</i>	81.8 ± 35.5A	0.46 ± 0.20A
		Plant + <i>L. terrestris</i>	131.2 ± 32.0A	0.73 ± 0.18A

^a BCF, bioconcentration factor = tissue concentration/soil concentration.

^b Within a given tissue, tissue concentrations and BCFs followed by different letters are significantly different ($p < 0.05$, One way repeated measures analysis of variance followed by a Student–Newman–Keuls multiple comparison test).

^c This value is significantly different at $p < 0.06$.

the presence of *L. terrestris* by nearly 2-fold relative to root uptake in plants grown without earthworms (the effect of *L. terrestris* is statistically significant at $p < 0.06$, one way repeated measures analysis of variance followed by a Student–Newman–Keuls multiple comparison test). Translocation of the compound was negligible in *C. maxima*.

3.2. Earthworm bioaccumulation

As measured by whole body concentration, bioaccumulation of *p,p'*-DDE was substantially higher for *E. foetida* than *L. terrestris* under all conditions and was influenced by the presence of the plants (Table 2). When grown in soil that contained no plants, tissue concentrations of *p,p'*-DDE were an order of magnitude higher for *E. foetida* than *L. terrestris*. For both earthworms, bioaccumulation factors (BAF; dry-weight ratio of contaminant concentration in the earthworm tissue to that in the soil) declined significantly in the presence of *C. maxima*. For *E. foetida*, although *C. pepo* had no statistically significant effect on uptake, bioaccumulation by earthworms grown with *C. maxima* declined by approximately 4-fold relative to that by earthworms grown without plants. Bioaccumulation of *p,p'*-DDE by *L. terrestris* in the presence of *C. pepo* was statistically indistinguishable from that by the earthworms grown

alone, but the presence of *C. maxima* reduced the BAF by nearly an order of magnitude.

3.3. Effect of species interactions on the amount of *p,p'*-DDE removed from soil

The absolute quantities of *p,p'*-DDE recovered from the tissues of the plants and earthworms grown under the different treatments are presented in Table 3. For the

Table 2

The effect of the presence of plants on the bioaccumulation of *p,p'*-DDE by the earthworms *Eisenia foetida* and *Lumbricus terrestris* from contaminated soil

Species	Treatment	Tissue concentration (ng/g)	BAF ^a
<i>E. foetida</i>	Worm only	2780.0 ± 827.3A ^b	15.6 ± 4.6A
	Worm + <i>C. pepo</i>	2072.0 ± 227.3A	11.6 ± 1.3A
	Worm + <i>C. maxima</i>	689.5 ± 140.0B	3.9 ± 0.8B
<i>L. terrestris</i>	Worm only	226.6 ± 111.5A	1.3 ± 0.6A
	Worm + <i>C. pepo</i>	134.7 ± 24.1A	0.75 ± 0.13A
	Worm + <i>C. maxima</i>	33.1 ± 12.3B	0.18 ± 0.07B

^a BAF, bioaccumulation factor = tissue concentration/soil concentration.

^b Within a worm, tissue concentrations and BCFs followed by different letters are significantly different ($p < 0.05$, one way repeated measures analysis of variance followed by a Dunn's multiple comparison test).

Table 3
Effect of species interactions on total *p,p'*-DDE recovery by plants and earthworms

Treatment	Tissue ^a (ng DDE)	Plant total ^b (ng DDE)	Earthworm ^c (ng DDE)	Total ^d (ng DDE)	Total plant biomass (g) ^e
<i>C. pepo</i>					
No worm					
Roots	1120 A ^f				
Stems	290 D				
Leaves	38.1 E	1450	NA ^g	1450	3.63
+ <i>E. foetida</i>					
Roots	2100 B				
Stems	330 D				
Leaves	66.4 E	2490	1740	4230	6.16
+ <i>L. terrestris</i>					
Roots	740 C				
Stems	230 D				
Leaves	63.2 E	1030	552	1580	2.34
<i>C. maxima</i>					
No worm					
Roots	1060 A				
Stems	49.0 C				
Leaves	81.1 E	1190	NA	1190	5.61
+ <i>E. foetida</i>					
Roots	510 B				
Stems	48.2 C				
Leaves	68.1 E	624	163	787	4.67
+ <i>L. terrestris</i>					
Roots	299 B				
Stems	13.2 D				
Leaves	60.8 E	371	201	572	2.63
<i>E. foetida</i>	NA	NA	4370	4370	NA
<i>L. terrestris</i>	NA	NA	274	274	NA

^a The amount of *p,p'*-DDE extracted from each tissue (means of replicate samples).

^b The total amount of *p,p'*-DDE extracted from plants per pot (sum of the mean tissue amounts).

^c The total amount of *p,p'*-DDE extracted from whole earthworms per pot (sum of means from replicate pots).

^d Sum of plant + earthworm uptake of *p,p'*-DDE per pot.

^e Total pooled plant biomass from all pots.

^f Within a tissue for a given plant species, values followed by different letters are statistically different ($p < 0.05$, one way repeated measures analysis of variance followed by a Student–Newman–Keuls multiple comparison test).

^g NA, not applicable.

roots of *C. pepo*, the presence of *E. foetida* increased the amount of pollutant taken up relative to that extracted by the plant grown in isolation, but *L. terrestris* led to a slight decrease in the amount of *p,p'*-DDE in the roots. The total amount of *p,p'*-DDE removed by *C. pepo* appeared to increase in the presence of *E. foetida* but, when comparing the total amount of contaminant removed by both species together to that by *E. foetida* alone, one can see that the values are approximately equal. It should be noted, however, that values given for total *p,p'*-DDE extracted per plant are sums of means

and cannot be compared statistically. The total quantity of *C. pepo* tissue present after 28 d of growth also seemed to increase in the presence of *E. foetida* and decline with *L. terrestris*. Since the replicate pots were composited prior to biomass determination, they are unreplicated. For *C. maxima*, the presence of either earthworm led to a decrease in the uptake by both the plant roots and plant biomass. For both plant species, the amount of *p,p'*-DDE extracted depended on the total plant biomass present.

The amount of the compound bioaccumulated by the earthworms was also affected by interactions. Uptake of *p,p'*-DDE by *E. foetida* grown with *C. pepo* was over 2-fold lower than that by the isolated earthworms. The presence of *C. maxima* caused a nearly 27-fold reduction in uptake by *E. foetida*. The total amount of *p,p'*-DDE extracted by both organisms (plant + earthworms) also appeared to decline when the organisms were grown together. The effects of the plants on total accumulation of the compound by *L. terrestris* were less dramatic. The presence of *C. pepo* may have doubled *p,p'*-DDE uptake by *L. terrestris*, whereas *C. maxima* had no effect on the bioaccumulation of the compound by the earthworm. As stated previously, since these values were derived by adding mean values, statistical analysis cannot be carried out. The data in Table 3 are presented to suggest trends and directions for future research only.

4. Discussion

The uptake of *p,p'*-DDE from soil is a function of both species differences and interactions among organisms. The observed patterns in bioconcentration of *p,p'*-DDE by *C. pepo* and *C. maxima* grown in isolation (i.e., without earthworms) are consistent with findings from previous studies. For example, under field conditions, *C. pepo* extracted more *p,p'*-DDE from soil than did *C. sativus* (cucumber), but when the plants were grown under dense and stressed conditions in small pots (as in the current experiment), a higher concentration of the compound was detected in the roots of *C. sativus* than in those of *C. pepo* (Wang et al., in press). However, under all conditions, translocation of the compound to stems and leaves by *C. pepo* was far more extensive than that seen in *C. maxima*. Similarly, the bioconcentration of *p,p'*-DDE and chlordane by *C. pepo* was higher than that by other plant species (White, 2001, 2002; Mattina et al., 2000). The unique physiology that enables *C. pepo* to extract certain soil pollutants more effectively than other plants could influence the phytoremediation of contaminated soil and is the subject of ongoing investigation.

Bioaccumulation by earthworms has also been shown to be highly species specific. Kelsey et al. (2005) observed significantly higher tissue concentrations of *p,p'*-DDE

in *E. foetida* than either *L. terrestris* or *A. caliginosa*, with bioaccumulation factors differing by an order of magnitude. The reasons for the observed variation among earthworm species are unclear, although differences in their processing of soil organic matter, ecological strategy, and lipid content are potential explanations (Kelsey et al., 2005). Importantly, as has been suggested elsewhere (Alexander, 2000), no single species should be used as a model for all organisms in the risk assessment of contaminated sites.

In addition to species differences between organisms grown in isolation, interactions between earthworms and plants appear to affect both tissue concentrations and absolute quantities of *p,p'*-DDE available to the organisms. The effect of earthworms on the bioconcentration factor (BCF) by the two plants was species specific. The presence of either earthworm led to a substantial increase in the root BCF of *p,p'*-DDE by *C. pepo* (Table 1). The total amount of *p,p'*-DDE in the plant roots (and possibly the whole plant) increased significantly in the presence of *E. foetida* (Table 3). *L. terrestris* led to a statistically significant decline in the quantity of the compound in roots of *C. maxima* (Table 3). The very low root biomass (data not shown) in the presence of *L. terrestris* is the probable explanation for the inconsistency between the effect of the earthworm on the tissue concentration (significantly increased relative to worm-free control, Table 1) and the absolute amount of the compound in the roots (significantly decreased relative to worm-free control, Table 3). The values for biomass in Table 3, although unreplicated, suggest a direct relationship between *p,p'*-DDE taken up and the amount of plant tissue present. The effects of *E. foetida* on the growth of *C. pepo* are consistent with those reported by a number of other researchers. The increase in nutrient availability that results from the activities of some earthworms has been shown to enhance plant growth (Lee, 1985; Stephens et al., 1994; Lavelle et al., 1998). Ma et al. (2002, 2003) noted an increase in the concentration of Pb and Zn in plant tissues when plants were grown with earthworms, indicating that the bioavailability of both essential and non-essential elements may be enhanced by earthworm activity. Earthworms also have been shown to release compounds that stimulate plant growth. Cannellas et al. (2002) observed improved root growth and other evidence of growth stimulation when plants were exposed to humic acids produced by *E. foetida*, and earthworm casts were shown to stimulate root growth in certain plant species (Tomati et al., 1988). Other reports indicate that the effect of earthworms on soil properties and plant activity are highly species specific (Derouard et al., 1997; Lavelle et al., 1998). It is not surprising, therefore, that *E. foetida* and *L. terrestris* affected the total amount of *p,p'*-DDE extracted by *C. pepo* in different ways. Additional research will be necessary to

determine the mechanism by which *E. foetida* and *L. terrestris* influence the phytoextraction of *p,p'*-DDE by *C. pepo*.

The data indicate that *C. maxima* interacts differently with the two earthworm species than does *C. pepo*. No change in the tissue concentration of *p,p'*-DDE occurred in the presence of *E. foetida*, and the BCF of the compound appeared to decline by nearly 2-fold when *L. terrestris* was added to the pots. However, when the absolute quantity of the *p,p'*-DDE taken up by the plants is considered, both species of earthworms appeared to exert a substantial effect on phytoextraction. As is evident in Table 3, the amount of the compound taken up by the roots was reduced by at least 2-fold when either earthworm was added to pots containing *C. maxima*. Furthermore, the total amount of *p,p'*-DDE extracted by the combined actions of the plants and earthworms was likely reduced when the organisms were grown together. Much more work will be required to elucidate the nature of the interaction between *C. maxima* and the two species of earthworms.

Generally, plants reduced tissue concentrations (i.e., BAF) of *p,p'*-DDE in the earthworms, but absolute quantities of the compound taken up by the worms varied by species and interaction. The presence of *C. maxima* reduced the concentrations of the compound in both earthworms. *C. pepo* may have also reduced BAF by the earthworms, but since the observed declines in the concentrations of the compound in the earthworm tissues were not statistically significant, no firm conclusions can be drawn. The total amount of the compound taken up by the earthworms followed a somewhat different trend than BAF. The total *p,p'*-DDE burden in *E. foetida* was reduced in the presence of either plant, whereas the amount of the compound in *L. terrestris* increased with *C. pepo* and was unaffected with *C. maxima*. Although the nature of the data in Table 3 makes this a somewhat speculative conclusion, it is possible that the adverse effects of a compound such as *p,p'*-DDE on earthworms and food webs could be mitigated by the presence of certain plants in contaminated soils. However, the effect is likely to be species specific. Our data for *E. foetida* are consistent with other published results that indicate plants can reduce the bioavailability of organic pollutants in soil. For example, Belden et al. (2004) observed a marked decline in the uptake of the herbicide pendimethalin by *E. foetida* after the contaminated soil had been used to grow prairie grass. Boucard et al. (2005) noted that the presence of ryegrass decreased the biodegradability and extractability of 2,4-dichlorophenol after the compound was aged in soil for 60 d. The authors cited root-induced changes in soil moisture and structure as the likely cause for enhanced sequestration. Banks et al. (2003) reported a similar reduction in the toxicity of petroleum hydrocarbons after phytoremediation by grasses, although

they suggested that aging of the pollutants in soil contributed to decreasing bioavailability as well. It should be noted that, in the earlier studies, earthworms were exposed to soil after plants had grown in it for a relatively long period of time (up to 2 years). In the current study, earthworms were added to pots after only 2 weeks of plant growth; the two species then coexisted for an additional 2 weeks.

A comparison of the absolute quantity of *p,p'*-DDE phytoextracted by *C. pepo* grown alone with that of *C. pepo* grown with *L. terrestris* suggests that the pool of biologically available compound present in the soil did not change in the two treatments. The total amount of the compound taken up by the plant alone was the same as the amount taken up by the combined actions of the plant and the worm. Similarly, the amount of compound accumulated by *E. foetida* grown alone was equal to the total amount taken up by *C. pepo* and *E. foetida* grown together. This finding is important because the presence of *E. foetida* may enhance the phytoextraction of persistent organic pollutants by *C. pepo*. On the other hand, interactions between *C. maxima* and either earthworm actually led to substantial declines in the total amount of *p,p'*-DDE taken up relative to that accumulated by any of the organisms grown alone. These results suggest an antagonistic relationship between plants and earthworms. Clearly, further study of the variables affecting plant–earthworm interactions and their effects on phytoremediation and earthworm toxicity is warranted.

5. Conclusions

The uptake of *p,p'*-DDE by plants and earthworms varied among different species and was highly dependent on the interactions between the organisms. When grown in isolation, bioaccumulation of the compound by *E. foetida* was far higher than that by *L. terrestris*. The total amount of the compound accumulated by *E. foetida* decreased dramatically in the presence of either plant, but the effect of the plants on uptake by *L. terrestris* was species specific. In the absence of worms, root concentrations of *p,p'*-DDE in *C. maxima* were higher than those of *C. pepo*, although translocation was much more extensive in *C. pepo*. The effect of the addition of worms on phytoextraction depended on the organisms involved. The presence of *E. foetida* led to an increase in the quantity of *p,p'*-DDE in *C. pepo* roots but a decrease in the amount of the compound in the roots of *C. maxima*. *L. terrestris* reduced contaminant uptake by both plants. Additional work will be required to explain the mechanisms by which the observed differences occurred. It appears that the prediction of both toxicity and phytoremediation of persistent organic pollutants in soil must take into

account all factors affecting biological availability, including the effects of species on each other.

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