CITRATE-MEDIATED INCREASE IN THE UPTAKE OF WEATHERED 2,2-BIS(p-CHLOROPHENYL)1,1-DICHLOROETHYLENE RESIDUES BY PLANTS

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Abstract—Experiments were conducted to determine the ability of citrate to enhance the plant uptake of weathered 2,2-bis(p-chlorophenyl)1,1-dichloroethylene (p,p’-DDE) from soil. Plots containing three rows of clover, mustard, hairy vetch, or rye grass were constructed in soils containing p,p’-DDE. On 11 occasions, the rows of each crop received water or sodium citrate (0.005 or 0.05 M). For each crop, there were significant reductions in p,p’-DDE concentration in the soil fractions (near root and rhizosphere) closely associated with the plant versus bulk soil. The roots of each crop accumulated 2 to 5 times more of the weathered contaminant (dry wt) than present in the bulk soil. Citrate (0.05 M) increased the concentration of p,p’-DDE in the roots of clover, mustard, and hairy vetch by 39% compared with vegetation that received water. In batch desorption studies, the release of weathered p,p’-DDE was significantly greater in the presence of 0.05 M citrate than in water. Citrate increased the extracted aqueous concentrations of five metal ions (Al, Fe, Ca, K, Mn) from soil by five- to 23-fold over distilled water. We hypothesize that citrate physically disrupts the soil through chelation of structural metal ions and release of bound humic material, facilitating p,p’-DDE availability and uptake by plants.

Keywords—Phytoremediation  Chelation  Sequestration  Bioavailability

INTRODUCTION

The bioremediation of natural solids contaminated with organic compounds is directly limited by pollutant bioavailability. Considerable data from both laboratory-prepared and field-weathered pollutants in soils indicate an inverse relationship between contaminant availability and residence time in the environment. This phenomenon has been termed sequestration and is mechanistically explained as a progressive entrapment of contaminant molecules within remote, inaccessible regions of the soil structure or within the soil organic matter itself [1]. A recent review of sequestration indicates that the phenomena may be demonstrated with physical and biological assays, including mild solvent extraction, toxicity to bacteria and insects, and bioavailability to bacteria and earthworms [1]. Many contaminants of environmental concern have been shown to undergo sequestration, including polycyclic aromatic hydrocarbons, polychlorinated biphenyls, triazine herbicides, and chlorinated pesticides [1]. Time-dependent declines in pollutant availability may explain the difficulty in remediating many field sites given that the soils received their contaminant burden years or even decades ago [1].

Phytoremediation is a promising new in situ technology involving the plant-assisted removal of contaminants from natural solids and occurs by one of three mechanisms [2]. The first mechanism is termed direct uptake and considers the plant as a biological pump-and-treat system where the flow of water toward the roots physically removes contamination from the soil and results in aqueous transport of the pollutants to the plant for absorption [3]. Direct uptake has been demonstrated for soils contaminated with metals including copper and zinc [4]; lead and nickel [5]; and for certain organic contaminants such as atrazine [6], trichloroethylene [7], and 2,4,6-trinitrotoluene [8]. A second potential mechanism of remediation involves in situ contaminant degradation from the enzymatic activity of material leaking out of the roots (root exudates) [3] and has been shown for pentachlorophenol [9] and chlorobenzoic acid [10]. Third, root exudates stimulate a large and active microbial community that may in turn biodegrade certain contaminants in the root zone. This last mechanism has been termed the rhizosphere effect and has been implicated in the removal of numerous contaminants from soil, including polycyclic aromatic hydrocarbons [11] and 2,4,5-trichlorophenoxyacetic acid [12].

Persistent organic pollutants (POPs) are a class of contaminants that present unique concerns relative to their presence in the environment and include organochlorine pesticides such as chlordane, dioxins, DDT, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons [13,14]. The half-lives of these compounds in soil are frequently measured in decades [15]. POPs have been linked to carcinogenicity [16] and endocrine disruption [17] in mammals, and concerns over toxicity are exacerbated by pollutant hydrophobicity, which results in bioaccumulation in fatty tissues [18,19] and biomagnification through food chains [20,21]. Enzymatic [22] and photolytic [23] reactions can rapidly convert DDT to p,p’-DDE. However, p,p’-DDE is also considered a POP and has been implicated in the egg shell thinning of fish-consuming birds in the Great Lakes, USA [24], likely through physiological alterations in the oviducts of the female birds [25].

The same physical properties that lead to POP longevity in the environment also make them difficult to remediate by tra-
Crimson clover (Trifolium incarnatum), mustard (Brassica juncea, southern giant), hairy vetch (Vicia villosa), and rye grass (Lolium multiflorum) seeds were purchased from Johnny’s Selected Seeds (Albion, ME, USA). Experimental plots were constructed at the Lockwood Farm (Hamden, CT, USA) in areas previously shown to contain significant levels of POPs in their tissues [26–29]. The utility and efficacy of phytoremediation in areas contaminated with highly sequestered, persistent, organic pollutants remains unknown. In an effort to investigate the mechanism by which certain plants remove highly sequestered POPs from soil, this study addresses the ability of four crops to remove p,p’-DDE from soil and assesses the effect of citrate amendments on that process.

**MATERIALS AND METHODS**

**Experimental plots**

Citrate-enhanced plant uptake of p,p’-DDE

Experimental plots were set up in an effort to investigate the mechanism by which certain plants may accumulate significant levels of POPs in their tissues [26–29]. The utility and efficacy of phytoremediation in areas contaminated with highly sequestered, persistent, organic pollutants remains unknown. In an effort to investigate the mechanism by which certain plants remove highly sequestered POPs from soil, this study addresses the ability of four crops to remove p,p’-DDE from soil and assesses the effect of citrate amendments on that process.

**MATERIALS AND METHODS**

**Experimental plots**

Crimson clover (Trifolium incarnatum), mustard (Brassica juncea, southern giant), hairy vetch (Vicia villosa), and rye grass (Lolium multiflorum) seeds were purchased from Johnny’s Selected Seeds (Albion, ME, USA). Experimental plots were constructed at the Lockwood Farm (Hamden, CT, USA) in areas previously shown to contain p,p’-DDE (no DDT or dichlorodiphenylchloroethane) at levels ranging from 50 to 600 ng/g (dry wt). Precise records were not available, but the area received routine applications of DDT from the late 1940s to 1970. The soil has an organic carbon content of 1.4% and is designated as a fine sandy loam (56% sand, 36% silt, 8% clay; pH 6.7). The experimental design consisted of duplicate plots of four separate species, with collection and analysis of three separate soil fractions, root, and shoot systems from each replicate plot. In May 2000, two separate plots (each 3 × 3 m) were constructed for each crop, and within each plot seeds were planted in three rows roughly 0.7 m apart. Approximately 100 to 125 seeds were used in each row. The plots were weeded and watered as necessary. On 11 occasions, two rows of all experimental plots received 0.005 or 0.05 M sodium citrate (pH adjusted to 7.0), and the third row received water. Approximate 1 L of solution was used per row for each crop. In August, the soil (bulk, near root, and rhizosphere) and vegetation (roots and shoots) were harvested and composited by crop type, plot number, and treatment regime. No p,p’-DDE-free soil could be located at the farm, so clean-soil control plots were not established.

**Soil extraction procedure**

The extraction procedure used was that of Mattina et al. [15] and has been described elsewhere [29,30]. Bulk soil samples were collected from the top 15 cm of individual plots, 0.75 m distant from vegetation at harvest. The near-root zone was operationally defined as the soil that fell off the roots at harvest, as well as the soil remaining within the volume occupied by the roots. This zone is well within the 0.75 m where the bulk soil samples were taken, since the root systems had minimal lateral growth. The depth of the near root zone was 12 to 15 cm depending on the plant species. The rhizosphere was operationally defined as the soil that remained attached to the roots at harvest.

Harvested root samples of all plants were air-dried for 1 to 2 hours, and the rhizosphere soil was removed with a fine bristle toothbrush. All soil fractions were air dried to ensure homogeneous moisture content and were sieved to 0.5 mm to remove particulates and provide sample uniformity. The moisture content of a representative soil sample of each treatment was determined (100°C for 24 h) for every three samples extracted. The moisture content of the air-dried soil (all fractions) ranged from 3 to 8% (by weight). Portions (3.0 g) of the soils from each plot and treatment were added to Teflon® PFA-lined digestion vessels from the CEM MES-1000 microwave solvent extraction system (CEM, Mathews, NC, USA) containing 50 ml of 2:3 (v/v) hexane/acetone (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA). Transnonachlor (500 ng) was added as an internal standard, and the sealed vessels were extracted in the CEM MES-1000 oven with the program of 100% power, 7 min ramp to 120°C, and 20 min hold time. The supernatant (and two additional hexane:acetone rinses of the soil) was decanted into Kuderna-Danish flasks fitted with 10-mL concentrator tubes containing a boiling chip. A Snyder column (Fisher Scientific, Springfield, NJ, USA) was fitted to the flask and the solvent was reduced to less than 1 ml in a 95°C water bath. Three ml of 2,2,4-trimethylpentane (Ultra-Resi-Analyzed, J.T. Baker) was added and the volume was again reduced to 1 ml. The flask was then removed and rinsed with an additional 3 ml of trimethylpentane. The extract (final volume 6–8 ml) was passed through a glass microfilter (0.2 µm, Laboratory Science, Sparks, NV, USA) prior to analysis. This procedure has been validated elsewhere [15,29,30].

**Vegetative extraction procedure**

Pylypiw’s [31] method was used to extract p,p’-DDE from the vegetation. No effort was made to analyze for the presence of p,p’-DDE metabolites within the plant tissues. All vegetation was washed with tap water to remove attached soil particles. One hundred gram samples (wet wt) of the roots were added to 300 ml of distilled water and sonicated for 15 min (FS30, Fisher Scientific) to ensure complete removal of residual soil. All vegetation was finely chopped and stored in a freezer in 500-ml amber glass bottles with Teflon-lined caps prior to extraction. Fifty-gram portions of the vegetation were then weighed into a 1-qt blender jar containing 50-ml of 2-propanol (Ultra-Resi-Analyzed, J.T. Baker) and 3 µg of transnonachlor as an internal standard. The sample was blended at high speed for 30 s and then amended with 100-ml volume of petroleum ether (Ultra-Resi-Analyzed, J.T. Baker). The sample was then blended at 40% of full speed for 4 min. The extracts were decanted through funnels containing glass wool and collected in a 500-ml glass separatory funnels with Teflon stopcocks. The solids were drained for 20 min, and 200 ml of distilled water and 10 ml of saturated sodium sulfate solution were added to each funnel. The funnels were capped, shaken gently for 1 min, and the phases were allowed to separate for 15 to 20 min. The water layer was drawn off and the petroleum ether was rinsed two additional times with 200 ml of distilled water. The final petroleum ether extract (~60–70 ml) was amended with 15 g of anhydrous sodium sulfate and allowed to sit for 2 to 3 h prior to analysis.

**Desorption to Tenax**

One-gram 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 sodium citrate solution (0.005, 0.05, or 0.50 M). All solutions were previously adjusted to pH 7.0 with 2 M hydrochloric acid. Each vial was then amended with 1 µg of transnonchlor (internal standard) and 300 mg of Tenax® beads (20/35 mesh, Supelco, Bellefonte, PA, USA), which served as an infinite.
The amount of \( p,p' \)-DDE in the trimethylpentane soil extracts and the petroleum ether vegetative extracts was determined on a Hewlett-Packard (Avondale, PA, USA) 5890 gas chromatograph with a \( 6^{3} \)Ni electron capture detector. The column (30 m × 0.53 mm 0.5 μm) contained a SPB-1 film (Supelco). The gas chromatograph program was 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% CH₄ in Ar at 20 ml/min. The electron capture detector was maintained at 325°C.

A stock of \( p,p' \)-DDE (100 μg/ml in methanol) was purchased from Chem Service (West Chester, PA, USA). The stock was transferred to either trimethylpentane (for soil extracts) or petroleum ether (for vegetative extracts) and diluted to prepare calibration standards of \( p,p' \)-DDE at 10, 25, 50, 100, 150, 250, and 500 ng/ml in each solvent. Each calibration level contained 100 ng/ml transnonachlor for internal standard calibration. The retention times of transnonachlor and \( p,p' \)-DDE were 20.95 and 23.95 min, respectively.

### Statistical analysis

All reported concentration values of \( p,p' \)-DDE are expressed on a dry-wt basis of either soil or vegetation and are the average of duplicate injections. The variation between duplicate injections was less than 3%. At harvest in the field, tissue types (roots or shoots) were composited by plot and citrate treatment and were subsampled for extraction. A one-way analysis of variance (ANOVA) followed by a Tukey multiple comparison test (at 0.05) was used to assess the statistical significance of differences in \( p,p' \)-DDE concentrations in soil or vegetation with regard to citrate treatment within individual plant species. Recovery of the internal standard was 102% (±9.5).

### Results and Discussion

**Soils**

Within each experimental plot, three separate soil fractions (bulk, near root, and rhizosphere) that varied in the influence exerted by the plant were collected from individual rows of vegetation. The concentration of \( p,p' \)-DDE in the three soil fractions from randomly selected plots containing clover, hairy vetch, mustard, and rye grass are shown in Figure 1. For all crops, all watering treatments (water or sodium citrate), and all plots, the concentration of \( p,p' \)-DDE in both the near root and rhizosphere was significantly less (ANOVA followed by Tukey multiple comparison at 0.05) than the amount of contaminant in the bulk soil. In some cases, the bulk soil concentrations in the duplicate plots for each crop varied significantly, making it difficult to express the soils data collectively. For example, the bulk soil concentrations in two plots containing hairy vetch were 228 and 125 ng/g soil (dry wt), respectively. Consequently, the data obtained from individual or replicate plots were normalized to the average bulk soil concentration for that specific plot. Thus for a given crop the data for all soil fractions (regardless of watering treatment) from the duplicate experimental plots were averaged and expressed as a fraction of the respective bulk soil concentrations (i.e., a fraction of 1.0). These results are shown in Table 1.

For plots containing clover and rye, the presence of sodium...
Table 1. Influence of crop type on the normalized concentration of 2,2-bis(p-chlorophenyl)1,1-dichloroethylene (p,p'-DDE) in the bulk, near root, and rhizosphere soil

<table>
<thead>
<tr>
<th>Crop</th>
<th>Bulk(^b)</th>
<th>Near root(^c)</th>
<th>Rhizosphere(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clover</td>
<td>1.0 (16) A(^e)</td>
<td>0.73 (18) B</td>
<td>0.74 (18) B</td>
</tr>
<tr>
<td>Vetch</td>
<td>1.0 (16) A</td>
<td>0.82 (18) B</td>
<td>0.81 (18) B</td>
</tr>
<tr>
<td>Mustard</td>
<td>1.0 (16) A</td>
<td>0.76 (15) B</td>
<td>0.87 (18) B</td>
</tr>
<tr>
<td>Rye</td>
<td>1.0 (15) A</td>
<td>0.74 (18) B</td>
<td>0.77 (18) B</td>
</tr>
</tbody>
</table>

\(^a\) Number of replicates (each replicate is the average of duplicate injections); all watering treatments (citrate or no citrate) are expressed collectively within each soil fraction.

\(^b\) Soil collected from vegetation-free areas before planting and after harvest. All values were normalized to the average bulk soil concentration.

\(^c\) Soil that falls off the roots upon harvest or is within the area encompassed by the roots. The values are expressed as a fraction of the average bulk soil concentration.

\(^d\) Soil that remains attached to the roots upon harvest. The values are expressed as a fraction of the average bulk soil concentration.

\(^e\) Within rows, values followed by the same letter are not significantly different (at 0.05 level) using a Tukey multiple comparison test after a one-way analysis of variance (ANOVA).

citrate at 0.005 or 0.05 M did not significantly effect the reductions in p,p'-DDE concentration observed in the near root and rhizosphere relative to the respective bulk soils. However, for both experimental plots containing hairy vetch the reductions in concentration observed in the near root and rhizosphere soil relative to the bulk soil value were significantly less in the rows that received either citrate treatment as compared with the vegetation amended with water. The percent reduction in p,p'-DDE concentration from the bulk soil level of plots receiving water, 0.005 M, and 0.05 M citrate were 40.5, 16.8, and 24.4% in the rhizosphere and 59.7, 14.6, and 14.1% in the near root zone, respectively. Similarly, the reduction in the rhizosphere and near root zone concentrations of p,p'-DDE (normalized to the bulk soil values) in plots containing mustard were significantly less (p < 0.05) in the presence of 0.05 M citrate than in those soil fractions amended with water.

The findings of plant-induced alterations in the p,p'-DDE concentration in soil fractions differing in their proximity to plant roots agrees with earlier findings. White [29,30] described similar reductions in the concentration of weathered p,p'-DDE in the soil of the root systems of alfalfa, spinach, pumpkin, and zucchini. The relationship between citrate treatment and relative amounts (i.e., percent reduction) of p,p'-DDE in the near root and rhizosphere as compared with the bulk soil concentration remains unknown. We have speculated that a plant-induced increase in pollutant availability mediated by the root exudates is followed by transport of the contaminant in the transpiration stream of water toward the roots [29,30]. These findings of reduced pollutant concentrations in the near root and rhizosphere soils are somewhat expected given the concurrent findings of measurable and sometimes significant levels of the contaminant within the vegetation. However, the effects we describe are with pesticide residues that have been sequestered/weathered in the soil for decades. Although the time course of p,p'-DDE bioavailability is unknown, the fact that uptake of a sequestered POP from soil occurs at all is significant. In addition, the ability to detect these losses after a single growing season within the remaining soil has relevance for the possible phytoremediation of POPs in soil.

Vegetation

The root and shoot systems of clover, hairy vetch, mustard, and rye grass were analyzed for p,p'-DDE. The concentration of p,p'-DDE in the roots from randomly selected plots receiving water or sodium citrate (0.005 or 0.05 M) are shown in Figure 2. On the left-hand portion of the figure the average bulk soil concentrations within the individual plots are shown. The roots of all plants contained significant levels of p,p'-DDE, with the water-amended roots containing 2 to 5 times the amount of contaminant found in the bulk soil. The variation in bulk soil concentration among the duplicate plots also led to similar variations in the root systems of specific crops. For example, the duplicate plots of clover had bulk soil p,p'-DDE concentrations of 158 and 113 ng/g, yielding root concentrations of 644 and 429 ng/g, respectively. Consequently, all root concentrations for a given plant were normalized to the average concentration of p,p'-DDE in the roots of the water treatment of that plot (Table 2). In addition, the concentration of p,p'-DDE in the shoot system of each crop was normalized to the total amount present in the roots of that specific treatment (water, 0.005 or 0.05 M citrate) and those data are also shown. As evident in Table 2 and Figure 2, treatment with 0.05 M citrate significantly (ANOVA followed by Tukey multiple comparison test at 0.05) increased the uptake of p,p'-DDE from soil by clover, vetch, and mustard as compared with vegetation receiving only water. At 0.005 M citrate, the results were mixed, with one crop (rye) showing reduced uptake of p,p'-DDE, two crops (vetch and mustard) showing no statistical effect (p < 0.05), and one (clover) showing enhanced contaminant uptake, all being compared with plants amended with water. The translocation of p,p'-DDE out of the root system ranged from 5 to 16% of the contaminant present in the roots for the four crops, yielding low concentrations ranging from 50 to 104 ng/g. No relationship was evident between citrate treatment and extent of p,p'-DDE transport to the aerial tissues.
Table 3. Effect of citrate amendment on the desorption of weathered 2,2-bis(p-chlorophenyl)1,1-dichloroethylene (p,p'-DDE) from soil to Tenax and release of metal ions to the aqueous solution

<table>
<thead>
<tr>
<th>Solution</th>
<th>p,p'-DDE desorbed (%)a (SD)b</th>
<th>Aqueous concentration of metals (mg/L)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (soil extract)</td>
<td>-</td>
<td>Al</td>
</tr>
<tr>
<td>0.005 M citrate (soil extract)</td>
<td>17.8 (1.82) A d</td>
<td>6.94</td>
</tr>
<tr>
<td>0.05 M citrate (soil extract)</td>
<td>21.6 (0.01) B</td>
<td>38.8</td>
</tr>
<tr>
<td>0.50 M citrate (soil extract)</td>
<td>22.3 (0.01) B</td>
<td>56.2</td>
</tr>
<tr>
<td>0.50 M citrate (aqueous solution)</td>
<td>19.5 (0.01) AB</td>
<td>32.2</td>
</tr>
</tbody>
</table>

a Soil concentration of p,p'-DDE was 526 ng/g (dry wt).
b SD = standard deviation of triplicate samples.
c Determined by inductively coupled plasma-atomic emission spectroscopy according to U.S. Environmental Protection Agency method 6010; detection limits for Al, Ca, Cu, Fe, K, and Mn were 0.2, 1.0, 0.1, 0.2, 0.2, and 0.2 mg/L, respectively.
d Values followed by the same letter are not significantly different (at 0.05 level) using a Tukey multiple comparison test after a one-way analysis of variance (ANOVA).
e Aqueous solution of 0.50 M citrate analyzed for background metals content.
f Not applicable.

The findings of significant levels of p,p'-DDE in the roots of clover, vetch, mustard, and ryegrass agree with previous studies. White [29,30] described moderate concentrations of p,p'-DDE in the roots of alfalfa, bean, ryegrass, and spinach and significant concentrations of the contaminant in the roots of pumpkin and zucchini. Similar findings have been reported for the uptake of chlordane by the roots of zucchini, pumpkin, cucumber, lettuce, and spinach [28]. Other POPs available for plant uptake include dioxins [27,33], dibenzofurans [27,33], aldrin [26], and heptachlor [26]. It is evident that considerable species variability exists in the extent to which weathered organic pollutants can accumulate in roots from soil. As early as 1960, Lichtenstein et al. [26] reported on the ability of certain cucurbits to readily mobilize and accumulate significant levels of organochlorine pesticides. White [29] observed levels of p,p'-DDE in the roots of zucchini and pumpkin that were 10 to 20 times that found in the soil (dry wt basis), whereas the roots of crops in the current study contained only 2 to 5 times the amount of pollutant in the soil. In a survey of 11 crops, Mattina et al. [28] reported root concentrations of chlordane that ranged from 0.24 (tomato) to 10 (zucchini) times the concentrations found in the soil. It is unclear if the extent of POP translocation to aerial tissues is also species-specific. Mattina et al. [28] observed little or no chlordane in the fruit of tomato and pepper, but significant (300–500 ng/g dry wt) concentrations in the fruit of zucchini and pumpkin [34]. However, for certain crops it appears that the low POP concentrations in the aerial tissues are a function of low concentrations absorbed by the roots. For example, the shoot concentrations of p,p'-DDE in rye and mustard ranged from 25 to 35 ng/g (dry wt) or from 6 to 11% of the concentration present in the roots. Conversely, White [29] reported concentrations of p,p'-DDE up to 400 ng/g (dry wt) in zucchini fruit, but these levels were only 3 to 8% of the concentration present in the roots. These types of comparisons are difficult to make because of the lack of information concerning the exact weight of the root and shoot systems, as well as information on other factors such as lipid content. However, it is possible that much of the observed variability in concentrations of POPs in aerial tissues may simply be the result of differential uptake to the roots, not crop-specific translocation through the shoot system. In addition, given the low levels of p,p'-DDE detected in the aerial tissues of all species, the contribution of atmospheric deposition may be significant. Jantunen et al. [35] described seasonal variations in the atmospheric concentrations of several organochlorine pesticides, including p,p'-DDE. There is also considerable evidence documenting the atmospheric deposition of such air-borne organic pollutants onto vegetation [27,36,37]. Given the rather low concentrations observed in the aerial tissues, it is possible that a significant fraction of the contaminant is of atmospheric origin, possibly explaining the loss of a citrate effect in moving from the roots to the shoots.

Desorption to Tenax

The desorption of weathered p,p'-DDE residues to an in situ infinite sink (Tenax) was determined in the presence of water or one of three citrate concentrations (Table 3). At 0.05 and 0.005 M citrate, the release of p,p'-DDE was significantly greater (ANOVA followed by Tukey multiple comparison at 0.05) than in water alone. The aqueous concentration of six metals was also measured in the presence of citrate (Table 3). The extraction of all detected metals from the soil increased dramatically at 0.005 and 0.05 M citrate as compared with distilled water. With the exception of K, that trend was reversed at the highest citrate concentration (0.50 M). The decrease in extracted metals at the highest citrate concentration is in line with the observed decrease in p,p'-DDE desorption at 0.50 M. In addition, the coloration of the supernatant became increasingly darker at 0.005 and 0.05 M citrate, an indication of the increased amount of extracted humic material from the soil. However, at 0.50 M citrate, the coloration of the supernatant was only slightly darker than that of the distilled water control, supporting the findings of a decrease in the citrate effect at high concentrations. The explanation for this loss of an effect at 0.50 M citrate remains unknown, but it is possible that the high ionic strength of the solution relative to the small soil amount is a confounding factor.

The findings of increased mobilization of certain soil constituents and contaminants within that structure are in line with
recent work of others. Huang et al. [38] demonstrated that amendments of citric, malic, and acetic acid significantly increased the uptake by plants of weathered uranium from soil. Although the authors cited direct chelation and mobilization of the uranium molecules as a possible mechanism, they also observed that organic acids may chelate structural metals from the soil, resulting in a partial dissolution of the solid and subsequent release of uranium. Similarly, Yang et al. [39] described the increased desorption of weathered polycyclic aromatic hydrocarbons in the presence of citric acid and other metal-chelating agents. They speculated that citric acid dissolved certain regions of the soil structure through the chelation of specific polyvalent metal ions, resulting in the release of previously bound humic acid. This structural opening or expansion of the soil organic matter subsequently increased the availability of the contaminants therein. In this study, citrate increased in the desorption of weathered \( \text{p,p}'\text{-DDE} \) and increased the extracted aqueous concentration of five polyvalent metals from the soil. In the field, clover, vetch, and mustard that received citrate (0.05 M) showed statistically significant increases in the uptake of \( \text{p,p}'\text{-DDE} \) to their roots. The presence of citrate likely mobilized the bound residues through chelation of critical structural metal ions and release of previously bound humic materials. The previously sequestered contaminants are likely still associated with the mobilized humic materials but will follow the flow of water to the vegetation, resulting in enhanced availability of \( \text{p,p}'\text{-DDE} \) to the roots. The precise amount of citrate influencing individual plants is unknown because within rows tissues were composited and subsampled, and the number of plants per row was not determined. However, on 11 separate occasions individual rows (3 m long × 0.1 m wide) of clover, mustard, vetch, and rye were amended with 1 L of 0.005 or 0.05 M citrate or approximately 16.2 or 162 g of citrate per row of vegetation, respectively. Some interesting observations can be made in analyzing the soils and root data together. The concentration of \( \text{p,p}'\text{-DDE} \) in the near root and rhizosphere of vetch and mustard receiving citrate (0.05 M) was significantly greater than in those soil fractions when water alone was applied, but in the roots the concentration of \( \text{p,p}'\text{-DDE} \) was nearly 40% greater in the presence of citrate. We hypothesize that these seemingly contradictory findings of increased contaminant concentration in the soil of plants absorbing more pollutant can be reconciled by citrate mobilizing \( \text{p,p}'\text{-DDE} \) from areas further away from the root zone. Quantitative mass balance studies are necessary to confirm this shift in the spatial distribution of \( \text{p,p}'\text{-DDE} \) contamination, and such studies are currently being planned.

There has been speculation that the rather significant uptake of certain POPs by specific plants is facilitated through the activity of root exudates [27,29,30,33]. Exudates refers to any and all material released by the plant roots to the rhizosphere, and these constituents are classified as low molecular weight compounds, high molecular weight compounds, and volatiles [40]. Included in the high molecular weight constituents are a range of proteins and enzymes, with the main volatile compound being carbon dioxide [40]. The low molecular weight exudates include organic acids such as citrate, malate, and succinate; amino acids; growth hormones; and simple carbohydrates such as glucose and fructose. Specific plants have been shown to differ significantly in exudate quantity and composition, and these species-specific differences in exudation may explain the observed variability in POP uptake from soil [41]. Campanella and Paul [33] isolated a protein-like molecule from the vegetation of zucchini and melon that they hypothesized was responsible for the enhanced uptake of weathered dioxin residues. Other exudate constituents have been implicated in micronutrient scavenging and have been described as phytosiderophores [42]. Their function is to chelate specific required metals (Fe, Zn, Cu, Mn) from soil, thereby making these nutrients available for uptake. For example, certain low molecular weight root exudates solubilize phosphorus in soil by complexing with and precipitating cations in the soil that are associated with the bound phosphorus [39]. Other plant exudates (citrate, salicylate, and succinate) have been shown to effectively chelate Fe with stability/binding constants on the order of \( 10^9 \) to \( 10^{11} \) [40]. Volker and Fikry [43] observed that when plants precultured under Fe deficiency were planted in soil contaminated with heavy metals, the uptake of not only Fe but also of Zn, Ni, and Cd was increased by 200% relative to plants cultured with adequate Fe. We maintain that it is not surprising that some of these same nutrient-scavenging exudates may promote the bioavailability of anthropogenic contaminants bound within the soil.

The results herein show that the roots of several species of plants accumulate weathered \( \text{p,p}'\text{-DDE} \) at levels 2 to 5 times in excess of the soil concentration. The observed moderate extent of contaminant uptake is restricted mainly to the roots and offers only modest promise as a phytoremediation technology. However, the findings that citrate, a model root exudate, increased the release of \( \text{p,p}'\text{-DDE} \) and several metal ions from soil in the laboratory and also the uptake of the contaminant by plant roots under field conditions provide insight into the mechanism by which certain plants mobilize highly sequestered organic pollutants. We hypothesize that certain root exudates chelate critical structural metal ions (Al, Fe, Ca) from soil, thereby promoting the partial dissolution of the soil organic matter and subsequent increase in the availability of anthropogenic pollutants therein. We find it particularly noteworthy that this hypothesized phenomena occurs with \( \text{p,p}'\text{-DDE} \), given its status as a POP. POPs are a group of contaminants that are of unique concern because of their longevity in the environment, as well as their potential for toxicity, bioaccumulation, and biomagnification. The risk posed by highly persistent residues in the environment is largely unknown, with the literature suggesting significant receptor variability. Their physical characteristics and resistance to biodegradation make them unamenable to commonly employed remediation technologies. In fact, from the viewpoint of remediation, POPs are similar to heavy metals. Data has begun to accumulate in the literature indicating that the exudates of certain plants may indirectly facilitate the accumulation of significant levels of POPs in their tissues. Fundamental investigations focusing on the mechanism by which this phenomenon occurs are necessary to evaluate the utility of phytoremediation in dealing with these types of pollutants. Controlled growth chamber studies to establish a quantitative mass balance of the contaminant in which the masses of all soil and plant compartments are known and air exchange is regulated are necessary to precisely describe the movement and translocation of the weathered contaminants through abiotic and biotic systems. Such experiments are currently being planned. Current studies are also focusing on the characterization and isolation of exudate constituents critical to contaminant mobilization from sequestered sites in the soil. Once the basic mechanisms are elucidated,
future studies can then address methodologies for maximizing and augmenting pollutant removal.

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