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Surfactants Differentially Impact *p,p′*-DDE Accumulation by Plant and Earthworm Species

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The effect of four surfactants (Triton X-100, Tween-80, rhamnolipids, cyclodextrin) at 100−1000 mg/L on *p,p′*-DDE phytoextraction by *Cucurbita pepo* (zucchini) under field conditions and *p,p′*-DDE bioaccumulation by earthworm species (*Eisenia fetida*, *Lumbricus terrestris*) under laboratory conditions was investigated. Abiotically, surfactants (except cyclodextrin) increased contaminant desorption from soil by 4-fold, with higher concentrations generally promoting greater release. Cyclodextrin had no effect on DDE desorption. DDE concentrations in unamended zucchini roots and stems were 30- and 7.8-fold greater than soil levels, respectively, and 1.6% of the contaminant was extracted from the soil. The surfactant effects were cultivar specific. Triton X-100 increased DDE uptake in "Costata" by 2.6-fold, yielding 5% contaminant phytoextraction. In "Goldrush," DDE accumulation decreased by 69% across all surfactants. Surfactants significantly increased DDE bioaccumulation by earthworms. For *E. fetida* with all surfactants and *L. terrestris* with Triton X-100 and cyclodextrin, DDE accumulation increased 2.5−7.2-fold, paralleling abiotic desorption. However, Tween-80 and rhamnolipids increased DDE accumulation in *L. terrestris* by 74 and 36 fold, respectively. These dramatic increases in contaminant bioaccumulation do not correlate with the increased availability observed abiotically. Surfactant-mediated increases in contaminant bioavailability are an unexpectedly complex process and clearly present unanticipated concerns over pollutant exposure to nontarget organisms.

Introduction

Persistent organic pollutants (POPs) such as dichlorodiphenyltrichloroethane (DDT) and its lesser chlorinated metabolite dichlorodiphenyldichloroethylene (DDE) have achieved global distribution (*1*, *2*). POPs are toxic and have been implicated in carcinogenicity and endocrine disruption (*3*, *4*). These pollutants are resistant to enzymatic breakdown and are extremely hydrophobic, with Log K_{ow} (octanol−water partition coefficients) in excess of 6.0. The recalcitrance is a function of the synthetic nature of the molecules; thus, organisms have not evolved enzymes capable of contaminant degradation. Consequently, POP half-lives in soils and sediments are frequently measured in decades (*5*, *6*). Contaminant hydrophobicity also results in intimate binding to soil organic matter, making remediation difficult and expensive (*6*, *7*). POP recalcitrance and hydrophobicity can result in contaminant accumulation within the fatty tissues of biota, as well as magnification through food chains (*8*). Last, POP semivolatility has led directly to global contamination, including arctic food chains (*8*, *9*).

Remedial options for POP-contaminated soils are limited and expensive. Phytoremediation can be an inexpensive and effective approach where plants remove organic and inorganic contaminants from the environment (*10*). Organic pollutants may be degraded in the rhizosphere by exuded plant enzymes or by the robust microbial community associated with the root zone (the "rhizosphere effect"). Contaminants with a Log K_{ow} of 1.5−3.0 may cross the plant root barrier, subsequently being degraded, transpired, or stored in plant tissues (*10*). The recalcitrance and hydrophobicity of POPs make successful plant-based remediation unlikely (*10*−*12*). However, early reports suggested that certain *Cucurbita* species accumulated weathered dioxins by a unique soil-to-plant mechanism (*13*). We have been investigating the accumulation of weathered POPs by *C. pepo* ssp *pepo* (zucchini, pumpkin), including DDT/DDE (*14*, *15*), chlordane (*15*), polychlorinated biphenyls (PCBs) (*16*), and polycyclic aromatic hydrocarbons (PAHs) (*17*). Zucchini stems and roots may accumulate POPs at concentrations 5−30-fold greater than present in the soil, removing 1−5% of the contamination in 2 months.

Not surprisingly, recent data indicates that DDE availability to *C. pepo* ssp *pepo* limits the rate of contaminant removal from soil. In preliminary studies, soil amendments designed to increase POP desorption from soil, including low molecular weight organic acids (*18*), mycorrhizal fungi (*19*), and a surfactant (*19*), may increase contaminant accumulation by zucchini. The current field study investigates four different surfactants at multiple concentrations so as to maximize contaminant extraction by *C. pepo* ssp *pepo*. Triton X-100, Tween-80, rhamnolipids, and cyclodextrin were selected for evaluation. These amendments have been shown to increase the apparent solubility of organic chemicals either through reducing interfacial tension or through molecular incorporation into surfactant micelles (*20*−*23*). For comparison, the effect of the surfactants on the abiotic desorption of DDE was determined. Last, to assess the impact of enhanced contaminant availability on nontarget organisms, the effect of surfactants on DDE bioaccumulation by two earthworm species was investigated.

Materials and Methods

Field Site. Field experiments were conducted at a site used in our previous studies; the soil is contaminated with weathered *p,p′*-DDE (DDE) at concentrations of 50−800 ng/g dry weight (*14*). The soil has an organic carbon content of 1.4% and is a fine sandy loam (56% sand, 36% silt, 8% clay) with a pH of 6.7. After covering the site with 1000 m^2 of black polyethylene plastic sheeting to minimize weed growth and water loss, 30 cm^2 squares were cut at 3.0 m^2 intervals. Each exposed area of soil would serve as a single replicate zucchini mound.

Three cultivars of *Cucurbita pepo* ssp *pepo* ("Raven," "Goldrush," and "Costata Romanesco;" all are true zucchini) were acquired from Johnny's Selected Seeds (Albion, ME) and were intended to serve as individual replicates. After pregermination in the laboratory, the seedlings were planted

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in May 2005. One day before planting, individual exposed mounds were amended with surfactant solutions (see below). Each replicate mound of vegetation contained four individual plants. The seedlings were protected from local fauna with row covers during the first two weeks of growth. Twenty-six mounds of each cultivar variety were grown; as described below, duplicates for each concentration of the four surfactants were used. Fruits were harvested throughout the summer. Plants were destructively harvested in August 2005 after all cultivars had been fruiting for several weeks.

Surfactants. Triton X-100 (10% solution) was purchased from Fluka BioChemika (Buchs SG, Switzerland). Tween-80 (70% solution) and β−cyclodextrin (crystals, 98% purity) were purchased from Sigma (Atlanta, GA). A rhamnolipid solution (JBR425, 25% surfactant in water) produced by a mixed culture of *Pseudomonas aeruginosa* strains was purchased from Jeneil Biosurfactant Co., LLC (Saukville, WI). Surfactants were prepared at 1000, 500, and 100 mg/L for use in various assays.

For field experiments involving zucchini cultivars, replicate mounds of soil were loosened with a hand trowel and one liter of surfactant solution was added the day before planting. For the earthworm and desorption assays, solutions were prepared 1 day prior to use.

DDE Extraction from Soil. The soil DDE content for all cultivar and surfactant combinations was determined as described previously (*14*). Briefly, soil cores were collected from each replicate mound prior to planting. Sieved and air-dried soil samples (3.0 g) were extracted with 15 mL of hexanes amended with 1 μg of *o*,*p*-DDE (internal standard, in 100 μL trimethylpentane) at 70 °C for 5 h. A portion of the supernatant was passed through a glass microfiber filter (0.2 μm, Laboratory Science Inc., Sparks, NV) prior to collection in a chromatography vial.

DDE Extraction from Vegetation. The zucchini cultivars were harvested and the tissues were extracted as described previously (*19*). The stem was severed at ground level and the leaves, stems, and fruit were separated. The soil containing the root system was excavated (1.0 × 1.0 × 0.25 m), and after careful removal of the roots, the fresh mass of all plant tissues was determined. After washing the fresh plant material thoroughly with water, the tissues were finely chopped, and archived at −4 °C by cultivar, surfactant concentration, and tissue. To extract DDE, the vegetation was mixed in an explosion-proof blender (Fisher Scientific, Springfield, NJ) with 25 mL of 2-propanol and 50 mL of petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ) amended with 1 μg of *o*,*p*-DDE. After blending for 5 min, the slurry was filtered through glass wool and rinsed several times with reverse osmosis water. The petroleum ether was collected in a graduated cylinder containing 10 g of anhydrous sodium sulfate, and a portion of the petroleum ether extracts was filtered through florisil. A preconditioned florisil cartridge (200 mg)(Alltech, Deerfield, IL) was amended with 1 mL of vegetation extract, followed by 6 mL of 6% diethyl ether in petroleum ether. The extract was collected in 8 mL glass vials and reduced to approximately 1 mL under nitrogen flow.

DDE Uptake by Earthworms. *Eisenia fetida* and *Lumbricus terrestris* were obtained from Carolina Biological Supply (Burlington, NC) and Lehigh Bait and Bow (Allentown, PA), respectively. The accumulation of DDE was determined as described previously (*24*). Five hundred grams DDE-contaminated soil was added to 600 mL beakers. The air-dried soils were amended with 100 mL of water or surfactant solution. Approximately 9 g (wet weight) of earthworm biomass was added to replicate soil samples; 30 individuals of *E. fetida* or three individuals of *L. terrestris*. The earthworms were washed with tap water prior to their addition to the soil. Beakers were covered with perforated aluminum foil and were stored in the dark at 22 °C. After 14 days, the worms were removed from the soil, washed with tap water, and transferred to clean petri dishes for depuration (24 h for *E. fetida* and 48 h for *L. terrestris*). The worms were divided into equal portions (*5*−*7*) by mass and transferred to 35 mL vials containing 10 mL of hexanes and 1 μg of *o*,*p*-DDE. The vials were sealed with Teflon-lined closures and were placed upright in an oven at 70 °C for 5 h. A 1 mL aliquot of the supernatant was passed through a glass microfiber filter (0.2 μm, Laboratory Science Inc., Sparks, NV) prior to analysis.

Abiotic Desorption of DDE. Forty-gram portions of soil were weighed into 250 mL amber bottles with Teflon-lined caps, and 220 mL of solution amended with 4 μg *o*,*p*-DDE was added. The solutions were either reverse osmosis water or surfactant solutions at 100, 500, or 1000. The bottles were placed on an orbital shaker at 100 rpm at approximately 26 °C for 3 d. The samples centrifuged at 500 rpm for 10 min, and the supernatant was decanted into a second 250 mL amber bottle. A pre-conditioned C-18 (octadecyl) disk (3M, St. Paul, MN) was added to each of the supernatant solutions, which were then placed back on the orbital shaker for 3 d. The C-18 disks were removed and transferred to 8-mL vials containing a layer of anhydrous sodium sulfate and 4 mL of hexanes. After 2 h at 70 °C, a 1 mL portion of the hexanes extract was transferred to a chromatography vial for analysis.

DDE Quantitation. The *p*,*p*′-DDE content in the soil or tissue or desorption extracts was determined on a Agilent (Avondale, PA) 6890 gas chromatograph (GC) with a ^{63}Ni micro-electron capture detector (ECD). An SPB-1 column (30 m × 0.53 mm × 0.5 μm) (Supelco, Bellefonte, PA) was used; 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 injection port was maintained at 250 °C and a 2 μL splitless injection was used. Hydrogen was the carrier gas, and the makeup gas was 5% CH_4 in Ar at 60 mL/min. The ECD was maintained at 325 °C.

Crystalline *p*,*p*′-DDE and *o*,*p*′-DDE were acquired from the EPA National Pesticide Standard Repository (Fort Meade, MD). Portions of *p*,*p*′-DDE were transferred to trimethylpentane and calibration standards were prepared at 10−500 ng/mL. One hundred ng/mL *o*,*p*-DDE was added to each calibration level as an internal standard. Concentrations of *p*,*p*′-DDE in the various tissue, soil, and desorption extracts were determined by internal standard calibration.

Statistical Analysis. All soils were extracted in quadruplicate. Triplicate vegetation extractions were conducted on the tissues of each cultivar for each surfactant level. The following indices were analyzed statistically by an ANOVA followed by either a Student−Newman−Keuls or a Dunn's Multiple Comparison test ($p < 0.05$): tissue (root, stem, leaf, or fruit) bioconcentration factors (BCFs), translocation factor (TF), biomass, and percentage of DDE phytoextracted. The tissue BCFs are the dry weight ratios of DDE concentration in the plant to that in the soil, and the TF is the stem BCF divided by the root BCF. To calculate the percent phytoextraction, the absolute DDE mass in the plant is obtained after multiplying concentration by dry biomass. The soil DDE mass is determined similarly, with the volume of soil impacted by the root being estimated by the parameters of destructive harvest; 1.0 × 1.0 × 0.25 m. Using a measured soil density (1.14 g/cm^3), the soil compartment is estimated at 290 kg. The absolute DDE content in the plant is then expressed as a percentage of that in the soil. For the abiotic desorption experiments, each concentration of each surfactant was evaluated in quadruplicate and statistical significance was determined with an ANOVA followed by a Studentn Newman−Keuls Multiple Comparison test ($p < 0.05$). For the earthworm assay, at least four replicates of each species were evaluated at each surfactant concentration, and statistical significance was determined as described above ($p < 0.05$).

TABLE 1. Effect of Four Surfactants on the Desorption of Weathered *p,p′*-DDE from Soil. Each Surfactant Was Tested at Three Concentrations; 100 mg/L (low), 500 mg/L (medium), and 1000 mg/L (high).

treatment	*p,p′*-DDE desorbed (ng/g)	treatment	*p,p′*-DDE desorbed (ng/g)
control[a]	5.3[a] (2.2)[b] A[c]	Rhamnolipid	
Triton		low	8.9 (1.4) B
low	15 (7.4) AB	medium	14 (0.58) C
medium	25 (2.9) B	high	23 (3.3) D
high	38 (14) B	Cyclodextrin	
Tween		low	5.1 (0.68) AB
low	11 (1.4) B	medium	3.2 (0.49) B
medium	21 (1.8) C	high	3.8 (0.75) AB
high	27 (1.6) D		

[a] The control is the average of 16 replicates (controls from each separate surfactant trial). All other values are the average of four replicates. [b] Values in parentheses are standard deviations. [c] Each surfactant is compared to the control only. Within a surfactant, values followed by different letters are significantly different (one way ANOVA with Dunns multiple comparison test).

Results and Discussion

DDE Content of the Soil. For the field experiments, the concentration of weathered DDE ranged from 47 to 330 ng/g. The DDE concentration in the soils used for the abiotic desorption and earthworm trials was 140 and 236 ng/g, respectively. These concentrations are in line with our previous findings (*14*) and are indicative of historical DDT usage in New England.

Abiotic Desorption of DDE. Table 1 shows the effects of surfactants on DDE desorption from soil. Water released 5.32 ng/g DDE or 3.77% of the pollutant. These results agree with our previous findings where similar experiments yielded 1.70% desorption of a weathered PCB from soil (*16*), as well as 8.4 and 0.01% release of weathered three and six ring PAHs, respectively (*17*). Excluding Triton X-100 at 100 mg/L, all concentrations of Triton X-100, Tween-80, and the rhamnolipid significantly increased the DDE desorption. For Tween-80 and rhamnolipid, the extent of contaminant desorption increased significantly with higher surfactant levels; a similar trend was evident with Triton X-100, although the effect was not statistically significant. Cyclodextrin did not increase DDE desorption from the soil; in fact, at 500 mg/L, the amount of contaminant released was significantly decreased.

It is widely known that surfactants can increase the apparent water solubility of organic compounds. Kile and Chiou (*20*) reported that a range of surfactants, including Triton X-100, increased the solubility of organochlorines such as DDT by up to 200-fold. Similarly, Zhang and Miller (*21*) observed that a microbially derived rhamnolipid increased octadecane solubilization by 4 orders of magnitude. Interestingly, Wang and Brusseau (*25*) showed that cyclodextrin increased the water solubility of DDT by 800-fold. Although the above studies (*20*, *21*, *25*) were in purely aqueous solutions, similar evidence of surfactant-enhanced solubility/availability of contaminants exists with soils (*26*). Under aqueous conditions, the mechanism of increased solubility is largely the result of contaminant encapsulation within surfactant micelles, although Kile and Chiou (*20*) did report that extremely hydrophobic compounds such as DDT may undergo partition to the nonpolar regions of the surfactant monomers. In soil based systems, the reactions are more complex, with competing processes of surfactant sorption and degradation, as well as enhanced contaminant binding to sorbed surfactant molecules, occurring (*22*). Yeom et al. (*26*) reported increased desorption of weathered PAHs in the presence of 6000 mg/L Triton X-100 and Tween-80; the amount of phenanthrene released from the soil was approximately 10%. In the current study, surfactant levels of 1000 mg/L resulted in 16−27% DDE release for Triton X-100, Tween 80, and rhamnolipids, although direct comparison between the two studies is complicated due to differences in contaminant properties and experimental design. The reason for a lack of increased DDE release with cyclodextrin is unknown. Tick et al. (*23*) reported in a field study that cyclodextrin at 15% (m/v) increased the amount of tetrachloroethene (PCE) leached from soil by 22-fold. Conversely, Cuypers et al. (*27*) showed that cyclodextrins increased the release of readily bioavailable PAH fractions but did not increase the overall magnitude of the available pool of contamination. Conversely, Triton X-100 directly interacted with the sediment structure, resulting in increased PAH bioavailability of resistant contaminant fractions and increased pollutant biodegradation (*27*).

TABLE 2. Biomass and DDE Accumulation of Three Zucchini Cultivars

parameter	Costata	Goldrush	Raven
biomass[a]	1390 (340)[d]	1660 (14)	676 (180)
root BCF[b]	9.96 (0.54)	47.5 (29)	33.6 (13)
stem BCF[b]	5.53 (0.19)	10.6 (0.31)	7.37 (1.5)
leaf BCF[b]	0.11 (0.01)	0.31 (0.05)	0.01 (0.005)
fruit BCF[b]	0.12 (0.01)	0.19 (0.08)	0.37 (0.04)
% phytoextracted[c]	1.17 (0.02)	2.87 (0.05)	0.720 (0.01)

[a] Average dry biomass (g) of all zucchini cultivars. [b] Ratio of tissue to soil *p,p′*-DDE content on a dry weight basis. [c] Ratio of total contaminant mass in the plant to that in the 290 kg of soil the plants are growing in. [d] Values in parentheses are the standard deviations of triplicate extractions.

DDE Content in Unamended Zucchini. DDE was detected in all tissues of all plants, with the highest contaminant concentration being consistently in the roots, followed by decreasing amounts in the stems, leaves, and fruit. The highest root and stem DDE content were 6810 and 2160 ng/g, respectively; contaminant concentrations in the leaves and fruit were typically 1−3 orders of magnitude lower. The plants accumulated 470 μg of DDE, with approximately 11% remaining in the roots. Of the translocated DDE, 85% was in the stems with approximately 4% in the other aerial tissues.

Differing DDE content in the soils of the various treatments make direct comparisons of tissue contaminant concentrations problematic. This confounding factor is eliminated by calculating BCFs as the dry weight ratio of DDE tissue content to that in the soil. In soils with a DDE concentration range of an order of magnitude or less, accumulation by *Cucurbita* species is linear and BCF determination is appropriate (*19*). The average total biomass of the un-amended zucchini cultivars was 1200 g (±490), with root and stem DDE BCFs of 30 (±23) and 7.8 (±2.4), respectively, and the average amount of contaminant phytoextraction was 1.6% (±1.1). Although the Costata, Raven, and Goldrush cultivars were intended to serve as replicate zucchini plants, there was a large amount of variability in many of the parameters evaluated; the standard deviation across the values was typically 56% of the mean. Table 2 shows the biomass, tissue BCFs, and percent phytoextraction for the individual zucchini cultivars.

These findings agree with our previous work and highlight the unique ability of *C. pepo* ssp *pepo* to accumulate weathered POPs from soil. As noted previously, Hülster et al. (*13*) suggested that zucchini accumulated weathered dioxins and furans by a soil-to-plant transfer pathway, and we have subsequently expanded upon that initial observation by demonstrating significant uptake and translocation of a range of weathered POPs (*12*, *14*−*16*). The wide variability among cultivars in the current study is somewhat unexpected and

TABLE 3. Effect of Surfactants on Total Plant Biomass, Tissue/DDE Bioconcentration Factors, the Translocation Factor (TF), and the Percent of Contaminated Phytoextracted from Soil. Each Surfactant Was Tested at Three Concentrations; 100 mg/L (low), 500 mg/L (medium), and 1000 mg/L (high).

treatment	biomass[a]	root BCF[b]	stem BCF[b]	TF[c]	% phytoextracted[d]
control	1200 (490) A[e]	30 (23) A	7.8 (2.4) A	0.26	1.6 (0.97) A
Triton					
low	1000 (510) A	28 (6.1) A	7.0 (2.8) A	0.25	1.4 (1.1) A
medium	1200 (270) A	21 (16) A	6.7 (3.1) A	0.32	1.1 (0.21) A
high	900 (750) A	15 (4.1) A	9.1 (7.8) A	0.61	2.0 (2.4) A
Tween					
low	1100 (250) AB	15 (7.3) A	6.6 (4.0) A	0.45	0.98 (0.43) AB
medium	680 (330) B	19 (5.2) A	6.7 (3.2) A	0.35	0.87 (0.64) AB
high	650 (280) B	19 (4.5) A	4.9 (3.4) A	0.25	0.49 (0.25) B
Rhamnolipid					
low	800 (270) B	15 (2.8) A	4.2 (0.59) B	0.29	0.55 (0.09) AB
medium	680 (470) B	14 (4.3) A	3.9 (1.9) B	0.27	0.53 (0.51) BC
high	300 (60) B	14 (2.7) A	4.0 (2.0) B	0.29	0.22 (0.04) C
Cyclodextrin					
low	640 (350) A	22 (2.0) A	8.5 (4.7) A	0.38	0.80 (0.46) A
medium	930 (530) A	24 (6.8) A	9.9 (2.8) A	0.41	1.5 (0.67) A
high	1200 (380) A	28 (18) A	8.9 (4.5) A	0.32	1.5 (0.95) A

[a] Average dry biomass of all zucchini cultivars [b] Tissue to soil dry weight concentration ratio for *p,p′*-DDE. [c] Translocation factor or TF, ratio of stem BCF to root BCF. [d] Ratio of total contaminant mass in the plant to that in the 290 kg of soil the plants are growing in. [e] Within a parameter (column) and surfactant, values followed by different letters are significantly different from each other or from the control.

problematic, given that these plants were designed as replicates of zucchini. However, stem BCFs of 5.5 and 0.72% contaminant extraction are still an order of magnitude above values observed for other plant species (*11*). Isolating the individual cultivars results in poor statistical power when analyzing only the unamended control plants; consequently, such analysis is reserved for comparing the different surfactant treatments.

DDE Content in Surfactant-Amended Zucchini. Table 3 shows the effects of the surfactants on the biomass and DDE accumulation of the grouped zucchini cultivars. Although cultivar variability limited the instances of statistically significant differences, a few points are worth noting. Tween-80 at 500 and 1000 mg/L, as well as the rhamnolipid at all concentrations, significantly decreased plant biomass. Rhamnolipids at all concentrations significantly decreased stem BCFs and at 500 and 1000 mg/L, reduced the amount of phytoextracted DDE. No statistically significant differences in contaminant uptake or plant mass are evident if the concentrations of a particular surfactant are combined or when all surfactants in general are grouped together for comparison against unamended plants.

Isolating the individual cultivars results in some noteworthy trends in the data. Figure 1 shows the percent DDE phyotextracted by the individual zucchini cultivars at the various surfactant concentrations; the data clearly show cultivar-level differences in the response to amendment. For example, Tween-80 at 1000 mg/L increased by 4.5-fold the DDE accumulation potential of Costata to a value of nearly 5% removal. For Raven and Goldrush, the same treatment resulted in an 80% decrease in contaminant uptake to values below 1% removal. Alternatively, 500 mg/L cyclodextrin decreased DDE uptake for Costata and Goldrush by 40−50% but nearly tripled the phytoextraction potential of Raven. Similar cultivar-level disparity in DDE accumulation is evident throughout the results. One useful transformation of the data is to combine the three concentrations of a particular surfactant and then compare the cultivar responses (Table 4). For ease of comparison among the multiple treatments, each value is normalized to its respective nonamended control. Goldrush values for biomass and DDE accumulation were consistently decreased relative to both the control and other cultivars. For example, Goldrush stem and root BCFs were decreased by 67 and 82%, respectively, across all surfactants. The rhamnolipid decreased DDE uptake of Goldrush by more than 90%. Translocation factor

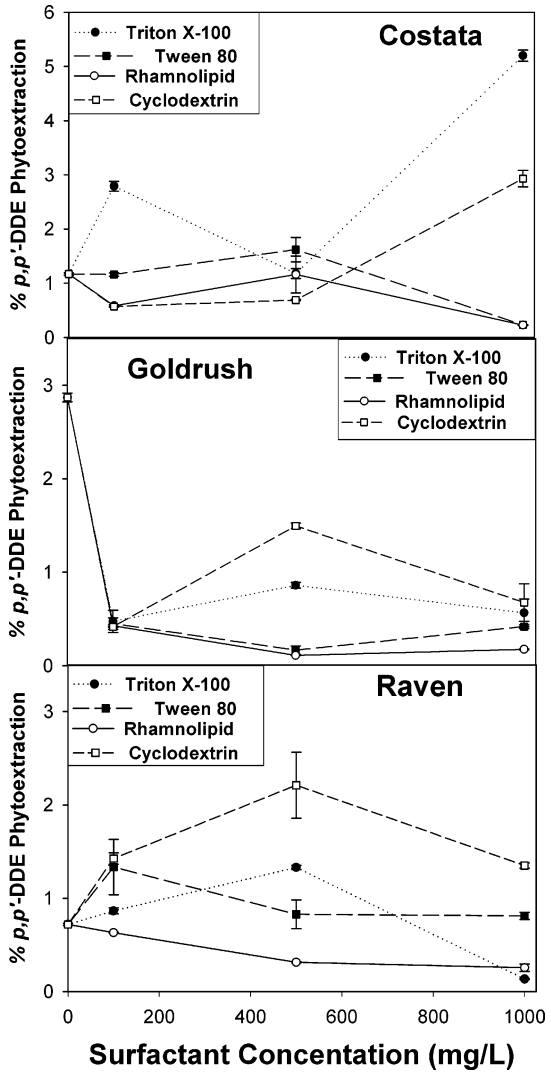

FIGURE 1. Influence of surfactants on DDE phytoextraction by 3 zucchini cultivars.

is the exception to these observations, but this is due to equivalent decreases in both the stem and root BCFs, thereby yielding values close to the nonamended controls. The only

TABLE 4. Effect of Four Surfactants on the Biomass and DDE Uptake of Three Zucchini Cultivars[a]

parameter	Triton[b]	Tween[b]	Rhamnolipid[b]	Cyclodextrin[b]
biomass[c]				
Costata	1.1 (0.16) A[d]	0.63 (0.33) A	0.60 (0.34) AB	0.63 (0.48) A
Goldrush	0.57 (0.26) A	0.38 (0.18) A	0.24 (0.11) A	0.47 (0.17) A
Raven	0.81 (0.53) A	1.3 (0.18) B	0.79 (0.44) B	1.7 (0.53) B
root BCF[c]				
Costata	1.8 (0.95) A	1.9 (0.87) A	1.5 (0.18) A	3.1 (1.7) A
Goldrush	0.32 (0.11) B	0.29 (0.04) B	0.22 (0.03) B	0.48 (0.14) B
Raven	0.92 (0.30) C	0.61 (0.10) B	0.51 (0.04) C	0.64 (0.15) B
stem BCF[c]				
Costata	2.2 (1.1) A	1.0 (0.52) A	0.78 (0.15) A	1.6 (0.51) A
Goldrush	0.30 (0.08) B	0.25 (0.04) B	0.24 (0.13) B	0.52 (0.26) B
Raven	1.1 (0.32) C	1.3 (0.23) B	0.73 (0.04) A	1.8 (0.23) A
TF[c]				
Costata	1.5 (0.86) A	0.70 (0.45) A	0.51 (0.07) A	0.58 (0.15) A
Goldrush	0.96 (0.18) A	0.87 (0.17) A	1.1 (0.51) B	1.0 (0.24) B
Raven	1.1 (0.24) A	2.2 (0.24) B	1.4 (0.24) B	2.8 (0.36) C
% phytoextracted[c]				
Costata	2.6 (1.5) A	0.86 (0.53) A	0.60 (0.38) A	1.1 (0.92) A
Goldrush	0.22 (0.07) B	0.12 (0.05) B	0.08 (0.05) B	0.30 (0.17) B
Raven	1.1 (0.72) AB	1.4 (0.43) A	0.56 (0.24) A	2.3 (0.62) C

[a] Values for each surfactant are averaged across the three concentrations tested. Each value is normalized to its respective control value. [b] For each surfactant, values of all three tested concentrations (100, 500, 1000 mg/L) are combined for this analysis. [c] All data is normalized to the control (no surfactant) value of each cultivar for the different parameters. [d] Within a parameter and surfactant (i.e, across cultivar), values followed by different letters are significantly different (one way ANOVA followed by a Holm−Sidak multiple comparison test).

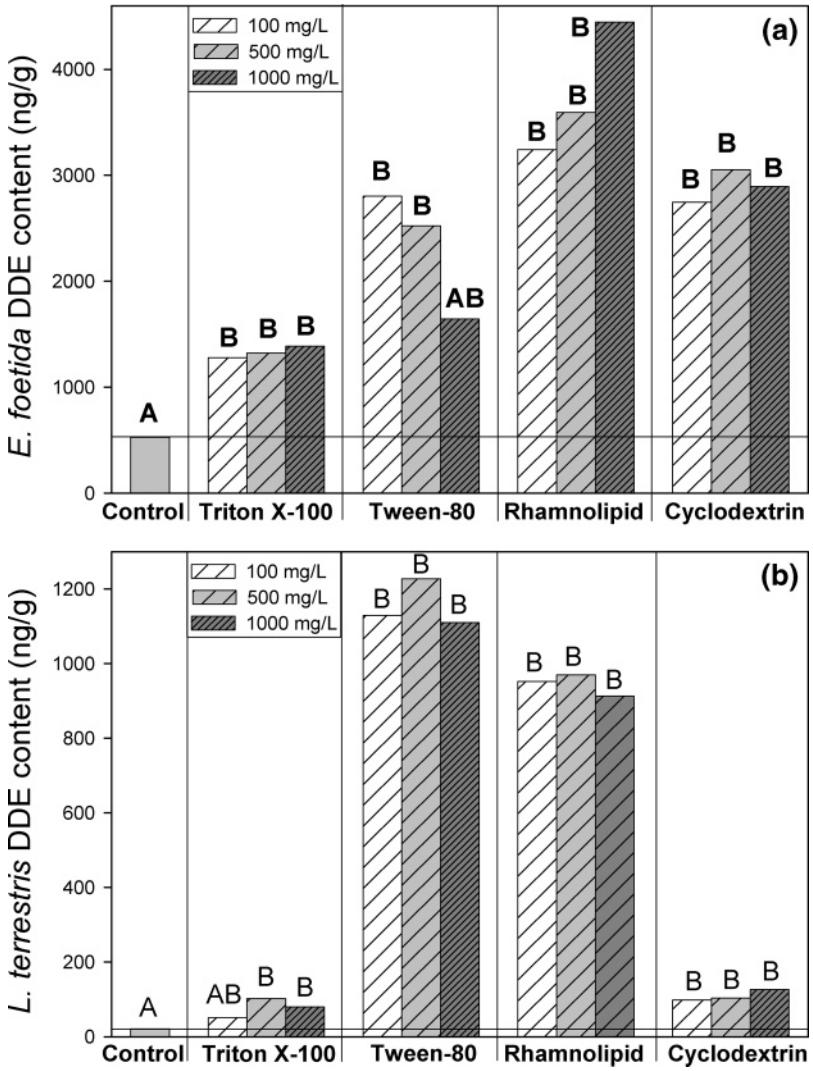

FIGURE 2. Effect of four surfactants at 100, 500, or 1000 mg/L on the accumulation of weathered DDE by *Eisenia fetida* (a) and *L. terrestris* (b). Within a surfactant, individual concentrations were tested against each other and the control, denoted as A. Different letters (i.e., B) indicate statistically significant difference from the control (One−way ANOVA followed by a Student−Newman−Keuls multiple comparison test, $p < 0.05$).

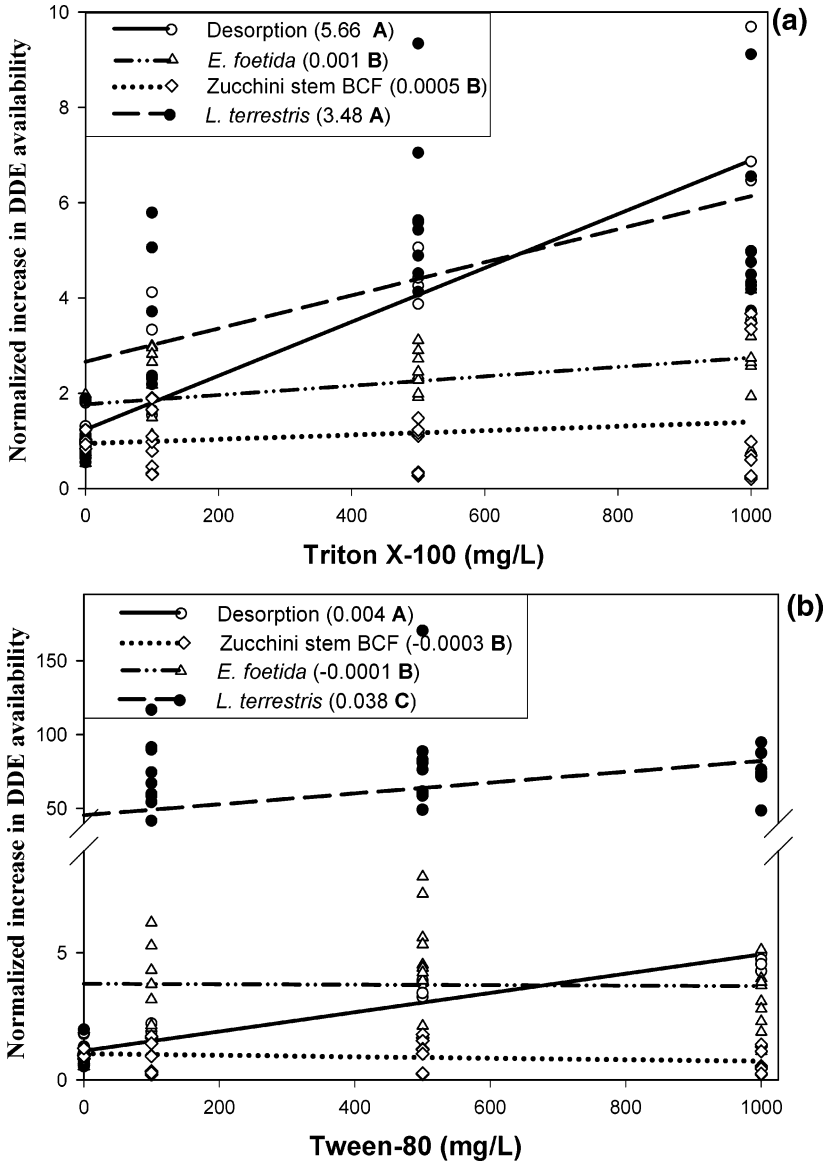

FIGURE 3. Effect of Triton X-100 and Tween-80 on the bioavailability of weathered DDE as determined by abiotic desorption and uptake by plant/worm species. Data are normalized to respective controls. Lines are regressed through individual assays; regression slopes are shown in parentheses in the legend. Slopes followed by different letters are significantly different as determined by the general linear model using surfactant and assay as variables. The model indicates statistically significant interactions ($p < 0.05$) between the linear response to surfactant among the assays (i.e., the slope).

other consistent trend in the data across all surfactants was increased root BCFs in Costata; 50−310% increases depending on the surfactant. In some instances, the difference in cultivar-specific response was particularly dramatic; 12-fold differences in phytoextraction potential upon amendment with Triton and Tween were observed. The reasons for the cultivar-level differences are unknown but are the focus of future studies.

There has been a significant amount of research on the use of soil amendments to enhance pollutant uptake by vegetation. Blaylock et al. (*28*) and others (*29*) observed significant increases in Pb uptake upon addition of the chelating agents such as EDTA. The toxicity of these chelating agents has led to interest in more biodegradable and less toxic alternatives, including low molecular weight organic acids and siderophores. The mechanism of increased availability with chelating agents is through direct binding to the pollutant. However, for POPs, amendments such as low molecular weight organic acids can also increase contaminant bioavailability and uptake (*16*, *18*, *30*). Here, organic acids chelate inorganic elements from the soil matrix, resulting in partial soil structure disruption and subsequent POP release to the aqueous phase. For surfactants, the apparent aqueous solubility of POPs is increased through incorporation into micelles or partitioning to hydrophobic monomers, effectively driving subsequent contaminant desorption/release by altering the sorption equilibrium.

In previous studies, we have shown that 1000 mg/L rhamnolipids could potentially increase DDE uptake by zucchini cultivars (*19*). Interestingly, of the five cultivars tested in the previous preliminary study, Goldrush was the sole strain that did not yield enhanced DDE uptake upon surfactant addition. In the current study, two synthetic/industrial surfactants and two biologically derived surfactants were compared. These amendments were chosen largely based on evidence in the literature that synthetic/industrial surfactants were more prone to cause toxic effects on biota (*31*, *32*). Makkar and Rockne (*33*) reported that biologically produced surfactants convey many of the same properties as their synthetic counterparts but cause less toxicity to exposed organisms. However, the current study suggests that this trend may not apply when using surfactants to increase

POP availability to plants. Table 3 shows that the two instances of reduced plant biomass occurred with Tween-80 and the rhamnolipids. Similarly, averaging the data in Table 4, the synthetic and biosurfactants yielded biomass values that were 80 and 74%, respectively, of the control values. These differences are neither significantly different from each other or that of the controls. Additional study is needed to elucidate the processes governing the surfactant-enhanced phytoextraction of weathered POPs. With potentially 450% increases in contaminant accumulation and overall remedial values of 5% removal in a single growing season, these studies are clearly warranted.

DDE Content in Earthworms. In soil with 236 ng/g weathered DDE, *L. terrestris* and *E. fetida* tissues contained 20.8 and 528 ng/g, respectively; the corresponding bioaccumulation factors (BAF; dry weight ratio in the tissue to that in the soil) were 0.088 and 2.27. In two previous studies, we reported BAFs for *L. terrestris* and *E. fetida* that ranged from 1.30 to 1.41 and from 10.6 to 15.6, respectively (*24*, *34*). The reasons for the lower accumulation values in the current study are likely the result of differences in experimental design, including earthworm maturity and density, but the order-of-magnitude difference between the species is still evident. The explanation for this species-specific difference in DDE accumulation is not known; the literature reports similar lipid content for these two species, ranging from 1.2 to 1.8% (*35*, *36*). The two species do have different ecological feeding strategies; *E. fetida* is epigeic and is present in surface litter, whereas *L. terrestris* is a soil-dwelling earthworm. Although not directly relevant to the current experiment (both were incubated in soil), these different feeding strategies may also manifest in different physiologies relative to the processing and digestion of soil organic matter. Further study is necessary to explore these species differences.

The effect of surfactants on the accumulation of weathered DDE by *E. fetida* is shown in Figure 2a. With the exception of Tween-80 at 1000 mg/L, all surfactants at all concentrations significantly increased DDE accumulation relative to the controls. For each of the four amendments, there was no effect of surfactant concentration on contaminant uptake. On average, Triton X-100, Tween-80, rhamnolipid, and cyclodextrin increased DDE uptake by 2.5, 4.3, 7.2, and 5.6 times, respectively. With the exception of rhamnolipid and cyclodextrin, these values are all significantly different (one way ANOVA on ranks with Dunns multiple comparison test, $p < 0.05$). Similarly, all surfactants at all concentrations, excluding Triton X-100 at 100 mg/L, significantly increased DDE accumulation by *L. terrestris* (Figure 2b). For each surfactant, the effect of concentration was nonsignificant. Triton X-100 and cyclodextrin increased DDE accumulation by 5.0 and 4.2 times, respectively; these values are not significantly different. The increased DDE uptake with Tween-80 and rhamnolipid was dramatic; 74 and 36 times, respectively. These values are significantly different from each other and from the control, Triton X-100, and cyclodextrin values (one way ANOVA on ranks, Dunns Multiple Comparison Test, $p < 0.05$).

We find no reports in the literature addressing the effect of surfactants on the increased availability and accumulation of POPs by earthworm species. In a study by von Westerhagen and Dethlefsen (*37*), a synthetic surfactant had no impact on Cd accumulation by fish and mussel species. For organic contaminants, there is a significant amount of research on the gut surfactant content of benthic and deposit feeding aquatic invertebrates; recent findings suggest that the surfactants associated with digestive processes are responsible for the greater than anticipated levels of PAHs and other organic contaminants in the tissues of such organisms (*38*, *39*). Conversely, Park et al. (*40*) described a micelle inhibition model for aquatic systems and observed that Tween-80 at increasing concentrations would decrease PAH uptake into aquatic invertebrates. Although of interest, the relevance of these studies to the current work is clearly tangential.

Some interesting findings become evident when comparing the surfactant effects on the different assays used in the current work. Figure 3 shows the impact of Triton X-100 and Tween-80 on DDE desorption, zucchini stem BCFs, and accumulation in earthworm tissue. The data was first normalized to respective control values for each assay. In certain cases, abiotic desorption correlates with the increase in contaminant bioavailability upon surfactant amendment; for example, DDE uptake into *L. terristris* with Triton X-100 followed the trends observed abiotically. In this instance, the slopes of the regressed lines are not significantly different ($p < 0.05$). In other instances, desorption significantly underestimates surfactant influences on bioavailability. As seen in Figure 3b, the slope of the regressed line through the *L. terrestris* data is 10-times greater than that of the desorption value. A similar finding was evident for the use of cyclodextrin, which had no impact on DDE desorption but significantly increased DDE accumulation in *L. terrestris* and *E. fetida*. Conversely, abiotic desorption consistently overestimated contaminant bioavailability to zucchini upon amendment with Triton X-100, Tween-80, and rhamnolipids. Clearly the impact of surfactants on the bioavailability of weathered POPs is governed by a complex series of processes, dependent upon both species-specific physiologies and surfactant properties. The significant increases in remedial potential, a 5-fold increase in DDE phytoextraction by certain zucchini, are of definite interest but the unexpected and dramatic increase in contaminant exposure to certain nontarget biota, a 74-fold increase in *L. terrestris*, is an issue of great concern.

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