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Role of organic acids in enhancing the desorption and uptake of weathered *p,p′*-DDE by *Cucurbita pepo*

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"Capsule": *The addition of low molecular weight organic acids to soil may enhance phytoremediation of persistent organic pollutants.*

Abstract

Experiments were conducted to assess the effect of seven organic acids [succinic, tartaric, malic, malonic, oxalic, citric, ethylenediaminetetraacetic (EDTA)] over a concentration range of two orders of magnitude (0.001–0.10 M) on the abiotic desorption of weathered *p,p′*-DDE and the extraction of polyvalent inorganic ions from soil. At 0.05 M all organic acids significantly increased contaminant desorption by 19–80%. Organic acids also increased the aqueous concentration of eight inorganic constituents extracted from soil, with at least a six-fold increase in the release of Al, Fe, Mn, and P at 0.001 M. Zucchini seedlings grown for 28 d in soil containing weathered *p,p′*-DDE (300 ng/g, dry weight) were periodically amended with distilled water, citric or oxalic acids (0.01 M). Plants receiving water removed 1.7% of the *p,p′*-DDE from the soil. Seedlings amended with citric or oxalic acids removed 2.1 and 1.9% of the contaminant, respectively, and contained up to 66% more contaminant in the shoot system than unamended vegetation. A second crop of untreated (distilled water) zucchini in the same soil removed more contaminant than the first crop (2.5%), although the addition of organic acids did not further enhance contaminant uptake. The data indicate that the addition of low molecular weight organic acids causes the partial dissolution of the soil structure through the chelation of inorganic structural ions, potentially enhancing bioavailability and having implications for the phytoremediation of persistent organic pollutants in soil.
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Keywords: Phytoremediation; Bioavailibility; Organic acids; Bioremediation

1. Introduction

Persistent organic pollutants (POPs) are a group of environmental contaminants that include polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), dieldrin, chlordane, and heptachlor. DDT, another POP, can be degraded microbially (Zayed et al., 1994) or abiotically (Hussain et al., 1994) to either DDD or DDE and both metabolites are considered to be POPs on their own accord. POPs have been singled out for elimination due largely to concerns over their toxicity and recalcitrance in environmental systems. Although the bioavailability of organic compounds is thought to decline with time (Alexander, 2000), there is evidence in the literature indicating the potential for bioaccumulation and biomagnification of POPs in faunal food chains (Kelly and Gobas, 2001). Kunisue et al. (2002) measured concentrations of persistent organochlorine compounds in the tissues of 16 migratory bird species in Russia and South Asia, noting that levels of DDT and its metabolites ranged from 42 to 17,000 ng/g lipid weight. Fraser et al. (2002) estimated the uptake and elimination of 27 pesticides in harp seals of the Barents sea and noted that *p,p′*-DDE had the highest values, with a biomagnification factor of 87 and a half-life of greater than 8 years. Bird et al. (1983) reported that the biomagnification of DDE resulted in structural abnormalities in the reproductive organs of piscivorous birds, likely yielding eggshell thinning and offspring inviability (Grasman et al., 1998). Similarly, Frank et al.

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(2001) detected DDE at levels exceeding 1000 ng/g in cormorant eggs in East Texas, USA, and speculated that such levels were sufficient to induce adverse health effects. Given the persistence of these contaminants in natural systems and the potential for accumulation and impact on sensitive organisms, investigations focused on novel means of remediation are warranted.

Phytoremediation is the use of vegetation to remove organic and/or inorganic contaminants from natural media. Plant-based remedial strategies are inexpensive, non-intrusive, socially desirable, and often highly successful, and several comprehensive reviews of the approach are available in the literature (Cunningham et al., 1996; White and Burken, 1998). Remediation can occur by direct uptake of the contaminant into the vegetative tissues, resulting in transformation by plant enzymes, sequestration within the plant, or transpiration through the leaves (Schnoor et al., 1995). The phytoremediation of heavy metals occurs largely through plant-facilitated mobilization of the contaminants from soil, followed by direct uptake of the metals into the plant and subsequent sequestration within vegetative tissues (Brown et al., 1994; Huang et al. 1998; Chen and Cutright, 2001). Similarly, certain organic contaminants such as trichloroethylene and 2,4,6-trinitrotoluene are taken into the plant with the transpiration stream and subsequently degraded by plant enzymes or transpired from the leaves (Anderson and Walton, 1995; Thompson et al., 1998). In addition, plant roots exude large amounts of carbon-based compounds (root exudates) that may directly induce contaminant degradation through inherent enzymatic activity (Schnoor et al., 1995). This abundance of usable carbon in the root zone or rhizosphere yields a diverse and active microbial community that may subsequently biodegrade the contaminants. The enhanced microbial activity of the rhizosphere has been linked to the phytoremediation of polycyclic aromatic hydrocarbons and 2,4,5-trichlorophenoxyacetic acid (Boyle and Shann, 1998; Banks et al., 1999).

The inherent resistance of POPs to enzymatic degradation presents a remediation challenge, potentially equating these contaminants to heavy metals. Because of the hydrophobic nature and highly weathered status, POP availability to plants for direct uptake is assumed to be minimal (Burken and Schnoor, 1998). Our laboratory and others have published data indicating that a narrow range of plants, largely within the genus *Cucurbita*, may have the potential to accumulate significant amounts of weathered organic pollutants from soil (Hülster et al., 1994; Mattina et al., 2000; White, 2001). Separately, evidence exists that amendments of citric acid may increase the availability of weathered POPs, including polycyclic aromatic hydrocarbons (Yang et al., 2001), *p,p′*-DDE (White and Kottler, 2002), and chlordane (White, unpublished data). This study addresses the influence of several organic acids on the availability, uptake, and translocation of weathered *p,p′*-DDE by zucchini.

2. Materials and methods

2.1. Soil extraction

Soil was collected from Lockwood Farm, the experimental facility of the Connecticut Agricultural Experiment Station, (Hamden, CT) in areas previously shown to contain 150–1200 ng/g *p,p′*-DDE (dry weight) (White, 2000, 2001; White and Kottler, 2002). Although precise records are not available, the farm received regular applications of DDT for insect control from the late 1940s to the early 1970s. No DDT or DDD has been detected in the soil. The soil has an organic carbon content of 1.4% and is designated as Cheshire fine sandy loam. All soils were air dried and sieved to 1.0 mm for particulate removal and to provide sample homogeneity. A representative portion (3.0 g) of each sample was placed in an oven at 100 °C for 24 h for moisture determination. To quantify *p,p′*-DDE concentration in soil, 3.0 g of the soil were transferred to 40 ml amber vials and were amended with 15 ml of methanol and 1000 ng of *trans*-nonachlor (United States Environmental Protection Agency National Pesticide Standard Repository, Forte Meade, MD) 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, 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.

2.2. Abiotic p,p′-DDE desorption and metals release

To measure the desorption of *p,p′*-DDE in the presence of organic acids, 1.0 g samples of *p,p′*-DDE-containing soil were added to 60 ml vials with Teflon-lined screw caps. The soils were amended with 55 ml of distilled water or 55 ml of an organic acid solution at one of the following concentrations: 0.001, 0.005, 0.01, 0.05, or 0.10 M. The acids used were succinic, tartaric, malic, malonic, oxalic, citric, or ethylenediaminetetraacetic acid (EDTA); each acid at each concentration was replicated four times. Organic acids were purchased from Sigma Aldrich (St. Louis, MO) as the molecular acid. All solutions were adjusted to pH 7.0 with 5 M NaOH. Each vial was then amended with 1000 ng of *trans*-nonachlor (internal standard) and 300 mg of Tenax™ beads (20/35 mesh, Supelco, Bellefonte, PA, USA), which served as an infinite sink for dissolved hydrophobic organic compounds (Pignatello, 1990). The vials were capped and placed on a rotary shaker at 21 °C for 7 d. The vials were then centrifuged at 870 *g*

for 10 min and the Tenax™ (which remained on the surface) was removed with a spatula. The Tenax™ beads were transferred to a screw-cap vial containing 5 ml of hexanes and approximately 0.5 g anhydrous sodium sulfate, and the vials were placed on a rotary shaker at 21 °C for 24 h. A portion of the hexanes was then analyzed by gas chromatography as described below.

To determine the effect of organic acids on the aqueous concentration of inorganic soil constituents, 10 g of *p,p′*-DDE-containing soil was added to 50 ml plastic centrifuge tubes and was amended with 40 ml of solution. The solution was either distilled water or one of the organic acids at 0.001, 0.005, 0.01, 0.05, or 0.10 M. The tubes were capped and placed on a rotary shaker 21 °C for 7 d. The tubes were centrifuged at 6500 *g* for 10 min and a portion of the supernatant was analyzed for aqueous concentrations of specific extracted inorganic ions (Fe, Al, Cu, Ca, K, Mg, Mn, P, S, Zn) by inductively coupled plasma–atomic emission spectroscopy (ICP–AES) (Perkin Elmer P-40) utilizing EPA methods 3010 (sample preparation) and 6010 (analysis).

2.3. Greenhouse trials

Rectangular plastic pots (8 × 13 cm) were lined with filter paper and amended with approximately 280 g (dry weight) of Lockwood farm soil. Zucchini seeds (*Cucurbita pepo* cv. Goldrush, Johnny's Selected Seeds, Albion, ME) were placed in moist germination paper and stored at room temperature for 3 d. A total of 10 germinated seeds were added to each of 24 pots in a greenhouse at approximately 26 °C. The pots were divided into three regimes; those to be treated with distilled water, 0.01 M citric acid, or 0.01 M oxalic acid. Unplanted control pots were established for each treatment. The plants were watered as necessary and seedlings were grown for 28 d, at which point they averaged approximately 12 cm in height. During the growing period, the pots were periodically (two–three times per week) treated with 1–3 ml of water, 0.01 M citric, or 0.01 M oxalic acid. The total amount of citric and oxalic acids added during a single growing period were 33 and 15 mg, respectively. At the end of the growing period (28 d), the vegetation was destructively harvested and leaf, stem, and root masses were determined for each pot. Each vegetative compartment was composited by treatment (water or organic acids) and washed thoroughly with tap water. All vegetative samples were finely chopped and were either extracted immediately or were stored in 250 ml amber glass bottles with Teflon-lined caps in a freezer. The soils were composited by treatment, air-dried, and sieved to 1 mm. Portions of the soil from each treatment and from unvegetated control pots were extracted to determine *p,p′*-DDE content. The remaining soils were re-weighed into rectangular pots for a second crop of zucchini. The watering regime, organic acid amendments, and vegetation harvesting protocols were identical to that described for the first crop of zucchini.

2.4. Vegetative extraction procedure

The method used for extraction of *p,p′*-DDE residues from various portions of the vegetation has been described previously (White, 2001). Portions of the vegetation (20–50 g) were weighed into a 1-quart blender container with 50 ml of 2-propanol (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ) and 3000 ng of *trans*-nonachlor as an internal standard. The sample was blended at low speed for 30 s in an explosion-proof blender (Fisher Scientific, Springfield, NJ). Then 100 ml of petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ) was added to each of the containers. The sample was blended on low speed 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 complete draining of the solids (approximately 20 min), 200 ml of distilled water and 10 ml of saturated sodium sulfate solution were added separately to each funnel. The funnel was capped, shaken gently for 10 s, and allowed to sit for 20 min to achieve phase separation. The water was drawn off and the petroleum ether was rinsed two additional times with distilled water. The final extract (approximately 60–70 ml) was then amended with 15 g of anhydrous sodium sulfate and allowed to sit for 3 h prior to GC analysis.

2.5. Quantitation

The amount of *p,p′*-DDE in the methanol soil extracts, hexane Tenax™ 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 make-up gas was 5% CH_4 in Ar at 20 ml/min. The ECD was maintained at 325 °C.

Crystalline *p,p′*-DDE purchased from Chem Service (West Chester, PA) was dissolved in either petroleum ether (for vegetative extracts), methanol (for soil extracts), or hexane (Tenax™ experiments) 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

trans-nonachlor as an internal standard. The retention times of *trans*-nonachlor and *p,p′*-DDE were 16.8 and 18.5 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 moisture content of the sample after at least 24 h at 100 °C. Tissue compartments (roots, stems, leaves) from composited individual treatments were extracted in triplicate. In the Tenax™ desorption studies, all acid treatments were replicated four times. To assess the statistical significance of tissue or treatment differences in contaminant concentration, either a student *t*-test ($P<0.05$) or a one-way analysis of variance (ANOVA) followed by a Tukey multiple comparison test (at 0.05) was employed. Recovery of the internal standard was 94.8% (±3.1%).

3. Results and discussion

3.1. Abiotic p,p′-DDE desorption and metals release

The concentration of *p,p′*-DDE in the soil utilized in the abiotic studies was 300±6.7 ng/g (dry weight). The extent of *p,p′*-DDE desorption from soil in the presence of organic acids, normalized to that observed in distilled water, is shown in Table 1. At concentrations of 0.01 M or higher, all amendments except succinic acid significantly enhanced contaminant desorption; at 0.005 M, all organic acids but malic, succinic, and tartaric were effective. EDTA, citric, and oxalic acids at the 0.05 M concentration level had the largest impact on desorption, with increases of 80, 58, and 45%, respectively. The consistent decrease in desorption noted at 0.10 M of all acids is likely related either to the high amount of Na added as NaOH to adjust the pH of the initially acidic solution or possibly due to large quantities of sorbing organic acids restricting the diffusion of weathered *p,p′*-DDE. We have previously noted that high concentrations of organic acids may possess confounding factors (White and Kottler, 2002).

The extraction of inorganic elements from soil was also quantified at all acid concentrations, and the values are expressed, again normalized to that observed from soil with distilled water, in Table 1. The aqueous concentration of eight of the 10 assayed inorganic soil constituents was increased substantially at nearly all organic acid concentrations tested. Al, Fe, and Mn seem particularly susceptible to extraction by organic acid amendments with, on average, greater than an order of magnitude increase in the aqueous concentration at the lowest level of acids tested. In certain cases, the release of P was increased by three orders of magnitude in the presence of the organic acids. Of the 35 organic acid variations examined, P was the most extracted element 71% of the time (expressed relative to the amount of P released by distilled water alone). It is notable that only three elements (Ca, Mg, S) follow the same pattern of release as *p,p′*-DDE desorption in the presence of organic acids (Fig. 1a–g), including the decrease in release at 0.10 M acid concentration. The explanation for this correlation is unknown.

The findings of increased desorption of organic contaminants and inorganic soil constituents in the presence of organic acids is not suprising. Boyle et al. (1967) observed that citric and oxalic acids produced an uncharacteristic weathering zone on particles of biotite and vermiculite, and speculated that acid treatment extracted and depleted this zone of metals such as Fe, Al, and Mn. The weathered zone was described as amorphous and fragile, due largely to the loss of structural and tetrahedral cations (Sawhney and Voigt, 1969). Kpomblekou and Tabatabai (1994) reported that citric and oxalic acids at 0.001 M solubilized significant amounts of P from phosphate rocks and correlated the increased solubilization with extracted levels of Ca and Mg. These findings agree with the present study in that both Ca and Mg were correlated with *p,p′*-DDE desorption at all organic acid concentrations tested. Several others have described the increased solubility of several heavy metals (U, Cd, Pb, Zn, Ni) and rare earth elements (La, Ce, Pr, Nd) in the presence of low molecular weight organic acids such as EDTA, citric, and oxalic acids (Krishnamurti et al., 1997; Huang et al., 1997; 1998; Shan et al., 2002), citing likely direct complexation, and subsequent mobilization, between the organic and inorganic constituents. Similarly, citric and malic acids are known to complex with Fe in soil, effectively solubilizing previously unavailable oxides (Jones, 1998). Others have reported that low molecular weight organic acids may increase the soil solution concentration of P by 1–3 orders of magnitude (Jones, 1998). The release is either due to the direct displacement of P from the soil surface or the secondary result of organic acid complexation with other inorganic soil-bound elements (Fe and Al) that effectively hold P within the soil matrix. Similarly, Yang et al. (2001) observed that several organic acids increased the desorption of weathered polycyclic aromatic hydrocarbons from soil. They speculated that a chelation of inorganic ions from the soil resulted in a release of previously bound humic acid and a subsequent increase in the availability of previously sorbed hydrocarbons. White and Kottler (2002) observed similar increases in the desorption of weathered *p,p′*-DDE in the presence of sodium citrate, noting that partial destruction and/or release of the sequestering soil matrix would likely yield increased bioavailability of the aged residue.

Table 1
Influence of organic acid amendments on the desorption of *p,p′*-DDE and release of structural metal ions from soil (all values are normalized to concentrations released from soil by distilled water)

Supernatant	Acid concentration (M)	Normalized *p,p′*-DDE desorption[a]	Al	Fe	Ca	Cu	Mg	Mn	P	S	Zn	K
Water	–	1.00 A	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Succinic acid	0.10	1.06 A	3.3	5.0	3.0	2.0	2.7	180	17	2.0	1.1	3.0
	0.05	1.19 B	0.33[b]	0.75	6.1	1.0	12	16	1.0	4.0	1.2	NA
	0.01	1.05 A	3.5	3.0	0.83	1.0	1.3	15	4.0	1.0	1.0	1.1
	0.005	1.01 A	7.0	7.5	0.46	2.0	0.60	1.7	33	1.0	1.3	0.89
	0.001	0.91 C	10	8.0	0.46	1.0	0.60	14	17	1.0	1.0	0.88
Tartaric acid	0.10	1.15 B	170	110	4.4	20	2.7	250	600	3.5	11	2.7
	0.05	1.27 C	4.4	7.0	4.0	3.3	10	17	22	8.0	0.60	NA
	0.01	1.07 D	4.8	4.5	0.96	4.0	1.3	20	6.0	1.5	1.3	1.3
	0.005	1.05 AD	9.5	10	0.55	5.0	0.60	17	33	1.5	3.3	1.0
	0.001	0.95 E	8.0	6.0	0.58	1.0	0.60	10	17	1.0	1.0	1.0
Malic acid	0.10	1.17 B	170	160	3.5	20	2.8	390	550	4.0	5.6	2.8
	0.05	1.31 C	13	11	4.8	6.7	10	20	38	11	0.60	NA
	0.01	1.11 BD	6.3	5.0	0.91	4.0	1.3	20	6.0	1.5	1.5	1.2
	0.005	1.06 AD	11	11	0.46	5.0	0.60	17	33	1.5	2.0	1.0
	0.001	0.95 A	14	12	0.42	2.0	0.60	18	33	1.0	1.3	0.88
Malonic acid	0.10	1.22 B	170	38	2.4	20	2.8	210	270	4.0	2.2	2.7
	0.05	1.36 C	10	5.9	3.3	10	11	19	18	12	0.60	NA
	0.01	1.13 B	14	7.0	0.61	6.0	1.3	20	10	2.0	2.5	1.1
	0.005	1.13 B	14	14	0.42	4.0	0.60	15	50	1.5	2.3	0.89
	0.001	0.94 A	11	10	0.46	1.0	0.60	16	17	1.0	1.0	0.89
Oxalic acid	0.10	1.32 B	210	120	0.19	40	3.2	230	780	6.0	2.2	2.8
	0.05	1.45 C	6.7	7.8	3.4	17	11	16	30	14	0.60	NA
	0.01	1.15 D	7.8	6.0	0.57	10	1.0	5.0	8.0	2.0	1.8	1.1
	0.005	1.16 D	8.5	10	0.42	3.0	0.60	17	33	1.5	1.7	1.1
	0.001	0.97 A	10	10	0.50	1.0	0.60	16	17	1.0	1.0	0.88
Citric acid	0.10	1.24 B	330	450	6.5	50	4.0	510	1400	6.0	22	3.6
	0.05	1.58 C	29	39	12	67	20	66	150	19	2.0	NA
	0.01	1.19 D	7.8	6.0	0.57	10	1.0	5.0	8.0	2.0	2.5	1.2
	0.005	1.19 D	29	16	0.63	10	0.80	33	66	2.0	2.7	1.1
	0.001	1.05 E	21	16	0.33	2.0	0.60	20	33	1.0	1.7	0.88
EDTA	0.10	1.25 B	280	250	7.6	100	4.0	480	1100	6.0	22	4.0
	0.05	1.80 C	24	19	27	130	11	71	140	19	6.0	NA
	0.01	1.22 B	42	37	5.5	100	1.3	140	86	4.0	50	1.5
	0.005	1.23 B	50	50	1.9	70	0.70	300	250	2.0	67	1.3
	0.001	1.03 A	29	30	0.58	40	0.60	40	33	1.0	33	1.0

[a] One g soil, 55 ml solution, 300 mg tenax. Shaken for 7 days and tenax is extracted with hexanes. Each values is the average of four replicates. Within a given organic acid, values followed by different letters are significantly different from each other and/or from the value obtained by water alone [one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test at 0.05].

[b] Metal ion concentration normalized to amount extracted in water solution control for a given organic acid concentration. Each value represents a single replicate.

3.2. *Effect of organic acids on* p,p′*-DDE uptake by zucchini*

Measurable concentrations of *p,p′*-DDE were detected in all vegetative tissues analyzed (Table 2). Concentrations were greatest in the roots, with values decreasing in the stems and leaves. Treatment with citric acid significantly increased the leaf biomass (2.1 g per pot, dry weight) of the vegetation relative to water alone (1.5 g per pot, dry weight), although the concentration of *p,p′*-DDE in this tissue was unaffected. The greater biomass in the presence of organic acids did yield greater total amounts of the contaminant in the leaf tissue. The concentration of *p,p*-DDE present in zucchini stems was significantly greater in the presence of citric and oxalic acids, again resulting in greater total

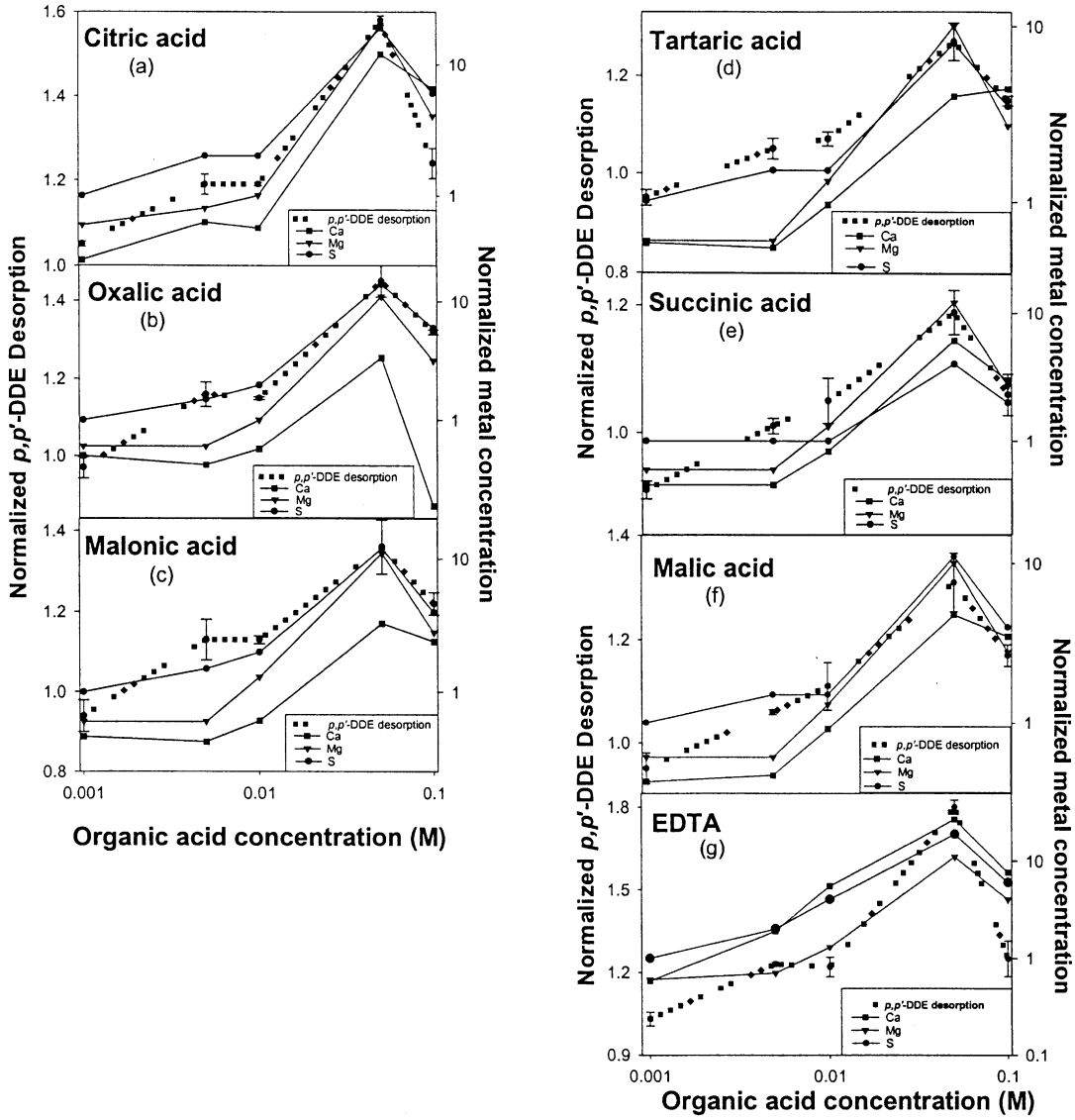

Fig. 1. Influence of organic acid amendments on the normalized desorption of *p,p'*-DDE or release of metal ions from soil. All values are normalized to those observed from distilled water and soil (note scale variation on both *y*-axes).

amounts of the contaminant in this tissue compartment. The presence of organic acids had no effect on the root biomass or on the amount of *p,p'*-DDE present in that tissue.

Interestingly, within a *second* crop of zucchini in the same soil produced different results (Table 2). Both the concentration and total amount of contaminant in zucchini leaves and stems were significantly reduced in the presence of citric acid. Similarly, amendments of oxalic acid resulted in decreased quantities of *p,p'*-DDE in leaf tissue but stem concentrations were not significantly affected.

In comparing the two crops directly, the biomass in crop two within all treatments was lower than in the first crop. Given that the reductions were particularly large for plants receiving organic acids (Table 3), it is likely that the cumulative effect of amendments either on the soil structure or on the plants themselves were detrimental to plant growth, thereby reducing *p,p'*-DDE removal from the soil. It is noteworthy that, regardless of treatment regime, the concentration of *p,p'*-DDE in nearly all tissue compartments was significantly greater in crop two than in crop one. Concentrations of *p,p'*-DDE in the tissues of plants receiving only water were increased by 1.9–4.3 times in the second crop of vegetation.

The concentration of *p,p'*-DDE in unvegetated control soil was unchanged during the two growing periods and unaffected by amendments of organic acids. Regardless of organic acid amendment, the concentration of *p,p'*-DDE in soil was not significantly reduced by a single crop of zucchini but after the second crop, all soil concentrations were significantly lower than initial levels. In pots receiving water, citric, or oxalic acids, the

Table 2
Influence of organic acid amendments on the uptake of DDE from soil (300 ng/g) by first and second crops of zucchini (Goldrush)

Tissue[a]	Treatment[b]	Crop number	ng/g DDE	Total DDE removed (ng)
Leaves	Water	1	27.9 A[b]a[c]	41.7 Aa
		2	120 Yb	145 Yb
	Citric	1	31.4 Aa	64.4 Ba
		2	80.3 Zb	89.2 Zb
	Oxalic	1	35.6 Aa	55.1 Ca
		2	87.5 Zb	99.6 Zb
Stems	Water	1	290 Aa	140 Aa
		2	606 Yb	283 Yb
	Citric	1	530 Ba	220 Ba
		2	424 Za	170 Zb
	Oxalic	1	380 Ca	200 Ba
		2	602 Ya	294 Yb
Roots	Water	1	1840 ABa	1310 Aa
		2	3530 Yb	1550 Yb
	Citric	1	2000 Aa	1450 Aa
		2	3020 Yb	1280 Zb
	Oxalic	1	1790 Ba	1310 Aa
		2	3260 Yb	1290 Za

[a] Pots received 1–3 ml of water, 0.01 M citric acid, or 0.01 M oxalic acid (17 ml over 28 day growing period; 33 mg of citric acid, 15 mg of oxalic acid).

[b] Within the same crop number (crop 1 or 2) and tissue, values followed by different *uppercase* letters are significantly different (Student *t*-test, $P<0.05$).

[c] Within different croppings (crop 1 and 2) and treatments, values followed by different *lowercase* letters are significantly different (Student *t*-test, $P<0.05$).

Table 3
Influence of organic acid amendments on the uptake of DDE from soil (300 ng/g) by first and second crops of zucchini[c]

Treatment	Crop number	Ratio of plant dry weight/soil dry weight [a]	Total ng removed per pot	% Removed[b]
Water	1	0.96 A	1490 A	1.72 A
	2	0.83 B	1980 B	2.53 B
Citric	1	1.2 A	1720 A	2.06 A
	2	0.79 B	1540 B	2.14 A
Oxalic	1	1.0 A	1570 A	1.92 A
	2	0.84 B	1690 A	2.30 B

[a] Average plant dry weight expressed as a percentage of the soil dry weight in which the vegetation was grown.

[b] Total amount of DDE in vegetative tissue in a pot divided by total ng present in the soil in that pot.

[c] Within crop number for each treatment, values followed by different letters are significantly different (Student *t*-test, $P<0.05$).

initial *p,p′*-DDE concentrations were 310, 299, and 298 ng/g and the final amounts were 284, 281, and 279 ng/g, respectively. These values are significantly different from the initial concentrations, within treatments ($P<0.05$, students *t*-test), but are not significantly different from each other.

We have previously shown that certain cucurbits can accumulate substantial amounts of weathered POPs, such as *p,p′*-DDE and chlordane, from soil (White, 2001; White et al., 2002; Mattina et al., 2002). These findings are somewhat surprising given the highly weathered nature of the organic residues. There is overwhelming evidence in the literature indicating a time-dependent decline in the bioavailability of organic compounds in soil as the contaminants become sequestered within the nanoporous soil structure (Alexander, 2000). Many organic compounds, including POPs, have been shown to undergo sequestration, resulting in decreased availability to a range of biotic and abiotic assays (Alexander, 2000). Indeed, the vast majority of plants seem to be unable to accumulate significant amounts of POP residues from soil. White (2000) and White and Kottler (2002) showed modest concentrations of *p,p′*-DDE in the roots of alfalfa, rye, pole bean, vetch, mustard, and clover grown in soil containing up to 900 ng/g of the contaminant. Hülster et al. (1994) and Mattina et al. (2000) report similar modest availability of weathered dioxins and chlordane, respectively, to a range of vegetable species. Most POPs are characterized by a log K_{ow} exceeding 3.0, a value at which Schnoor et al. (1995) states that contaminant hydrophobicity will minimize the potential for uptake by plants. Nevertheless, Lichtenstein et al. (1965) measured considerable uptake of chlorinated hydrocarbon insecticides into various plants, although the residues of these contaminants were not highly weathered. In addition, Hülster et al. (1994) reported significant uptake and translocation of weathered polychlorinated dibenzo-*p*-dioxins and dibenzofurans from soil in the aerial tissues of zucchini and pumpkin. To attempt to compare and quantify the degree of uptake of weathered POPs in our work, we have previously proposed a root:soil index where the concentration of contaminant in the roots (dry weight) is divided by the amount in the soil (dry weight). We have reported root:soil indices for various species of zucchini and pumpkin ranging from 12 to 18 for *p,p′*-DDE and chlordane, whereas values for other plants typically range from 0.5 to 2 (White, 2001, 2002; White et al., 2002). Root:soil indices for plants in this study range from 5.9 to 6.8 and 10 to 11 in the first and second crops, respectively. Values for this variety of zucchini had been estimated at 16 previously (White, 2002), although it is possible the lower ratio of accumulation in the present study is due to the short growing period and immature status of the vegetation.

3.3. Implications for phytoremediation

In addressing the phytoremediation of organic compounds in soil, most studies focus either on the enhanced degradative abilities of the rhizosphere or on the uptake and subsequent transformation or transpiration of the contaminant. In discussing the phytoremediation of heavy metals, the extraction and sequestration of contaminants within plant biomass is the focus. We have hypothesized that there is potential for remediating weathered persistent organic pollutants by a similar phytoextraction approach. In fact, remedial values associated with *p,p′*-DDE (Table 3) are similar to those described for heavy metals. Here, *Cucurbita pepo* cv Goldrush removed 1.5 μg of *p,p′*-DDE from 280 g of soil in 28 d, corresponding to a removal of 1.7%. Lasat et al. (1998) described the phytoextraction of radiocesium at 3% per year as practical. Brown et al. (1994) demonstrated that *Thlaspi caerulescens*, a known heavy metal hyperaccumulator, phytoextracted from 0.1 to 0.6% of the Zn and 0.3 to 1.3% of the Cd from a contaminated soil. Also using *T. caerulescens*, Lombi et al. (2001) demonstrated removal of 2.0 and 6.3% of the Zn and Cd from a contaminated soil after a 4-month growth period. Alternatively, in a discussion of the phytoremediation of Pb, Blaylock et al. (1997) demonstrated ratios of the contaminant in the shoot (mg/kg dry weight) to that in the soil (mg/kg) of less than one. Huang et al. (1997, 1998) showed that in the phytoremediation of Pb and U by hyperaccumulating species, the shoot:soil concentration ratios were at most 3 and 6, respectively. Similarly, Brown et al. (1994) reported shoot:soil concentration factors of 1.7 and 1.1 for Cd and Zn accumulation by *T. caerulescens*. Brakefern (*Pteris vittata*), a hyperaccumulator species for weathered As and perhaps the premier example of heavy metal phytoremediation, reaches shoot:soil contaminant concentration ratios of 17 (Ma et al., 2001). In the present study, the stem:soil values are approximately 1 but are likely conservative given the short growth period and immature status of the vegetation. We have previously reported stem:soil ratios ranging from 1.5 to 6.7 for chlordane by zucchini (Mattina et al., 2002; White et al., 2002) and values from 8.8 to 22 for *p,p′*-DDE (White, 2001) by pumpkin and zucchini, respectively. The major weakness to phytoremediation with cucurbits is their small and diffuse root system, resulting in less than optimal root to soil contact, in comparison to other species used in the remediation of heavy metal contaminated soils. In a field study with *Cucurbita pepo* cv. Goldrush in *p,p′*-DDE-contaminated soil, the estimated ratio of root mass to soil mass was 4.4×10^{-4}, yielding a phytoextraction of 0.40% in 100 days (White, 2002). In the greenhouse study described here (same soil and zucchini variety), by design the root to soil mass ratio was increased significantly to a value of 2.5×10^{-3}, yielding a phytoextraction of 1.7% in only 1 month.

One approach to overcoming the limitations imposed by root to soil contact is the introduction of chelating agents to disrupt soil structure and increase the availability of the organic contaminants. The addition of various chelating agents has been described for several heavy metals. Barona et al. (2001) increased the availability of Pb, Ni, and Zn in soil with EDTA. Jarvis and Leung (2001) observed that lead chelated with H-EDTA or EDTA was more efficiently transported to the shoots of certain plants relative to unchelated lead. Similarly, Blaylock et al. (1997), Huang et al. (1998), and White and Kottler (2002) describe the enhanced plant uptake of Pb, U, and *p,p′*-DDE, respectively, in the presence of citric acid. One negative effect from the addition of chelates to heavy metal contaminated soil is the increased mobility of the metal–chelate complex and potential movement to groundwater (Römkens et al., 2002). This concern over leaching to groundwater is not likely a concern for weathered persistent organic pollutants given the indirect mechanism of mobilization (i.e. no direct contaminant–chelate complex) and the highly hydrophobic nature of the compounds. However, these reports of enhanced contaminant availability are in line with the findings of this study. Perhaps most significant here was the observed 66 and 55% increase in contaminant burden in the shoot system when treated with citric and oxalic acids, respectively. One could speculate that under field conditions, where root to soil contact is minimized relative to the experimental design in this study, that the effects of organic acid amendments could be greater.

It is noteworthy that the percent of contaminant removed from the soil was significantly increased in a second crop of vegetation. Brown et al. (1994) and Lombi et al. (2001) both observed increases in the removal of Cd by *T. caerulescens* in successive croppings but reported constant or decreasing removal of Zn under similar treatment. In this study, the influence of organic acid amendments was somewhat mixed, with definite enhanced contaminant uptake in crop one but likely toxicity in crop two. Chen and Cutright (2001) observed similar findings with EDTA and HEDTA promoting increased concentrations of certain heavy metals in plant tissue but also yielding overall reductions in plant biomass relative to untreated vegetation. We have also previously observed that in the field repeated treatment of zucchini with 0.05 M citric acid resulted in decreased plant biomass and lower remediation potential (unpublished data). Clearly, the addition of organic chelators to promote contaminant availability is a complex issue, with potential conflict concerning maximum contaminant release from soil and maintaining plant health and uptake potential.

The findings of this study are of interest in that they indicate that certain plants may accumulate quantities of field-weathered POPs such as *p,p′*-DDE at levels approximating those observed in heavy metal phytoremediation. Similar to such phytoextraction approaches, the weathered POPs are not degraded but are sequestered within the plant biomass. Consequently, depending on the contaminant concentration and toxicity, the residual plant material may have to be managed through techniques similar to those utilized in heavy metal phytoremediation. The data also indicate that under the appropriate treatment regime, the application of low to moderate quantities of low molecular weight organic acids may increase the availability of weathered POPs through partial destruction of the sequestering soil matrix, resulting in enhanced availability and uptake of the contaminants. Future studies are being designed to address the effects of such amendments under field conditions, the implementation of treatment regimes that maximize contaminant removal, and the overall efficacy of phytoremediation over many growing seasons.

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