Available online at www.sciencedirect.com

SCIENCE DIRECT®

Microchemical Journal 81 (2005) 148–155

MICROCHEMICAL JOURNAL

www.elsevier.com/locate/microc

Uptake of weathered *p*,*p*′-DDE by plant species effective at accumulating soil elements

Jason C. White[a,*], Zakia D. Parrish[a], Mehmet Isleyen[b], Martin P.N. Gent[c], William Iannucci-Berger[b], Brian D. Eitzer[b], Mary Jane Incorvia Mattina[b]

[a]*Department of Soil and Water, Connecticut Agricultural Experiment Station (CAES), 123 Huntington Street, New Haven, CT 06504, United States*
[b]*Department of Analytical Chemistry, CAES, United States*
[c]*Department of Forestry and Horticulture, CAES, United States*

Received 21 January 2005Accepted 22 January 2005
Available online 10 March 2005

Abstract

Ten plant species previously shown to accumulate inorganic elements effectively from natural solids were grown under field conditions in *p*,*p*′-dichlorodiphenyldichloroethane (*p*,*p*′-DDE) contaminated soil. The plant species, which included rye, mustard, canola, vetch, pigeonpea, clover, peanut, and 3 cultivars of white lupin, represented both monocots and dicots, as well as two major families within the dicots: the Brassicaceae and the Fabaceae. The plants varied widely in their ability to phytoextract and translocate weathered *p*,*p*′-DDE. The percentage of contaminant phytoextracted ranged from 0.06% (white lupin) to 0.22% (clover, vetch), and the translocation factors (TF; contaminant concentration ratio of stems to roots) ranged from 0.04 (clover, white lupin) to 0.37 (canola). An inverse relationship exists between the amount of contaminant in the roots as measured by the root BCF (bioconcentration factor; dry weight contaminant concentration ratio of root to soil) and the TF. Duplicate mounds of each species were periodically amended with nitrogen (N), phosphorus (P), nitrogen and phosphorus together (N/P); a minus phosphorus treatment involved the addition of $AlSO_4$ to the soil prior to planting. The effect of nutrient regime on plant biomass, *p*,*p*′-DDE uptake and translocation, and inorganic element content varied greatly among the 10 plant species. For some species (rye, vetch, pigeonpea, clover, white lupin), reductions or non-significant changes in *p*,*p*′-DDE uptake were observed under the nutrient treatments and were not correlated with plant biomass effects. For mustard, canola, and peanut, the percentage of *p*,*p*′-DDE phytoextracted in the various treatments was more than doubled and was directly correlated with a two-fold increase in total plant biomass. Although it is generally assumed that fertilizer amendments will enhance the phytoremediation of organic and inorganic pollutants, the data here suggest that such effects are highly species specific and in some cases may actually decrease remediation potential.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Phytoextraction; *p*,*p*′-DDE; Persistent organic pollutants; Phytoremediation

1. Introduction

Phytoremediation is a promising in situ technology where vegetation is used to remove organic and inorganic contaminants from soils and sediments. This technology has received much attention due to its modest cost, low impact on the natural landscape, and successful application under specific conditions [1]. However, for both engineered applications and basic research, phytoremediation has been strongly dependent on contaminant type: inorganic or organic pollutants. The phytoremediation of inorganic contaminants such as Cu, Zn, Pb, Ni, Cd, Se, and As has been shown to occur by direct uptake or phytoextraction mediated by the flow of water into the plant, followed by sequestration in vegetative tissues [2–5]. For organic contaminants, a variety of plant-mediated remedial processes are possible. Plant roots may exude enzymes into the root zone or rhizosphere that may degrade susceptible organic pollutants [2,6]. Alternatively, plant roots release large quantities of carbon; some estimates reach 20% of the total carbon fixed by photosynthesis. This input of carbon

* Corresponding author.
E-mail address: jason.white@po.state.ct.us (J.C. White).

0026-265X/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.microc.2005.01.010

into the rhizosphere stimulates a microbial community that may be 1–3 orders of magnitude larger than in non-vegetated soil. In turn, this enhanced microbial community may break down susceptible organic compounds [2]. A wide range of organic pollutants have been shown to be degraded to a greater extent in the rhizosphere than in plant-free soil, including pesticides such as atrazine and 2,4,5-trichlorophenoxyacetic acid [7,8], polychlorinated biphenyls [9], chlorinated solvents such as trichloroethylene [10], explosives such as 2,4,6-trinitrotoluene [11], and polycyclic aromatic hydrocarbons [12,13]. Lastly, relatively hydrophilic compounds (Log K_{ow} values 1.0–3.5) may be effectively transported into vegetative tissues with the flow of water, where plant-based enzyme systems may facilitate degradation, storage, or transpiration of the pollutant [2,14]. It has been assumed that more hydrophobic pollutants would not desorb from soil organic matter effectively and that the interior hydrophobic portion of the cell membrane lipid bilayer would exclude more hydrophilic contaminants [2,15].

As described above, compartmentalization of research and technologies for organic and inorganic contaminants has dominated the field of phytoremediation since its inception. However, we have shown that *Cucurbita pepo* ssp. *pepo* phytoextracts large amounts of highly weathered persistent organic pollutants (POPs) from soil and translocates significant quantities to aboveground tissues [16–18]. This has proven to be a unique system given the abundance of literature indicating that highly weathered hydrophobic organic compounds will have very low bioavailability and that diffusion of these contaminants out of their sequestered sites in soil organic matter will be a highly rate-limited process, regardless of what plant or animal species is present in the soil [19]. In addition, we have noted that *C. pepo* and other species shown to more moderately access POPs, *Spinacia oleracea* and *Lactuca sativa*, also contain higher than expected levels of certain soil elements, including Cd, Zn, and As [20]. We hypothesize that uniquely evolved nutrient acquisition mechanisms for plants such as *C. pepo* may lead to a convergence rather than divergence in the phytoremediation of inorganic and organic pollutants, and the ability of *C. pepo* to concurrently phytoextract weathered POPs and heavy metals is the subject of ongoing investigations.

The purpose of the current study was to explore the possibility that plant species shown to accumulate soil elements may actually have unforeseen potential to phytoextract weathered *p*,*p*′-DDE. A range of plant species was chosen based on reports in the literature indicating accumulation of certain heavy metals or an ability to effectively acquire soil nutrients: mustard (*Brassica juncea*) [21], canola (*Brassica napus*) [22], pigeonpea (*Cajanus cajan*) [23,24], peanut (*Arachis hypogaea*) [24,25], and 3 cultivars of white lupin (*Lupinus albus*) [26]. Crimson clover (*Trifolium incarnatum*) was included because it is a legume and is commonly used in phytoremediation studies [27]; perrenial ryegrass (*Lolium multiflorum*) and hairy vetch (*Vicia villosa*) were included because of their use in agriculture and phytoremediation studies [27,28]. In addition, the effect of various nutrient amendments on the *p*,*p*′-DDE phytoextraction potential by all of these plant species was investigated.

2. Materials and methods

2.1. Field plot

The following plant species were purchased from Johnny's Selected Seeds (Albion, ME): perrenial ryegrass (*L. multiflorum*), mustard (*B. juncea*), canola (*B. napus*), hairy vetch (*V. villosa*), pigeonpea (*C. cajan*), crimson clover (*T. incarnatum*), and peanut (*A. hypogaea*). In addition, 3 cultivars of white lupin (*L. albus*) were used; *L. albus* cv "Homer" was acquired from Dr. E. van Santen (Auburn University) and *L. albus* cv "Amiga" and "Weibit" were acquired from Sudwestaat GbR (Rastatt, Germany). The field plots were established at The Connecticut Agricultural Experiment Station's Lockwood Farm (Hamden, CT). Previous studies have found weathered *p*,*p*′-DDE residues at concentrations ranging from 90 to 1200 ng/g (dry weight). The soil is a fine sandy loam (56% sand, 36% silt, 8% clay) with a pH of 6.7 and an organic carbon content of 1.4%. The inorganic element content of the soil in mg/kg is as follows: Cd (0.15), Cu (8.0), Mn (300), Ni (9), Pb (37), Zn (39), Ca (1100), K (720), S (200), P (670), Al (11,000), Mg (2300), and Fe (8300). The entire field plot was covered with 1250 m^2 of polyethylene black plastic to minimize potential volatilization and subsequent aerial deposition of *p*,*p*′-DDE onto vegetation. Thirty cm^2 squares were cut out of the plastic at 2.0-m intervals. In late May 2003, approximately 5–30 seeds of each species were directly seeded into 2-cm deep furrows in the middle of each square. There were 5 nutrient treatments for each species and duplicate squares or mounds for each treatment. Commencing 1 week after planting, the nutrient treatments began and continued on a weekly basis until destructive harvest (August 2003). The individual treatments were 500 mL of water only (control), nitrogen (200 mg/L KNO_3), phosphorus (400 mg/L K_2HPO_4), nitrogen/phosphorus (200 mg/L KNO_3 and 400 mg/L K_2HPO_4), and minus phosphorus. The minus phosphorus treatment consisted of a one-time 1-L addition of 1 g/L $AlSO_4$ added to the soil prior to planting to bind available P. All solutions were pH adjusted to 6.7–7.0 prior to application. For the first 3–4 weeks, nutrient amendments were made to the soil in which the seedlings were growing; as the plants increased in size, the amendments were made to the shoot system of the plants with spray bottles. Over 107 days of growth, the plants received nutrient treatments on 12 occasions. Other than nutrient amendments, the vegetative plots were watered and weeded as necessary.

2.2. Quantitation of p,p′-DDE in soil

Four soil cores (2.5-cm diameter, approximately 10-cm depth) were collected from each replicate square or mound for all treatments prior to seeding. The duplicate samples were composited and stored at room temperature in 250-mL amber glass bottles sealed with Teflon-lined screw caps. All soils were air dried for 12–24 h and were sieved to 0.5 mm to remove non-soil debris and to facilitate homogeneous sampling. Soil moisture was determined by placing a 3.0-g portion of each sample in an oven at 100 °C for 24 h. In addition, up to five 3-g samples of soil from each treatment were transferred to 40-mL amber vials containing 15 mL of hexane. Trans-nonachlor (1 μg in hexane) was added as an internal standard. The vials were sealed with Teflon-lined caps and placed in an oven at 70 °C for 5 h. Prior to analysis, a 1-mL portion of the supernatant was passed through a glass microfiber filter (0.2 μm, Laboratory Science, Sparks, NV) for particulate removal.

2.3. Quantitation of p,p′-DDE in vegetation

After 100–110 days of growth, all plant species were destructively harvested and the mass of separate tissue compartments of all replicate mounds was determined on site with an Ohaus field balance (Fisher Scientific, Suwanee, GA). Clover, rye, and vetch consisted of roots and shoots. Pigeonpea, mustard, canola, and white lupin consisted of roots, stems, and leaves. Peanut consisted of roots, stems, leaves, and fruit. At harvest, separate plants from replicate mounds of vegetation were composited by treatment and all vegetation was washed thoroughly with water to remove attached soil. Each plant tissue type was separately finely chopped and stored by treatment in a freezer in 250-mL amber glass bottles with Teflon-lined caps prior to extraction.

Extraction of *p*,*p*′-DDE from vegetation was conducted as described by Pylypiw [29]. Ten to thirty grams of vegetation (wet weight) was added to 1-quart blender jars containing 30 mL of 2-propanol and 3 μg of trans-nonachlor (internal standard). The sample was blended for 30 s, 60 mL of petroleum ether was added, and the sample was blended for 5 min. The extract was poured through a funnel packed with glass wool and was collected in a 500-mL glass separatory funnel with Teflon stopcocks. After settling for 20 min, 100 mL of reverse osmosis (r.o.) water and 10 mL of saturated sodium sulfate solution were added and the funnels were capped, shaken gently for 10 s, and the solvent phases were allowed to separate for 20 min. After drawing off the water layer, the petroleum ether was rinsed with 100 mL of r.o. water two additional times, once with 50 mL of distilled water, and once with 10 mL of saturated sodium sulfate solution. The petroleum ether was collected in a graduated cylinder containing 10 g of anhydrous sodium sulfate, and a portion was passed through a glass microfiber filter (0.2 μm, Laboratory Science, Sparks, NV) into a chromatography vial prior to storage in a freezer.

Stocks of crystalline *p*,*p*′-DDE and trans-nonachlor were acquired from the EPA National Pesticide Standard Repository (Fort Meade, MD) and portions were transferred to the appropriate solvent: petroleum ether (vegetation) or hexane (soil). The *p*,*p*′-DDE stock solution was diluted to prepare calibration standards at 10, 25, 50, 100, 150, 250, and 500 ng/mL and each calibration level was amended with 100 ng/mL trans-nonachlor as an internal standard. The *p*,*p*′-DDE content in the solvent extract 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×0.53 mm×0.5 μm) contained an SPB-1 film (Supelco, Bellefonte, PA) and the GC program was 175 °C initial temperature ramped at 3.5 °C/min to 225 °C, then ramped at 25 °C/min to 250 °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 °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 °C. The retention times of trans-nonachlor and *p*,*p*′-DDE were 9.9 and 10.3 min, respectively.

2.4. Extraction of inorganic elements

Individual tissue samples (roots, stems, leaves, fruit) from each treatment of each species were extracted to quantify concentrations of 10 inorganic elements (N, Al, Mn, B, Mg, Cu, Zn, P, Cd, Pb). The vegetation was dried at 100 °C for 24 h and a portion (0.5 g) was digested with 10 mL concentrated HNO_3 on a hot plate for 30 min. All inorganic element concentrations (except Cd and N) were determined by Inductively Coupled Plasma Emission Spectroscopy (ICP-OES) using the Atom Scan 16 (Thermo-Jarrell Ash, Franklin, MA). Cadmium was analyzed with a PE 5100PC graphite furnace atomic absorption spectrometer (GFAAS; Perkin Elmer, Wellesley, MA, USA), and N was analyzed by a LECO FP-528 Nitrogen Analyzer utilizing an induction furnace and thermal conductivity (Leco, St. Joseph, MI).

2.5. Statistical analysis

All reported *p*,*p*′-DDE and inorganic element concentrations are on a dry weight basis. Differences in *p*,*p*′-DDE or inorganic element content of the different species′ tissues under the different nutrient regimes were assessed either by a Student's *t*-test or by a two-way analysis of variance (two-way ANOVA) followed by a Student–Newman–Keuls multiple comparison test using SigmaStat 3.0 (SPSS, Chicago IL).

3. Results and discussion

3.1. Soils

Five to eight replicate bulk soil samples were extracted for each treatment of each plant species. The *p*,*p*′-DDE

content of the soil was similar to previously reported values [16,18], ranging from 98 (pigeonpea plus N) to 610 ng/g *p,p′*-DDE (white lupin cv "Homer" control).

3.2. Control vegetation *p,p′*-DDE and inorganic element content

Detectable residues of *p,p′*-DDE were observed in all tissues of all plant species analyzed. In all species except pigeonpea, the concentration of *p,p′*-DDE was greater in the roots than in the aerial tissue. For pigeonpea, the concentration among roots, stems, and leaves was low and not significantly different. Concentrations of *p,p′*-DDE in the roots ranged from 57 ng/g (pigeonpea) to 910 ng/g (clover) and shoot concentrations ranged from 16 ng/g (white lupin cv "Weibit" leaves) to 110 ng/g (peanut leaves). Fig. 1 shows the total micrograms of *p,p′*-DDE in the root and shoot tissues of all 10 plant species; it is evident that the ability of these plant species to remove the contaminant from the soil and to translocate it to aerial tissues varied widely.

As indicated above, the concentration of *p,p′*-DDE within the plot varied by a factor of 6 and as such, direct comparison of contaminant concentrations in the vegetation is problematic. Bioconcentration factors or BCFs for *p,p′*-DDE were calculated as the dry weight contaminant ratio of tissue to soil (Table 1). The average root BCFs for *p,p′*-DDE ranged from 0.44 (pigeonpea) to 6.0 (clover). Both clover and vetch stand out, with the former accumulating 6 times more *p,p′*-DDE in its roots than present in the soil. Translocation factors or TF can be calculated by dividing the stem or shoot BCF by that of the soil and these values are also reported in Table 1. Similar to BCFs, TFs vary widely among the plant species, ranging from 0.04 to 0.37. Interestingly, an inverse relationship seems to exist with regard to the concentration of contaminant in the roots and the amount translocated to the stems (Fig. 2). This suggests that plants containing higher concentrations of *p,p′*-DDE either have much of the contaminant in the root system associated with the external surfaces of that tissue or that an inability to effectively translocate the pollutant results in an accumulation in or on the roots. These findings agree in part with those of White and Kottler [30] where as part of a separate study *p,p′*-DDE uptake into rye, vetch, clover, and mustard was measured. When root BCFs are calculated from those data, the values for rye, vetch, clover, and mustard are 3.0, 3.0, 4.0, and 2.7, respectively, yielding TFs of 0.06, 0.16, 0.11, and 0.10, respectively.

The total dry weight of the plant species is shown in Table 1 and ranges from 27 to 430 g. Because of the large amount of scatter, the data fail an ANOVA but clearly pair-wise differences would be significant. Interestingly, similar variability exists in the fraction of total plant biomass present

Table 1
DDE uptake characteristics and biomass of plant species shown to accumulate heavy metals

Plant species	Root BCF[a]	Translocation factor (TF)[b]	Total biomass (g, dry weight)	% Phytoextracted[c]
Rye	2.0 A[d]	0.09 AB	65 A	0.14 AB[e]
Mustard	0.71 AB	0.12 B[f]	150 A	0.10 AB
Canola	0.70 AB	0.37 C[f]	94 A	0.03 A
Vetch	3.6 C	0.16 BD	79 A	0.22 B
Pigeonpea	0.41 B	0.29 D	430 A	0.20 B
W.L. Homer	0.97 A	0.06 A	74 A	0.06 A
W.L. Amiga	1.2 A	0.04 A	100 A	0.12 AB
W.L. Weibit	1.8 A	0.04 A	52 A	0.08 AB
Clover	6.0 D	0.04 A	46 A	0.22 B
Peanut	1.3 A	0.18 D	27 A	0.09 AB

[a] BCF or bioconcentration factor; dry weight ratio of DDE concentration in roots to soil.
[b] TF or translocation factor; ratio of stem BCF to root BCF.
[c] % Phytoextracted; total amount of DDE in vegetation as a percentage of that in the soil compartment. Soil compartment mass calculated based on volume of soil occupied by the root system.
[d] With a column, values followed by different letters are significantly different as determined by a one-way repeated measures analysis of variance (one-way RM ANOVA) followed by a Student–Newman–Keuls multiple comparison test).
[e] For % phytoextracted, the one-way RM ANOVA was followed by a Dunn's multiple comparison test due to different numbers of replicate samples.
[f] Leaf BCF used as stems were reproductive structures that appear only during flowering.

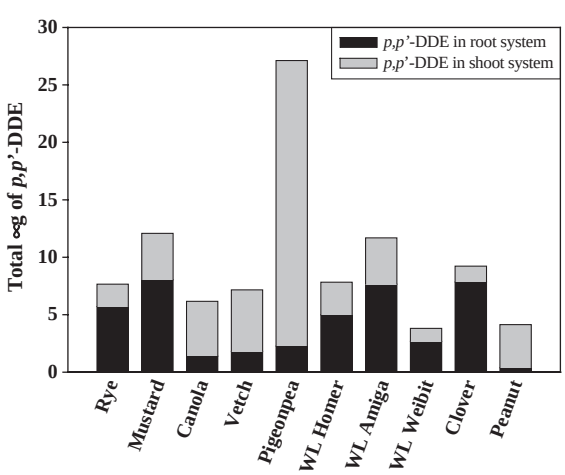

Fig. 1. Uptake of weathered *p,p′*-DDE in to roots and shoots of various plant species.

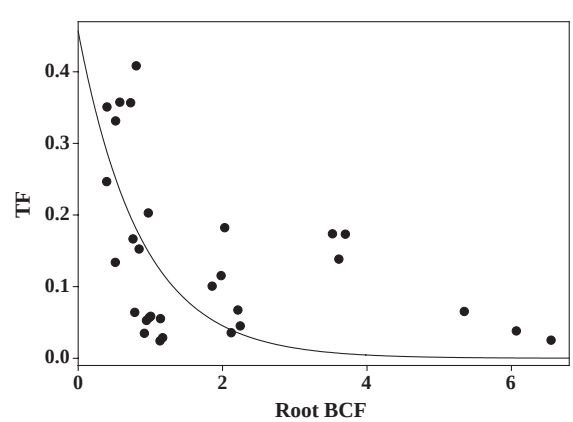

Fig. 2. Inverse relationship between root bioconcentration factors (BCF) for weathered *p,p′*-DDE and the translocation factor (TF).

in the root system, ranging from 3.0% (peanut) to 21% (rye). To calculate the absolute quantity of *p*,*p*′-DDE removed, the mass of the soil compartment for each species had to be estimated. This was done based on the volume of soil occupied by the root system of each plant species at destructive harvest and was converted to mass with a measured soil density of 1.14. The range in soil compartment mass was from 22 kg (white lupin cultivars) to 110 kg (pigeonpea and canola). Based on these values, the percentage of contaminant phytoextracted from the soil was calculated (Table 1) and the values differ by up to 7-fold. As evidenced by Fig. 1, the relative distribution of total *p*,*p*′-DDE among the root and shoot systems varied widely for these plant species, with clover and pigeonpea translocating 15 and 92% of the contaminant to the shoot system, respectively.

The concentrations of 10 inorganic elements were determined in the tissues of all plant species. Lead was detected periodically and only at trace quantities in certain tissues and is therefore excluded from further discussion. The average concentration of inorganic elements in the tissues of the various plant species is shown in Table 2. Because only duplicate extractions were performed for each tissue of each species, the statistical power of the analysis is somewhat lacking, but the standard error of the mean is shown in parentheses. Even without the appropriate statistical rigor, some differences are clearly evident. For example, one would assume that an Al content of 2600 mg/kg in rye roots would be significantly greater than values of 110 mg/kg in canola or pigeonpea roots were the analysis done again with greater replication. Among the noteworthy observations is the extremely effective translocation of Mn to the leaves of white lupin cultivars; this is a characteristic for which these cultivars were selectively bred (Sudwestaat GbR, Rastatt, Germany). Also of note are the Cd levels in the roots of rye, vetch, and peanut. Although this soil contains only 0.19 mg/kg Cd, these species accumulate nearly 6 times that concentration in their roots. Of particular note is the effective translocation of Cd by peanut, with 2.0 and 0.86 mg/kg in the stems and leaves, respectively. However, across the entire group of plant species, there was no discernable relationship between any element content in roots or shoots and that plant's ability to access and translocate weathered *p*,*p*′-DDE. Conversely, some interesting observations become evident when the 10 plant species are grouped on the basis of plant morphology; i.e.; those with stem compartments throughout their entire life cycle (white lupin cultivars, mustard, canola, pigeonpea, and peanut) as compared to those that have stems only at flowering (rye, vetch, clover). Although the biomass of these two groups of plants is not significantly different, a

Table 2
Inorganic element content (μg/g vegetation, dry weight) of tissues of plant species grown in *p*,*p*′-DDE contaminated soil (standard error)

Tissue	Mn	Zn	B	Al	P	Cu	Mg	Cd	N (%)
Roots									
Rye	170 (8.1)	59 (0.23)	1.0 (0.31)	2600 (140)	1400 (21)	24 (0.42)	880 (52)	1.1 (0.01)	0.84 (0.06)
Mustard	39 (2.9)	45 (1.8)	9.4 (0.41)	510 (62)	2800 (130)	18 (1.3)	1300 (63)	0.62 (0.01)	0.82 (0.11)
Canola	27 (1.1)	28 (0.83)	17 (0.20)	110 (7.6)	4500 (64)	5.0 (0.14)	1500 (50)	0.25 (0.01)	2.5 (0.02)
Vetch	73 (2.9)	48 (1.8)	18 (2.2)	1100 (77)	5500 (350)	29 (1.5)	1100 (54)	1.23 (0.11)	3.9 (0.09)
Pigeonpea	16 (0.46)	23 (0.79)	8.6 (0.39)	110 (6.7)	2000 (84)	10 (0.17)	1400 (50)	0.19 (0.09)	1.1 (0.09)
W.L. Amiga	190 (24)	30 (1.9)	11 (0.21)	640 (73)	4800 (420)	6.7 (0.51)	1200 (85)	0.18 (0.09)	1.9 (0.15)
W.L. Weibit	160 (15)	32 (5.7)	7.9 (0.63)	420 (130)	4100 (97)	8.3 (1.8)	1100 (57)	0.19 (0.04)	2.7 (0.21)
Clover	42 (4.7)	26 (2.7)	17 (0.40)	740 (140)	3900 (420)	21 (2.4)	4500 (420)	0.32 (0.03)	2.2 (0.11)
Peanut	29 (1.1)	24 (0.5)	15 (0.21)	310 (16)	1400 (74)	13 (0.62)	4000 (181)	1.2 (0.10)	2.0 (0.04)
Stems									
Mustard	19 (0.42)	33 (0.64)	13 (0.53)	41 (3.4)	2200 (30)	4.4 (0.29)	2000 (120)	0.44 (0.02)	1.3 (0.13)
Canola	35 (0.83)	36 (0.04)	23 (0.53)	7.7 (0.22)	4400 (120)	4.7 (0.20)	3700 (98)	0.25 (0.01)	3.7 (0.06)
Pigeonpea	34 (1.6)	52 (2.3)	14 (0.85)	24 (1.0)	2100 (64)	21 (0.59)	1600 (65)	0.49 (0.02)	1.9 (0.01)
W.L. Amiga	480 (55)	22 (2.1)	20 (0.84)	93 (20)	3800 (140)	4.5 (0.33)	1800 (140)	0.16 (0.03)	2.0 (0.05)
W.L. Weibit	600 (56)	21 (1.6)	15 (0.19)	69 (10)	3000 (370)	4.8 (0.01)	1700 (180)	0.07 (0.01)	1.4 (0.09)
Peanut	51 (0.17)	34 (0.31)	24 (0.53)	240 (2.9)	2200 (5.5)	9.8 (0.01)	7400 (77)	2.0 (0.01)	2.3 (0.07)
Leaves									
Rye	110 (0.85)	37 (0.12)	6.2 (1.9)	130 (5.9)	3000 (12)	9.0 (0.10)	2300 (45)	0.25 (0.01)	2.9 (0.04)
Mustard	67 (0.31)	51 (0.16)	28 (0.82)	45 (2.8)	3800 (11)	9.8 (0.17)	2100 (2.5)	0.51 (0.02)	2.4 (0.05)
Canola	77 (1.3)	49 (0.44)	31 (1.0)	9.8 (0.07)	4900 (34)	1.0 (0.21)	4600 (69)	0.59 (0.05)	4.4 (0.03)
Vetch	130 (0.01)	63 (0.65)	33 (1.3)	850 (1.2)	4000 (43)	11 (0.26)	2300 (40)	0.10 (0.01)	3.6 (0.09)
Pigeonpea	120 (1.1)	26 (0.50)	22 (0.06)	35 (0.76)	2900 (44)	14 (0.31)	2700 (31)	0.20 (0.01)	4.2 (0.03)
W.L. Amiga	3500 (77)	58 (0.90)	27 (0.60)	96 (4.2)	5000 (180)	8.0 (0.14)	3200 (140)	0.21 (0.01)	4.2 (0.01)
W.L. Weibit	2700 (70)	53 (0.03)	31 (2.8)	145 (4.1)	4300 (2.0)	8.9 (0.07)	2300 (120)	0.08 (0.01)	3.1 (0.01)
Clover	76 (1.5)	33 (0.28)	28 (0.16)	300 (19)	2300 (24)	12 (0.38)	3800 (59)	0.07 (0.01)	3.1 (0.07)
Peanut	92 (0.45)	33 (0.18)	31 (0.10)	150 (3.1)	2200 (4.5)	8.3 (0.06)	3500 (20)	0.86 (0.01)	3.2 (0.04)
Fruit									
Peanut	20 (0.04)	29 (0.47)	19 (0.23)	98 (0.48)	3200 (8.5)	12 (0.24)	2400 (52)	0.16 (0.04)	3.7 (0.06)

Table 3
Differential uptake of elements and weathered *p,p′*-DDE as a function of plant morphology and/or development

Parameter	Plants with stems only at flowering	Plants with stems
% Phytoextracted	0.19 A[a]	0.10 B
Root BCF	3.9 A[b]	1.0 B
Stem BCF	0.34 A[b]	0.12 B
Root Al content	1500 A[b]	350 B
Root Zn content	44 A[a]	31 B
Root Cu content	24 A[a]	10 B
Root Cd content	0.90 A[b]	0.44 B

[a] Values followed by different letters across a row are significantly different (one-way ANOVA followed by Student–Newman–Keuls multiple comparison test).
[b] Values followed different letters across a row are significantly different (one-way ANOVA on ranks followed by Dunn's multiple comparison test).

number of significant differences exist with regard to *p,p′*-DDE and element uptake (Table 3). Plant species that form stems only at flowering are better able to effectively accumulate and translocate weathered *p,p′*-DDE and a range of inorganic elements than do plants that have stems throughout their growth cycle. The reasons for this phenomenon are unclear but are not based on phylogenetic relationships. The plant species represent both classes of flowering plants; the monocotyledons (rye) and the dicotyledons (remaining species). Within the dicots, two separate families are represented, including the Brassicaceae (canola, mustard) and the Fabacea (lupins, peanut, pigeonpea, vetch, and clover).

Hydrophobic organic compounds undergo a time-dependent reduction in bioavailability in soils and sediments that has been termed sequestration [19]. Assays such as solvent extractability, biodegradation/mineralization, and toxicity/uptake studies to biota have demonstrated reduced availability of persistent organic pollutants (POPs) such as DDT, dieldrin, heptachlor, PCBs, and PAHs [31–35]. However, the species used in the current study were selected on the basis of their ability to scavenge inorganic elements in heavy metal contaminated or nutrient-poor soils. Mustard (*B. juncea*) is commonly used in the phytoremediation of metals such as Zn and Pb [21]. White lupin cultivars (*L. albus*) have shown to accumulate heavy metals from wastewater [26] and have served as a model system for acquisition studies of P and other required elements from nutrient-poor soils [36]. Similarly, canola (*B. napus*; [22]), peanut (*A. hypogaea*; [25]), and pigeonpea (*C. cajan*; [23]) have all been studied for their unexpected abilities to access P from soils deficient in that nutrient. Lastly, clover (*T. incarnatum*), rye (*L. multiflorum*), and vetch (*V. villosa*) have been used in phytoremediation studies with PAHs [27] and in nutrient uptake investigations [28].

The ability of these plant species to phytoextract and translocate weathered *p,p′*-DDE did vary significantly but as a group, levels of the contaminant removed from soil were modest and agree with the predicted minimal bioavailability of this type of contaminant. The average root and stem BCFs for *p,p′*-DDE of these plants was 1.2 (±1.7) and 0.18 (±.16), respectively. These values are in line with other studies from our group focused on plant species denoted as non-uptakers of weathered persistent organic pollutants. For example, White [16] reported *p,p′*-DDE root and stem BCFs of 1.4 (±0.89) and 0.41 (±0.33) for non-uptaker cucurbits; White et al. [18] observed root and stem BCFs of 2.4 (±1.9) and 0.45 (±0.027) for similar non-uptaker species. Alternatively, in both those studies *C. pepo* ssp. *pepo* had *p,p′*-DDE root and stem BCFs of 13 (±3.8) and 6.1 (±1.4) [16], respectively, and of 7.2 (±3.0) and 5.4 (±3.0), respectively [18]. As previously stated, for inorganic elements there were no discernable relationship between the content of a given element and the uptake of weathered *p,p′*-DDE. There are however a few interesting differences between the non-uptaker plants used in the current study and *C. pepo* ssp. *pepo* data from other investigations. For example, White et al. [37] reported leaf B, Zn, and Mg concentrations of 65, 150, and 8500 μg/g; these values are significantly greater (Student's *t*-test, $p<0.01$) than the average values of the 10 plants reported in the current study. The reasons for these differences are not known but given the possible relationship to more effective phytoextraction and translocation of *p,p′*-DDE by *C. pepo* ssp. *pepo*, further study on this issue is planned.

3.3. Impact of nutrient amendments

The effect of various nutrient amendments on the uptake and translocation of weathered *p,p′*-DDE and inorganic elements was determined. It should be noted that interpretation of minus P treatment is somewhat problematic. For example, in certain instances the root zone of the vegetation "out-grew" the region treated with $AlSO_4$. In addition, significant quantities of soluble Al and S are being added to the soil and the effect is unknown. Because of the variability in biomass among duplicate mounds of vegetation of the 10 species of plants, total biomass was not

Table 4
Effect of nutrient amendments on the phytoextraction of weathered *p,p′*-DDE by various plant species

Plant species	Control	+N	+P	+N/P	−P
Rye	1.0[a] A[b]	1.2 B	0.97 A	1.3 C	0.78 D
Mustard	1.0 A	1.2 B	1.8 C	2.3 D	3.3 E
Canola	1.0 A	1.8 BC	1.4 AB	1.7 ABC	2.4 C
Vetch	1.0 A	0.97 A	0.56 B	0.44 C	0.83 D
Pigeonpea	1.0 A	1.2 B	0.62 C	0.70 C	0.61 C
W.L. Homer	1.0 A	1.6 B	1.6 B	1.9 C	0.83 D
W.L. Amiga	1.0 A	0.85 B	0.50 C	1.6 D	1.4 E
W.L. Weibit	1.0 A	0.80 ABC	0.71 B	0.73 AB	1.2 C
Clover	1.0 A	0.87 B	1.1 C	1.5 D	1.7 E
Peanut	1.0 A	3.4 B	1.7 C	0.93 A	2.3 D

[a] For a given plant species, the percentage of *p,p′*-DDE phytoextracted under the control treatment is set equal to 1.0 and all other values for the different nutrient treatments are normalized to the control.
[b] Within a row, values followed by different letters are significantly different (one-way ANOVA followed by a Student–Newman–Keuls multiple comparison test).

significantly influenced by any of the nutrient treatments. However, several parameters describing the uptake and translocation of *p*,*p*′-DDE were affected by the amendments. Table 4 shows the effect of nutrient treatments on the percentage of *p*,*p*′-DDE phytoextracted from the soil. For each plant species, the amount of contaminant removed under the control treatment is set equal to 1.0 and all other values across the nutrient treatments are normalized to the control. Of the 40 possible instances, there are 33 cases where the nutrient amendment resulted in a value of *p*,*p*′-DDE removal that was significantly different than the controls and in 22 of those instances, the effect was increased phytoextraction. Although biomass was not significantly affected by the nutrient amendments, there was a weak correlation (r^2=0.353) between the normalized (treatment to control) plant biomass and percentage of *p*,*p*′-DDE phytoextracted by the treated plant species. There were no treatments that consistently increased or decreased phytoextraction across all 10 plant species or between plants differing morphologically (i.e., with stems throughout the life cycle or at flowering only). However, some of the individual effects were quite large, including the 3.4-fold increase in *p*,*p*′-DDE phytoextraction observed with peanut amended with N and the 3.0-fold increase of mustard under minus P conditions. The effect of the nutrients on bioconcentration factors was also mixed but instances of statistical significance were far less numerous. In fact, the only consistent effects within a plant species were increased root BCFs for pigeonpea under all nutrient amendments and decreased translocation factors (TFs) for canola under all treatments. For the root BCFs, there were only 10 instances of significant difference between the control and the treatments and 7 of those were increases. For the stem BCFs, there were 14 instances of significant difference and 7 of those were increases. For TFs, there were 17 instances of significant difference and 11 of those were increases.

The influence of nutrient amendments on the inorganic element content of all species was also determined. Again, there were no consistent effects on content of a given element across all plant species, and in general, the effects were quite small and not of statistical significance. For example, the average relative element content of the roots of plants receiving N, P, N/P, and minus P treatments were 1.0, 1.2, 1.1, and 1.2 times that of the respective control vegetation and for the stems, the treated plants had values 1.0, 1.0, 1.0, and 0.92 times that of controls. Interestingly, amendments of N and P, as well as minus P, did not significantly influence the content of those elements in the plants. For example, the average root N content of plants amended with N was 0.99 that of the controls, with a maximum value of 1.1 for clover, and in the shoots of plants receiving N, the average value was 0.96 that of the controls with a maximum value of 1.4 for mustard leaves. For plants receiving P amendments, the average relative P content of the roots and shoots of all species was 1.0, with maximum values of 1.2 in the roots (white lupin cv Amiga) and 1.6 in the shoots (mustard leaves). The N/P and minus P treatments also resulted in generally non-significant effects on the content of those elements in the plant tissues. The reason for a lack of increased nutrient content in the tissues of plants receiving amendments of those elements is not known but the results agree with White et al. [37] where the element content of cucurbits amended with similar nutrients were also not significantly impacted. Given these findings, it is possible that the levels of N and P are sufficient in the Lockwood Farm soil to support growth or that some other required nutrient may be limiting.

We have hypothesized that the remarkable ability of *C. pepo* ssp. *pepo* to accumulate weathered persistent organic pollutants is a secondary effect from unique modes of nutrient acquisition from soil. Thus, the primary goal of the current study was to investigate the corollary: do plant species known to effectively scavenge nutrients from soil have the ability to phytoextract weathered persistent organic pollutants? If so, what will be the effects of nutrient amendments on that uptake potential? In general, it is assumed that fertilizing plants with various nutrients will enhance overall remediation potential of both organic and inorganic compounds as larger, healthier plants are more likely to impact levels of the contaminant in the soil. White et al. [38] found that amendments of inorganic fertilizers increased plant biomass and resulted in greater degradation of crude oil in the soil than for unamended plants. Similarly, Hutchinson et al. [39] observed that two grasses amended with N and P degraded more petroleum sludge than did control vegetation. However, for certain heavy metals, antagonistic relationships may exist that preclude enhanced remediation potential. For example, As utilizes the P-uptake system and substrate competition results [40]. Similarly, Cd uptake by certain plant species has been shown to be related to Zn and Fe [41,42]. In addition, for plants that do not form mycorrhizal symbioses, a direct relationship between plant nutrient status and the release of root exudates may exist [36]. Plant species such as white lupin are known to form specialized proteiod or cluster roots which exude large quantities of low molecular weight organic acids (LMWOA) to facilitate nutrient availability under stressed conditions [36]. Ishikawa et al. [23], Tanaka et al. [25], and Hoffland et al. [22] described similar enhanced LMWOA exudation of pigeonpea, peanut, and canola, respectively, under nutrient deficient conditions. Alternatively, Ae and Shen [24] could not attribute the superior P-scavenging ability of peanut and pigeonpea to patterns of root exudation, and they speculate on the existence of P-solubilizing components within the root cell membranes of these species in a process described as “contact reaction.”

The data in the current study indicate that plant species vary in uptake and translocation of weathered *p*,*p*′-DDE but on the whole and much as predicted, bioavailability of the contaminant is quite low. An ability to effectively scavenge inorganic elements from soil does not correlate well with the ability to phytoextract weathered *p*,*p*′-DDE. Overall,

nutrient amendments to these plant species did not increase plant biomass or *p*,*p*′-DDE uptake, indicating that fertilization during phytoremediation trials may not result in greater remediation.

Acknowledgements

This work was funded partially through EPA STAR Grant R829405. We thank Lydia T. Wagner, Craig Musante, Terri Arsenault, and Lauren Pasniewski for technical assistance.

References

[1] J.L. Schnoor, Ground Water Remediation Technologies Analysis Center, 2002, Tech. Eval. Rep. 02-01.
[2] M.J. Blaylock, D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley, I. Raskin, Environ. Sci. Technol. 31 (1997) 860.
[3] S.D. Ebbs, L.V. Kochian, J. Environ. Qual. 26 (1997) 776.
[4] L.Q. Ma, K.M. Komar, C. Tu, W. Zhang, Y. Cai, E.D. Kennelley, Nature 409 (2001) 579.
[5] M.M. Lasat, J. Environ. Qual. 31 (2002) 109.
[6] C. Kazunga, M.D. Aitken, A. Gold, Environ. Sci. Technol. 33 (1999) 1408.
[7] J.G. Burken, J.L. Schnoor, J. Environ. Eng. 122 (1996) 958.
[8] J.J. Boyle, J.R. Shann, J. Environ. Qual. 27 (1998) 704.
[9] M.B. Leigh, J.S. Fletcher, X. Fu, F.J. Schmitz, Environ. Sci. Technol. 28 (2002) 1110.
[10] T.A. Anderson, B.T. Walton, Environ. Toxicol. Chem. 14 (1995) 2041.
[11] P.L. Thompson, L.A. Ramer, J.L. Schnoor, Environ. Sci. Technol. 32 (1998) 975.
[12] W. Aprill, R.C. Sims, Chemosphere 20 (1990) 253.
[13] H.H. Liste, M. Alexander, Chemosphere 40 (2000) 11.
[14] J.G. Burken, J.L. Schnoor, Environ. Sci. Technol. 31 (1997) 1399.
[15] A.C. Dietz, J.L. Schnoor, Environ. Health Perspect. 109 (2001) 163.
[16] J.C. White, Chemosphere 49 (2001) 143.
[17] M.J.I. Mattina, J.C. White, B.D. Eitzer, W. Iannucci-Berger, Environ. Toxicol. Chem. 21 (2002) 281.
[18] J.C. White, X. Wang, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, N.P. Schultes, M. Arienzo, M.J.I. Mattina, Environ. Sci. Technol. 37 (2003) 4368.
[19] M. Alexander, Environ. Sci. Technol. 34 (2000) 4259.
[20] M.J.I. Mattina, W. Iannucci-Berger, C. Musante, J.C. White, Environ. Pollut. 124 (2003) 375.
[21] S.E. Ebbs, L.V. Kochian, Environ. Sci. Technol. 32 (1998) 802.
[22] E. Hoffland, G.R. Findenegg, J.A. Nelemans, Plant Soil 113 (1989) 161.
[23] S. Ishikawa, J.J. Adu-Gyamfi, T. Nakamura, T. Yoshihara, T. Watanabe, T. Wagatsuma, Plant Soil 245 (2002) 71.
[24] N. Ae, R.F. Shen, Plant Soil 245 (2002) 95.
[25] H. Tanaka, N. Adachi, T. Isoi, Soil Sci. Plant Nutr. 41 (1995) 389.
[26] P. Ximinez-Embun, Y. Madrid-Albarran, C. Camara, C. Cuadrado, C. Burbano, M. Muzquiz, Int. J. Phytoremediat. 3 (2001) 369.
[27] A. Porta, N. Filliat, N. Plata, in: A. Leeson, B.C. Alleman (Eds.), 5th International in Situ and On-site Bioremediation Symposium. San Diego, CA April 19–22, 1999, vol. 6, 1999, p. 51.
[28] G.A. Pederson, G.E. Brink, T.E. Fairbrother, Agron. J. 94 (2002) 895.
[29] H.M. Pylypiw, J. AOAC Int. 76 (1993) 1369.
[30] J.C. White, B.D. Kottler, Environ. Toxicol. Chem. 21 (2002) 550.
[31] R.G. Nash, E.A. Woolson, Science 157 (1967) 924.
[32] B.K. Robertson, M. Alexander, Environ. Toxicol. Chem. 17 (1998) 1034.
[33] D.E. Morrison, B.K. Robertson, M. Alexander, Environ. Sci. Technol. 34 (2000) 709.
[34] K.M. Carroll, M.R. Harkness, A.A. Bracco, R.R. Balcarcel, Environ. Sci. Technol. 28 (1994) 253.
[35] P.B. Hatzinger, M. Alexander, Environ. Sci. Technol. 29 (1995) 537.
[36] G. Neumann, E. Martinoia, Trends Plant Sci. 7 (2002) 162.
[37] J.C. White, Z.D. Parrish, M. Isleyen, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, M.J.I. Mattina, Environ. Toxicol. Chem., in press.
[38] P.M. White Jr., D.C. Wolf, G.J. Thoma, C.M. Reynolds, Int. J. Phytoremediat. 5 (2003) 381.
[39] S.L. Hutchinson, M.K. Banks, A.P. Schwab, J. Environ. Qual. 30 (2001) 395.
[40] C.J. Asher, P.F. Reay, Aust. J. Plant Physiol. 6 (1979) 459.
[41] S.L. Brown, R.L. Chaney, J.S. Angle, A.J.M. Baker, J. Environ. Qual. 23 (1994) 1151.
[42] K.D. Rodecap, D.T. Tingey, E.H. Lee, J. Environ. Qual. 23 (1994) 239.