Chemosphere 144 (2016) 1428–1434

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Bioavailability assessments following biochar and activated carbon amendment in DDT-contaminated soil

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HIGHLIGHTS

- GAC and biochar were added *in situ* and *ex situ* to soil contaminated with DDT.
- Biochar significantly reduced the uptake of DDT into worms.
- None of the carbon amendments significantly reduced DDT accumulation in plants.
- The known DDT phytoextractor exhibited a concentration threshold effect at >10 μg/g.
- POM passive samplers accurately predicted worm but not plant bioavailability.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form 30 September 2015
Accepted 7 October 2015
Available online 23 October 2015

Handling editor: Ian Cousins

Keywords:
Biochar
Activated carbon
Bioavailability
Polyoxymethylene equilibrium passive sampler
Phytotechnologies

ABSTRACT

The effects of 2.8% w/w granulated activated carbon (GAC) and two types of biochar (Burt's and Blue-Leaf) on DDT bioavailability in soil (39 μg/g) were investigated using invertebrates (*Eisenia fetida*), plants (*Cucurbita pepo* spp. *pepo*) and a polyoxymethylene (POM) passive sampler method. Biochar significantly reduced DDT accumulation in *E. fetida* (49%) and showed no detrimental effects to invertebrate health. In contrast, addition of GAC caused significant toxic effects (invertebrate avoidance and decreased weight) and did not significantly reduce the accumulation of DDT into invertebrate tissue. None of the carbon amendments reduced plant uptake of DDT. Bioaccumulation of 4,4′DDT and 4,4′-DDE in plants (*C. pepo* spp. *pepo*) and invertebrates (*E. fetida*) was assessed using bioaccumulation factors (BAFs) and compared to predicted bioavailability using the freely-dissolved porewater obtained from a polyoxymethylene (POM) equilibrium biomimetic method. The bioavailable fraction predicted by the POM samplers correlated well with measured invertebrate uptake (<50% variability), but was different from plant root uptake by 134%. A literature review of *C. pepo* BAFs across DDT soil contamination levels and the inclusion of field data from a 2.5 μg/g DDT-contaminated site found that these plants exhibit a concentration threshold effect at $[DDT]_{soil}$ > 10 μg/g. The results of these studies illustrate the importance of including plants in bioavailability studies as the use of carbon materials for *in situ* contaminant sorption moves from predominantly sediment to soil remediation technologies.

Abbreviations: AC, Activated carbon; DDT, Dichlorodiphenyltrichloroethane; POM, Polyoxymethylene; HOCs, Hydrophobic organic contaminants; BAFs, Bioaccumulation factors; PPNP, Point Pelee National Park; CEC, Cation exchange capacity; GAC, Granular activated carbon; RMC, Royal Military College of Canada; MAE, Microwave assisted extraction; ANOVA, One-way analysis of variance; DDE, Dichlorodiphenyldichloroethylene; DDD, Dichlorodiphenyldichloroethane; ASU, Analytical Services Unit.

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http://dx.doi.org/10.1016/j.chemosphere.2015.10.029
0045-6535/

1. Introduction

Soils contaminated with persistent, bioaccumulative, and toxic organic chemicals occur worldwide and pose a substantial challenge to environmental risk assessment and management. Alternative 'greener' remediation approaches than traditional soil excavation and transport for hydrophobic organic contaminants (HOCs) are being sought in order to reduce risk to both the environment and human health. Carbon amendments such as activated carbon (AC) and biochar have been successful in immobilizing contaminants via sorption in sediment (Ghosh et al., 2011), and more recently soil (Denyes et al., 2013; Chai et al., 2012; Wang et al., 2013) systems when added at ca 3% (w/w). The sorption of organic contaminants by carbon amendments is a result of two separate processes; – i) *ab*sorption into amorphous organic matter, and ii) *ads*orption onto the surface (Cornelissen et al., 2005). Immobilization of organic contaminants *in situ* reduces bioaccumulation of these compounds in plants, invertebrates and fish, reducing risk to higher trophic organisms. Contaminant toxicity decreases as a consequence, and the overall health of the ecosystem improves as measured by increased plant and invertebrate biomass (Denyes et al., 2013).

Over the past few decades, extensive work has been conducted on measuring bioavailability via biomimetic methods. Equilibrium passive sampling devices such as those based on the polymer, polyoxymethylene (POM), can be used to determine the chemical activity and thus bioavailability of HOCs (Beckingham and Ghosh, 2013; Oen et al., 2011). In environmental systems, chemical activity is measured by the freely dissolved equilibrium porewater concentration and is often used as an analogue for invertebrate bioaccumulation, as they accumulate HOCs via ingestion and diffusion in the gut. Biomimetic methods such as POM-based samplers have been used to measure the effect of activated carbon on contaminant bioavailability. One study (Sun and Ghosh, 2008) showed that POM derived sediment equilibrium porewater values were related to PCB congener concentrations in *Lumbriculus variegatus*, a freshwater oligochaete for both AC treated and untreated sediments. The relationship was linear for tetra- and penta-chlorinated congeners (40–50% chlorinated by weight) over a range of 0.33–84.7 μg/g (Sun and Ghosh, 2008). These authors concluded that this biomimetic method provided a "convenient and accurate" method for monitoring sediment remediation via AC amendment. Other studies further supported the biological basis for using passive samplers to monitor the success of AC remediation in PCB-contaminated sediments in freshwater (Beckingham and Ghosh, 2013) and soil (Paul and Ghosh, 2011) invertebrates.

In a recent review of methods to assess bioavailability (Cui et al., 2013), the following gaps in literature were identified: i) a lack of studies exploring naturally (field) contaminated sediments and soils, ii) field scale scenarios, and iii) contaminant accumulation in higher trophic levels. The current study investigates the effects of field scale AC and biochar addition to soils that have been field-contaminated (and naturally weathered) with high levels (39 μg/g) of the organochlorine insecticide dichlorodiphenyltrichloroethane (DDT). Bioavailability is assessed using a common soil invertebrate (*Eisenia fetida*) and the plant species *Cucurbita pepo* spp. *pepo*, which is known to accumulate DDT (White, 2002; Whitfield Åslund et al., 2008; Lunney et al., 2004). The primary objective of the current study is to compare *E. fetida* and *C. pepo* uptake to a POM-based biomimetic method. In addition, for plant studies, bioaccumulation data from an area with a lower DDT soil concentration (2.5 μg/g) is included as well as a literature review of *C. pepo* bioaccumulation factors (BAFs) across various DDT soil concentrations.

2. Materials and methods

2.1. Site details

In situ experiments were conducted at Point Pelee National Park (PPNP) located immediately south of the town of Leamington, Ontario, Canada. The area has historical significance as Canada's first National Park and is comprised of a unique Carolinian ecosystem making it renowned worldwide for its influx of endangered migratory birds (Smits et al., 2005). As a result of PPNP's former use as orchard land, legacy DDT contamination exists at levels greater than the agricultural guideline of 0.7 μg/g set by the Canadian Council of Ministers of the Environment. Due to the historical significance of the park, as well as the sensitivity of many species of birds (Smits et al., 2005) traditional remediation approaches such as soil excavation and off site transport are not viable due to their detrimental effects on the ecosystem.

2.2. Soil and materials

The soil at PPNP is classified as sandy and contains DDT contamination, composed predominantly (90% ± 12%) of 4,4′-DDE (dichlorodiphenyldichloroethylene) and 4,4′- DDT, which have weathered in place for over 40 years. Total DDT (ΣDDT) concentration refers to the sum of 2,4′- and 4,4′- DDT, DDE, and DDD isomer concentrations in the sample. Experiments were conducted from June–September 2012 and 2013 at two former agricultural areas of the park. The 2012 plot was established in soil containing an average total DDT concentration of 2.5 ± 0.03 μg/g. This soil had a cation exchange capacity (CEC) of 11.2 cmol/kg, a pH of 7.7 and contained 3.5% organic matter. In 2013, plots were established in soil with a mean total DDT concentration of 39 ± 1.8 μg/g, a CEC of 5.8, a pH of 7.9 and 3.1% organic matter.

Two types of biochar and one granular activated carbon (GAC) were obtained for experimentation. Full details of each carbon amendment characterization can be found in Supporting Information Table 1. A detailed discussion of biochar production and characterization (including methods) for the purposes of remediation is available in Denyes et al. (2014). Particle size distribution was performed in triplicate on all three carbon materials. Briefly, the carbon amendments used increased in their relative proportions of coarse particles (≥0.5 mm) from BlueLeaf biochar (86%) < GAC (96%) < Burt's biochar (98%). To provide an environmentally relevant approach to remediation the particle sizes were not altered or sieved and reflect the products produced at that specific retailer. A detailed discussion of the effects of particle size on sorption kinetics and substrate improvements can be found in Denyes et al. (2013).

All plant experiments were conducted in both soil concentrations, however invertebrate and POM-based studies were conducted in the 39 μg/g DDT-contaminated soil only. All studies i.e. greenhouse, lab, and field, were conducted in field-contaminated, naturally weathered soil from/at PPNP.

2.3. Invertebrate (worm) experiments

Worms (*E. fetida*) purchased from "The Worm Factory" (Westport, ON), were tested for DDT bioaccumulation, toxicity and avoidance in the Phytotechnology Laboratory located at the Royal Military College of Canada. In all cases worms were maintained in dark aluminium containers, at a temperature of 21 °C (±3 °C), at approximately 35% moisture.

Two toxicity experiments were performed in 39 μg/g DDT-contaminated soil mixed with 0%, 2.8% GAC, 2.8% Burt's biochar and 2.8% BlueLeaf biochar amendments. Twenty-five worms were

added immediately after mixing to the soil/amendment mixtures and the number of surviving worms counted and weighed at ~50 days. This method has shown to provide a sufficient amount of time to demonstrate bioavailability reductions in PCB-contaminated soil (Denyes et al., 2013). Surviving worms from the second experiment were washed using a container of clean water and depurated for 72 h at 4 °C, dried for 24 h at 25 °C, stored in individually labelled Whirlpak® bags, and frozen until analysed for DDT concentration.

Selection for invertebrate avoidance assays was based on the method described by Li et al. (2011) (Li et al., 2011a), and worms weighing 0.3–0.6 g were used. Avoidance wheels were constructed using a modified design from Environment Canada's Acute Avoidance Test (Denyes et al., 2014). Each of the six compartments was filled with 120 g of DDT-contaminated soil or DDT-contaminated soil/carbon amendment mixture, with every other compartment serving as an unamended control. During testing, wheels were covered with aluminium foil to prevent worm escape and to maintain moisture. Testing was done in triplicate for each amendment and 30 worms were exposed (i.e. 10/replicate) for a period of 48 h. Refer to Denyes et al., 2014 for detailed illustrations and video of avoidance testing.

2.4. Plant experiments

For both field sites (2012 and 2013) native vegetation was removed and four plots, 200 cm long by 50 cm wide and 20 cm deep were established manually using a shovel. Plots were a minimum 50 cm apart. The appropriate carbon amendment (0% (control), 2.8% w/w – GAC, Burt's biochar, or BlueLeaf biochar) was weighed then added to the respective plot and mixed thoroughly to ensure homogeneity. DDT concentrations did not differ between treatments (Mean = 38.6 ± 12.5, n = 4). Each plot received nine pumpkin (*C. pepo* ssp. *pepo* cv. Howden) seeds purchased from the 'Ontario Seed Company' (Waterloo, ON) in June, however extra seedlings were removed at ca. three weeks, such that each plot contained only three growing plants, evenly spaced. Pumpkin plants were harvested at 65 days. In the 2013 experimental design only, all the treatments (i.e control, GAC, Burt's and BlueLeaf) were planted again with another nine pumpkin seeds, and plants were grown in triplicate for another 60 days. Plants were also re-planted in triplicate in the control (0%) treatment in the area containing 2.5 μg/g DDT. Plants were watered two times per week regardless of precipitation.

The *in situ* field experiment in 2013 was replicated in triplicate in the greenhouse located at the Royal Military College of Canada (RMC) using DDT-contaminated soil collected from site in PPNP. The amendment mixture was placed in bottom perforated six-inch diameter planting pots (total soil weight per planter of 500 g). Pumpkin plants were measured for plant growth on a weekly basis and harvested at 60 days. Greenhouse temperature was maintained at 27 °C (±7 °C) and the pumpkins were grown under a 14:10 h (day:night) fluorescent photoperiod. Planters were top and bottom watered to maintain sufficient moisture.

All pumpkin plants (field and greenhouse) were harvested by cutting the shoot of the pumpkin with acetone rinsed scissors as close to the soil surface as possible. Root samples were then collected and roots and shoots rinsed clean with water. Plant tissues (shoots and roots) were patted dry, weighed, and biomass was used to assess plant health. Plant samples were then placed in individually labelled Whirlpak® bags and frozen prior to analysis for DDT concentration.

2.5. Polyoxymethylene (POM) passive sampling experiment

A thin sheet of POM (76 μm thick) was purchased from CS Hyde Company (Lake, Villa, IL) as this product has been commonly used to determine porewater concentrations of HOCs (Oen et al., 2011; Chai et al., 2012). The partition coefficients (K_{POM}) for 4,4-DDT and 4,4-DDE were previously determined by Endo et al. (2011). Using the K_{POM} value, the soil porewater concentration (C_w) was calculated based on the equation, $C_w = C_{POM}/K_{POM}$, where C_{POM} is the calculated concentration in the polymer. The POM sheets were cut into 9 × 2 cm strips (200 mg each) and cleaned via immersion in a series of hexane, methanol and double distilled water containing 200 mg/L NaN_3 (a biocide) with gentle shaking (100 rpm) for 24 h each. All carbon amendments were added at 2.8% (w/w) to DDT-contaminated soil (39 μg/g) and mixed for 1 h at 30 rpm. Treatments were tested in triplicate by adding 10 g (dry wt.) of soil or soil/amendment to 300 mL amber glass vials. Thirty mL of water (25 mg/L NaN_3) and 200 mg POM were added to each vial. Bottles were shaken on an orbital shaker at 25 rpm for 31 days. This length of time has been shown sufficient to reach equilibrium in PCB and PAH experiments which have similarly high sorption coefficients and low diffusivities in condensed phases as DDT (Endo et al., 2011; Hawthorne et al., 2009). POM strips were removed, rinsed with double deionized water, gently wiped dry and extracted as described below. Although only one contaminant concentration was tested, linear sorption to POM has been reported from the pg/L range and upward (Endo et al., 2011; Hawthorne et al., 2009).

2.6. Analytical procedures

2.6.1. DDT concentrations in soil, worm, plant and POM samples

All soil, worm and plant samples were dried at 25 °C for 24 h immediately prior to analysis. POM samplers were patted dry with clean tissue. Plant root and shoot samples were analysed by microwave-assisted extraction (MAE) at RMC. MAE was performed at a temperature of 120 °C for 35 min in 30 mL of 1:1 hexane:acetone mixture using a Milestone Ethos SEL microwave extraction system. Following extraction, sample extracts were concentrated using a Syncore, the solvent exchanged for hexane, and then extracts were applied to a Florisil column for cleanup.

DDT concentrations in soil and worm tissues and POM samplers were analysed via Soxhlet extraction, at the Analytical Services Unit of Queen's University. Worm samples were finely chopped using metal scissors (rinsed with acetone between samples) and homogenized. Chopped worm samples were dried at room temperature for approximately 12–18 h, and then soil and worm samples were ground with sodium sulphate and Ottawa sand. Soil and worm and POM samples were extracted in a Soxhlet apparatus for 4 h at 4–6 cycles per hour in 250 mL of dichloromethane and 250 mL of a 1:1 hexane:acetone mixture, respectively.

Sample extracts were analysed for DDT and its key metabolites (i.e DDE and DDD), using an Agilent 6890 Plus gas chromatograph equipped with a micro-^{63}Ni electron capture detector (GC/μECD), a SPB™-1 fused silica capillary column (30 m, 0.25 mm ID × 0.25 μm film thickness) and HPChem station software. The carrier gas was helium, at a flow rate of 1.6 mL/min. Nitrogen was used as the makeup gas for the electron capture detector (ECD). Detection limits were 1.0 ng/g. All values were reported as μg/g dry weight, and DDT concentration unless otherwise specified refers to the sum of DDT and its metabolites.

2.6.2. Quality assurance/quality control (QA/QC)

One analytical blank, one control and one analytical duplicate sample were prepared and analysed for every nine samples analysed by Soxhlet or MAE. Ottawa sand was used as the control sample and was spiked with a known amount of Supelco Appendix IX pesticide mixture. Decachlorobiphenyl (DCBP) was added to each sample as a surrogate standard prior to extraction. None of the

analytical blanks contained DDT at concentrations above detection limits (1.0 ng/g for total DDT) and all control samples were between 80 and 110% of the expected value. Relative standard deviations between the samples and their analytical duplicate were below 14% for all results and the average surrogate recovery for samples was 89%.

2.7. Statistical analyses

The tissue concentration data were analysed by one-way analysis of variance (ANOVA) followed by a post hoc Tukey comparison. All residuals of the data were determined to be normally distributed by a Kolmogorov Smirnov test, and the homogeneity of the variances was determined to be equal using a Levene's test. Tests were performed with three degrees of freedom among, and eight within the groups. Individual p values were reported when significant, using a level of $\alpha = 0.05$ for all tests, and results were recorded with the standard deviation of the mean.

3. Results and discussion

3.1. Invertebrates

The type of carbon amendment had a significant effect on *E. fetida* weight ($F(3,8) = 20.3$, $p = 0.001$), but not survivorship ($F(3,8) = 0.43$, $p = 0.76$). For worms exposed to unamended DDT-contaminated soil (control), Burt's, and Blueleaf biochars, there were no significant differences in earthworm final weight (relative to initial weight) (Fig. 1). In these treatments, earthworms either maintained or increased their weight by the end of the exposure (Fig. 1). However, worms exposed to the 2.8% GAC decreased in weight over the trial (by 59–82%), resulting in a significantly lower final weight (relative to initial weight) that the other treatments (Fig. 1).

In the avoidance study, 84% and 80% of *E. fetida* significantly avoided the 2.8% GAC ($F(1,4) = 31.5$, $p = 0.005$) and BlueLeaf ($F(1,4) = 50.0$, $p = 0.002$) amended soil respectively (Supporting Information Table 3), similar to other studies (Li et al., 2011b). However, the worms preferred the 2.8% Burt's biochar amended soil over the unamended soil highly contaminated with DDT ($F(1,4) = 2.0$, $p = 0.230$). Soil amended with Burt's and BlueLeaf biochar resulted in worms accumulating significantly ($F(3,8) = 7.64$, $p = 0.01$) lower DDT concentrations relative to the control (49% and 36% respectively). Although not significant, GAC also reduced DDT bioavailability (as measured by concentration) by 29%. There were no significant differences between amendments as indicated by a post-hoc Tukey comparison ($p > 0.05$). Bioavailability reductions of HOCs to invertebrates as a result of carbonaceous amendment are well reported (Gomez-Eyles et al., 2011; Sun and Ghosh, 2007; Paul and Ghosh, 2011; Millward et al., 2005; Denyes et al., 2012, 2013), and are explained by strong sorption of the contaminant molecule to the carbonaceous sorbent particle. This study was conducted over a relatively-short period of time and treatment benefits may be delayed as a result of spatially heterogeneous AC particles, minimizing DDT/carbon amendment contact (Denyes et al., 2013; Cho et al., 2007; Werner et al., 2006; Cho et al., 2012). Thus even greater reductions in DDT uptake may be achieved overtime and a monitoring component should be included in future field-scale studies.

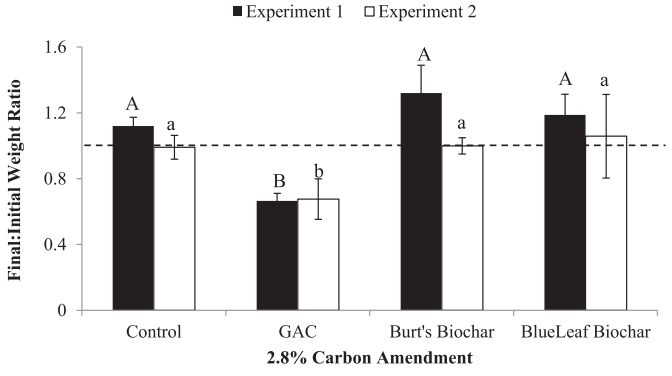

Fig. 1. Ratio of final to initial weights (g) of *Eisenia fetida* exposed to unamended DDT-contaminated soil (control) or 2.8% (w/w) GAC, Burt's biochar or BlueLeaf biochar for both toxicity experiments. Values < the dashed 1.0 line represent a loss in worm weight. Error bars represent one standard deviation. Upper-case letters (first experiment) and lower-case letters (second experiment) indicate statistically significant differences between treatments ($p < 0.05$) via Tukey post hoc analysis.

Soil invertebrates are essential for maintaining soil health and represent an important food source for many avian species at PPNP. Hence, maintaining healthy soil invertebrates while minimizing contaminant uptake is especially important at PPNP. It has been suggested that carbon amendments may cause adverse effects such as weight loss to soil/sediment invertebrates (Millward et al., 2005) as a result of strong nutrient sorption to the AC particle, particle interference within the gut of the organisms, or changes to pH and soil salinity as a result of biochar containing elevated amounts of magnesium and sodium ions (Liesch et al., 2010). In the current study, the pH of the soil (10) was very similar to the pH of GAC, Burt's and BlueLeaf biochars (9, 9, and 10, respectively) and our amendments contained magnesium and sodium levels two orders of magnitude less than the toxic levels observed in Liesch et al. (2010). Additionally, the particle size distribution of all amendments were similar (86%–98% > 0.5 mm) and thus also unlikely to explain the observed differences. Thus, the effects on worm behaviour and weight (i.e. reduced toxicity) are likely explained by reductions in DDT bioavailability due to biochar addition. Subsequently when the GAC amendment failed to reduce DDT bioavailability, the worms had the lowest body weights and significantly avoided (when given the opportunity) the treatments.

3.2. Plants

Plants grew well in soils contaminated with 2.5 and 39 μg/g DDT, showing no signs of toxicity, and there were no differences in plant biomass between treatments (2.5 μg/g *soil*: $F(3,8) = 1.26$, $p = 0.351$, 39 μg/g *soil*: $F(3,32) = 1.46$, $p = 0.245$). Earlier studies by Denyes et al. (Denyes et al., 2012, 2013) reported increased plant growth as a result of the addition of biochar to intensely degraded Brownfield soil. In this study the PPNP soil was not degraded and therefore substrate improvements such as CEC, increased particle size distribution and nutrient additions as a result of biochar amendment were negligible.

No significant reductions in plant DDT concentrations (i.e uptake) as a result of AC and biochar amendments in either soil concentration were achieved (Shoot: $F(3,47) = 0.05$, $p = 0.99$, Root: $F(3,47) = 0.58$, $p = 0.63$) (Supporting Information Table 2). These results were not expected as the same GAC and BlueLeaf biochar and a later batch of Burt's biochar were previously reported to reduce PCB (Aroclor 1254/1260) uptake to *C. pepo* by up to 74% (Denyes et al., 2013). In the current and PCB studies, seeds were sown immediately following carbon amendment and plants grown for 60 days. Even after 120 days post amendment in the current study plants did not show reduction in DDT uptake. Significant differences in plant shoot ($F(1,49) = 148.39$, $p = 2.2 \times 10^{-16}$) and root ($F(1,49) = 182.93$, $p < 1 \times 10^{-16}$) bioaccumulation factors ($BAF = [DDT]_{plant/worm/POM}/[DDT]soil$) were observed between the two study sites. Plants grown in 2.5 μg/g DDT-contaminated soil had mean shoot and root BAFs of 4.6 and 13.1 (2012) and 2 and 15 (2013), respectively (Supporting Information Table 2). These were

significantly higher than the shoot and root BAFs of the plants grown in 39 μg/g DDT-contaminated soil which were 0.26 and 0.92, 0.002 and 0.99, and 0.06 and 1.95 for the first and second field harvests and the greenhouse study, respectively (Supporting Information Table 2). When comparing BAFs from the current study and those from literature (Supporting Information Fig. 1), a trend emerges showing decreasing BAFs with increasing DDT soil concentrations, particularly in plant shoots indicating a concentration threshold effect. Plots of the $\log BAF_{shoot}$ and $\log BAF_{root}$ versus $\log[DDT]_{soil}$ (Supporting Information Fig. 2) show linear correlations having R^2 values of 0.6 and 0.4, respectively. These results in combination with the significant difference in plant DDT concentration between sites suggests that the ability of plants to accumulate high levels of DDT is dependent on soil concentration and may be indicative of a concentration threshold effect in soils with DDT concentrations >10 μg/g. The lack of literature BAF data for field-contaminated soils with DDT concentrations between 10 and 40 μg/g makes it difficult to accurately identify the exact threshold concentration for *C. pepo*. However this finding offers an explanation for the lack of significant bioavailability reductions in the amended and control plants in the soil containing 39 μg/g DDT. In the 2.5 μg/g DDT-contaminated soil, the inability of the carbonaceous sorbents to effectively immobilize the DDT contamination may be related to the unique ability of *C. pepo* to facilitate DDT uptake via root exudates, a process known to be even more effective than for PCB uptake (Whitfield Åslund et al., 2010). Also, the act of incomplete mixing of the carbon amendments with the DDT-contaminated soil may be delaying treatment benefits similarly to the invertebrate study. It is well reported in literature that thorough mixing (i.e. rototiller, laboratory techniques such as end over end for 24 + hrs) promotes smaller particle sizes which in turn increases the soil/carbon amendment contact time, improves the homogeneity of the mixture and offers a greater number of particles per unit volume of soil (Cho et al., 2007; Denyes et al., 2013; Hale and Werner, 2010).

3.3. Comparison of accumulation in invertebrates and plants with predicted bioavailability using passive samplers in 39 μg/g DDT-Contaminated soil

Soil porewater concentrations were calculated from the POM based passive samplers using partition coefficients for 4,4′-DDT and 4,4′-DDE from Endo et al., (Endo et al., 2011) given that PPNP soil is predominately (90%) composed of these metabolites. The proportion of these two compounds relative to total DDT extracted was 84.7% ± 4.9%, and did not differ significantly between sample type (i.e. soil, shoot, root, worm and POM, $F(5,108) = 20.19$, $p = 3.54 \times 10^{-14}$). Reductions in the soil porewater concentrations of the combined total of 4,4′-DDT and 4,4′-DDE, as a result of AC and biochar soil amendments, are compared to the corresponding measured reductions in worms, plant roots and plant shoots in Fig. 2. Porewater concentrations significantly ($F(3,8) = 7.69$, $p = 0.01$) decreased by 29% and 31% as a result of GAC and BlueLeaf biochar amendments, respectively. The POM-biomimetic method adequately predicted DDT accumulation reductions in worms in all three carbon amendments, as also shown by other studies (Paul and Ghosh, 2011; Chai et al., 2012). None of the carbon amendments reduced uptake of 4,4′-DDT and 4,4′-DDE into the plant roots ($F(3,8) = 0.079$, $p = 0.969$) or shoots ($F(3,8) = 1.04$, $p = 0.427$), and soil porewater concentrations determined by the POM based passive sampler did not successfully predict treatment effectiveness of carbon amendments to plants.

In Fig. 3a–c POM BAFs (determined in amended and unamended 39 ppm total DDT soils) are compared to the corresponding worm, shoot and root BAFs in a similar manner as by Gomez et al. (Gomez-Eyles et al., 2012) and Brennan et al. (Brennan et al., 2014). Soil porewater concentrations generally underestimated the concentration of 4,4′DDT and 4,4′-DDE accumulated into worm tissue (Fig. 3a), but mean worm BAFs for unamended and amended soils were within 50% of the POM-derived BAFs. Studies have shown better POM-predicted and measured bioavailability correlations for invertebrates in sediment systems using linear regression models ($R^2 \sim 0.9$) (Beckingham and Ghosh, 2013; Sun and Ghosh, 2008) in compounds with similar levels of chlorination. However in soil systems, contamination is more heterogeneous than in sediments potentially limiting mass transfer (Denyes et al., 2013; Hale and Werner, 2010). Gomez et al. (Gomez-Eyles et al., 2012) have suggested one order of magnitude error in the biomimetic method (Fig. 3) is appropriate due to soil heterogeneity.

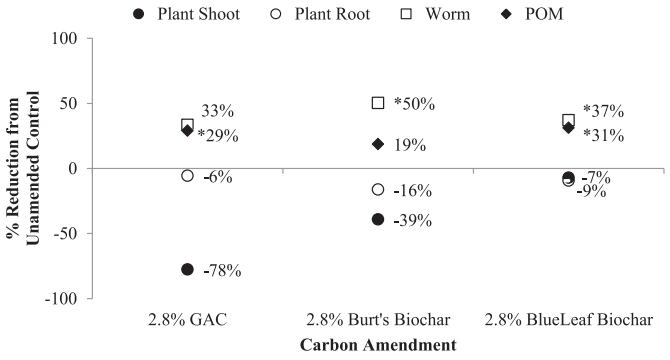

Fig. 2. Measured plant and invertebrate- and POM-predicted bioavailability reductions of Σ4,4′-DDE and 4,4′-DDT following 2.8% carbonaceous amendment to DDT-contaminated soil (39 μg/g). Data labels indicate the percent reduction from the relative control in each experiment as a result of that particular AC or biochar amendment. * indicate a significant reduction ($p < 0.05$). Negative values represent no reduction in plant, invertebrate or POM DDT uptake.

The POM biomimetic method clearly over-predicted the actual accumulation in plant tissues (Fig. 3b and c). As expected bioavailability in roots is better predicted and is within an order of magnitude (134 ± 18%). Gomez et al. (Gomez-Eyles et al., 2012) observed an under-prediction of PAH bioavailability in plants via POM-based extraction methods, founded on the ability of root exudates to act as biosurfactants and increase the mobilization of PAHs from the soil matrix. A similar result was expected in this study as DDT is readily mobilized by exudates of *C. pepo*. The high DDT soil concentration leading to a concentration threshold effect likely prevented this from occurring.

There are a lack of studies comparing equilibrium aqueous concentrations and bioavailability in organisms other than invertebrates, especially in highly contaminated systems. This is the first study to compare predicted bioavailability as determined by POM passive samplers to actual plant bioavailability from samples collected *in situ*. Plant shoot ($F(1,22) = 1.49$, $p = 0.234$) and root ($F(1,22) = 0.004$, $p = 0.949$) 4,4′-DDT and 4,4′-DDE concentrations did not differ significantly between soil concentrations, despite having significantly higher BAFs (Shoot: $F(1,22) = 82.31$, $p = 6.88 \times 10^{-9}$, Root: $F(1,22) = 56.93$, $p = 61.54 \times 10^{-7}$) when grown in lower DDT contamination. This confirms that (in this study) *C. pepo* uptake is inhibited at high soil DDT concentrations, and a threshold effect is occurring. Plant shoot and root BAFs from the 2.5 μg/g total DDT soils (4.3 ± 1.6 μg/g and 11.8 ± 5.1 μg/g, respectively) are more comparable to POM BAFs (4.9 ± 0.9 μg/g), suggesting potential for this method at lower DDT soil concentrations (i.e. < 10 μg/g). Future experiments of POM samplers in a range of soils having lower DDT contamination are required to confirm this. Also, a POM-based biomimetic sampling method may be appropriate for use in soil systems where plant uptake is not controlled by soil contaminant concentration.

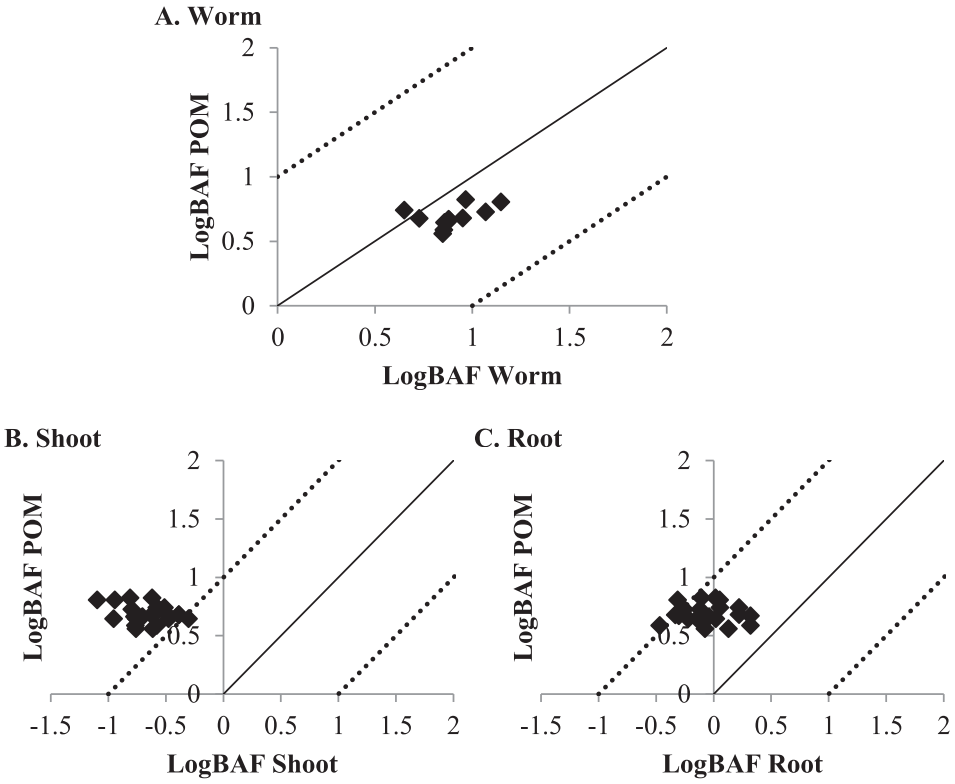

Fig. 3. Relationship between predicted bioavailability of 4,4′-DDT and 4,4′-DDE as determined by POM bioaccumulations factors (BAFs) and BAFs in A) Worm, B) Shoot, and C) Root tissues from experiments performed in [DDT]soil = 39 μg/g. The solid black line indicates 1:1 relationship, where the dotted black lines delimit one order of magnitude deviation intervals.

4. Conclusions

Biochar and activated carbon show potential to be used as an *in situ* management strategy for HOC-contaminated soils, by minimizing bioavailability. These materials must be applied in a site-specific manner to avoid detrimental effects to soil invertebrates. Further research is required to determine the exact concentration at which the threshold effect limiting DDT uptake in *C. pepo* is occurring, and if the POM-biomimetic method can accurately predict the effect of carbon amendments on contaminant bioavailability in plants including those which are not phytoextractors or known to exhibit a concentration threshold effect.

Acknowledgements

This work was funded by the NSERC Canada Research Chair program to Dr. Barbara A. Zeeb. Special thanks to Drs. Sarah Hale and Gerard Cornelissen from the Norwegian Geotechnical Institute for their correspondence and insight with respective to the POM-based passive samplers.The authors would like to thank the Analytical Services Unit (ASU) at Queen's University, A.C. Carbone Inc., Burt's Greenhouses, BlueLeaf Inc., and the site owners for their ongoing support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2015.10.029.

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