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Influence of plant–earthworm interactions on SOM chemistry and *p,p′*-DDE bioaccumulation

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ABSTRACT

Laboratory experiments assessed how bioaccumulation of weathered *p,p′*-DDE from soil and humic acid (HA) chemistry are affected by interactions between the plants *Cucurbita pepo* ssp. *pepo* and ssp. *ovifera* and the earthworms *Eisenia fetida*, *Lumbricus terrestris*, and *Apporectodea caliginosa*. Total organochlorine phytoextraction by ssp. *pepo* increased at least 25% in the presence of any of the earthworm species (relative to plants grown in isolation). Uptake of the compound by ssp. *ovifera* was unaffected by earthworms. Plants influenced earthworm bioaccumulation as well. When combined with *pepo*, *p,p′*-DDE levels in *E. fetida* decreased by 50%, whereas, in the presence of *ovifera*, bioconcentration by *L. terrestris* increased by more than 2-fold. Spectral analysis indicated a decrease in hydrophobicity of HA in each of the soils in which both *pepo* and earthworms were present. However, HA chemistry from *ovifera* treatments was largely unaffected by earthworms. Risk assessments of contaminated soils should account for species interactions, and SOM chemistry may be a useful indictor of pollutant bioaccumulation.

1. Introduction

Bioaccumulation of persistent organic pollutants (POPs) from soil may adversely affect individual organisms, food webs, and human health. To best asses and manage these risks, an understanding of factors influencing the availability and uptake of such contaminants is necessary. The effects of soil chemistry, pollutant properties, and compound residence time in soil on POP bioavailability have been the subject of a number of studies (Alexander, 2000; Vasseur et al., 2008). How POP uptake is affected by biological factors such as organism interactions has received little attention, though, as research in this area has focused mainly on inorganic elemental contaminants. Earthworms, for example, have often been shown to change the availability of inorganic pollutants to plants (Sizmur and Hodson, 2009). In separate studies, *Lumbricus terrestris* and *Eisenia fetida* increased metal uptake by plants (Abdul Rida, 1996; Ruiz et al., 2009) from contaminated soil. The addition of earthworm mucus also enhanced cadmium uptake by tomato seedlings grown hydroponically (Zhang et al., 2010). In contrast, bioconcentration of metals by plants grown in sewage-sludge amended soil was reduced in the presence of *E. fetida* (Liu et al., 2005). Analogously, through a variety of processes that includes uptake, volatilization, metabolism, and stimulation of microbial degradation, plants can be used to reduce the bioavailability of pollutants to earthworms (Pilon-Smits, 2005).

Still largely unexplored are the effects of interactions among species on POP availability to multiple organisms exposed simultaneously. Soil microcosms were used to study the effects of the pesticide carbendazim (Burrows and Edwards, 2004) on a number of plants, animals, and microorganisms, but organism-to-organism effects were not directly measured. Similar combinations increased removal of hydrocarbons and metals from soil relative to that seen when plants were grown alone (Bianchi and Ceccanti, 2010). Work done in our laboratory showed that the uptake of the persistent pesticide metabolite *p,p′*-DDE by plants and earthworms from soil was influenced by co-occurrence of the organisms, although the outcome depended on the species involved (Kelsey and White, 2005).

The mechanisms associated with interaction-specific bioaccumulation remain largely unknown. Variations in processes associated with the acquisition of essential nutrients are likely responsible for differences in pollutant uptake among organisms, and interactions have the potential to produce unexpected synergistic or antagonistic effects. Earthworms can affect soil physicochemical properties through different metabolic and ecological strategies (Scheu, 2002; Brown and Doube, 2004; Brown et al., 2004; Liu et al., 2005), and these differences could have an impact on the accumulation of POPs by other soil-dwelling biota. Similarly, plants affect soil chemistry, structure, and the growth of other organisms in species-specific ways (Pilon-Smits, 2005; Perez-Bejarano et al., 2010). Plants also vary dramatically in their

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ability to extract pollutants (White, 2002; Pilon-Smits, 2005; Chhikara et al., 2010).

Many physical and chemical properties subject to modifications by organisms are potentially relevant to soil toxicology. Since soil organic matter (SOM) plays a critical role in the absorption of non-polar compounds (Gunasekara et al., 2003), biological activity affecting its chemistry is particularly important to the fate of organic pollutants. The current study was undertaken to continue our investigation of the effects of co-occurrence of soil organisms on bioaccumulation of weathered *p,p′*-DDE. Of interest was the way changes in humic acid (HA) chemistry induced by interactions can be linked to patterns of uptake observed previously (Kelsey and White, 2005). Data presented herein will enhance our understanding of the toxicology, exposure assessment, and remediation of soil pollutants.

2. Materials and methods

2.1. Soil

Soil (56% sand, 36% silt, 8.0% clay, 1.4% organic carbon) was collected from Hamden CT, USA. Residues of *p,p′*-DDE from historical application of DDT persist in this soil. After air drying, the soil was manually sieved (<2.0 mm) and homogenized. Eight replicate 4-g soil samples were transferred to 40-mL vials and amended with 15 mL of *n*-hexane and 1 μg of *o,p′*-DDE (in all cases, DDE was obtained from ChemService, West Chester, PA, USA) in 100 μL of *n*-hexane as an internal standard. Vials were sealed with Teflon®-lined caps and soil was extracted at 65 °C for 5 h. Samples were then cooled. For these and all extractions (soils and tissues), a 1-mL aliquot was filtered (0.45 μm) into chromatography vials for quantitative analysis (Section 2.6). This extraction method has previously been validated by comparison with microwave assisted extraction (MAE) of *p,p′*-DDE contaminated soil (White, 2002). Contaminant concentration was 200.8 ± 10.9 ng *p,p′*-DDE g^{-1} soil. Soil pH was raised from 4.9 to 6.5 according to the Adams–Evans Buffer Method (Adams and Evans, 1962) and maintained at approximately 20% moisture for all experiments.

2.2. Preparation of experimental pots

Four hundred and ten grams of the dried, sieved soil were mixed with 50 g of perlite and added to 55 square 12.5 cm × 10.5 cm plastic pots lined on the outside with Al foil (punctured at the bottom for irrigation). Next, 1000 mL of deionized (DI) water was used to leach excess salt and residual $CaCO_3$ (from pH adjustment, Section 2.1). Soils were homogenized periodically as they were air-dried for 1 week. Pots were then randomly sampled for use in eleven separate treatment regimes. Five treatments contained a single plant or earthworm, and six contained two species (see Sections 2.3 and 2.4). All treatments consisted of six replicates. Plants were harvested after 28 d, and worms were removed after 14 d (in experiments containing two organisms, earthworms were added 14 d after seedlings were planted, and the two organisms inhabited the soil simultaneously for the next 14 d). Each pot was covered with plastic wrap to prevent worms from escaping while plants grew.

2.3. Earthworms

Three species of earthworms representing three feeding strategies (Curry and Schmidt, 2007) were used: *E. fetida* (epigeic), *L. terrestris* (anecic), and *Apporectodea caliginosa*, (endogeic). Earthworms were added to replicate soil samples as follows: 2 juvenile *L. terrestris*, 20 adult *E. fetida*, or 25 adult *A. caliginosa*. Earthworms were washed with tap water and added to the tops of the soil in each designated pot, and burrowed within 8 h of the start of incubation.

After 14 d, earthworms were removed from pots, transferred to moistened filter paper on petri plates for depuration (48 h for *L. terrestris*, 24 h for the others), washed with tap water, dried, and transferred to 40-mL amber glass vials. Ten milliliters of *n*-hexane and 1 μg of *o,p′*-DDE were added to each vial. The vials were sealed with Teflon®-lined closures and agitated at 180 rpm at 65 °C for 5.5 h.

2.4. Plants

Two species of plants were used: *C. pepo* (spp. *pepo*, a known DDE accumulator) and *C. pepo* (spp. *ovifera*, a known non-accumulator) (Johnny's Selected Seeds, Winslow, ME, USA). Seeds were treated with a cleaning solution (2% Alconox, 50% ethanol, 0.1% Triton-X 100) solution prior to germination to reduce the growth of fungi during experiments. Three 10-d old seedlings of either plant were transferred to each pot, and 4 mL of NH_4NO_3 (100 mg L^{-1} solution) was added to each seedling. Artificial light was provided for 24 h on the first day and 14 h on subsequent days (at 22 °C) with 40-W aquarium grow lamps (four light tubes per palette of approximately 12 pots). After 7 d of growth, one plant was removed from each pot. The remaining two plants grew for an additional 21 d. After 28 d, plants were destructively harvested. Earthworms were removed from the soil and analyzed as described in Section 2.3. Oven assisted extraction (OAE) (Slizovskiy et al., 2010) was used to recover *p,p′*-DDE residues from washed vegetation.

2.5. Soil organic matter (SOM) chemistry

After removal of organisms, soil was recollected, air-dried, sieved (<2 mm) to remove perlite particles, pooled to obtain an aggregate sample from all replicates for each treatment, transferred to amber glass vials, and stored in desiccators. Humic acid (HA) was chosen as a representative fraction of the SOM and extracted using the modified MIBK technique (Kohl and Rice, 1998). This method isolates HA from other constituents in soil, including perlite, due to solubility differences in acids and bases. Samples were filtered (0.45 μm) to remove any residual particles. Three replicate HA samples were produced from each treatment and analyzed by Fourier transform infrared spectroscopy (FTIR) (see Section 2.7). All HA samples were stored in glass amber screw-capped vials in desiccators and were cryogenically preserved until further analysis.

2.6. DDE quantitation

The *p,p′*-DDE content in tissue and soil extracts was determined using an Agilent 5890 gas chromatograph with a 30 × 0.32 mm × 0.25 μm HP-5 column and a ^{63}Ni electron capture detector. The GC program used was 165 °C initial temperature ramped at 10 °C min^{-1}–225 °C then ramped at 5 °C min^{-1}–250 °C with a hold time of 0.8 min. Temperature was then ramped at 25 °C min^{-1}–280 °C with a hold time of 3 min. Total run time was 16 min. A 3-μL splitless injection was used, and the injection port was maintained at 250 °C. The carrier gas was H_2, and the make-up gas was N_2 at 60 mL min^{-1}. The detector was maintained at 325 °C. Retention times of *o,p′*-DDE and *p,p′*-DDE were 6.2 and 6.7 min, respectively. All six-point calibration solutions (formulated with spectrophotometric-grade ACS solvents) differed by < 3% for *o,p′*-DDE and <5% for *p,p′*-DDE ($r^2 = 0.999$) between each set of calibration curves. Stocks were freshly made prior to their use in all phases of experiments and were periodically analyzed for statistical validation ($p < 0.05$). Method blanks and spike standards