Activated Carbon Immobilizes Residual Polychlorinated Biphenyls in Weathered Contaminated Soil

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Activated carbon (AC) has recently been shown to be effective in sequestering persistent organic pollutants (POPs) from aquatic sediments. Most studies have demonstrated significant reductions of POP concentrations in water and in aquatic organisms; however, limited data exist on the possibility of using AC to immobilize remaining POPs at terrestrial contaminated sites. Under greenhouse conditions, pumpkin *(Cucurbita pepo* ssp *pepo* cv. Howden) were grown, and red wiggler worms (*Eisenia fetida*) were exposed to an industrial contaminated soil containing a mixture of polychlorinated biphenyls (PCBs), i.e., Aroclors 1254 and 1260) treated with one of four concentrations of AC (0.2, 0.8, 3.1, and 12.5%) for 2 mo. The addition of AC to contaminated soils virtually eliminated the bioavailability of PCBs to the plant and invertebrate species. There were reductions in PCB concentrations of more than 67% in *C. pepo* ssp *pepo* and 95% in *E. fetida*. These data suggest that AC could be included as part of comprehensive site closure strategy at PCB-contaminated sites.

Amorphous organic matter, black carbon, coal, and kerogen are known to adsorb organic chemicals (reviewed in Cornelissen et al., 2005; Koelmans et al., 2006). Carbonaceous geosorbents and synthetic activated carbon (AC) have a much stronger adsorption affinity for organic contaminants, in particular at low solute concentrations than amorphous organic carbon (e.g., humic substances). Numerous studies have demonstrated the efficiency of AC to reduce the bioavailability of organic pollutants, such as polychlorinated biphenyls (PCBs) (McLeod et al., 2008; Sharma et al., 2009), dichlorodiphenyltrichloroethane (DDT) (Tomaszewski et al., 2008), and polycyclic aromatic hydrocarbons (Cornelissen et al., 2006), in aquatic environments. Treatments of freshwater sediments with 2.5% AC reduced PCB concentrations by more than 97% in water (McLeod et al., 2008). Similar reductions in PCB uptake have been reported in many aquatic organisms. For example, a 92% decrease has been measured in California blackworm (*Lumbriculus variegates*) (Sun and Ghosh, 2007), a 51% decrease in Bent-nosed clam (*Macoma nasuta*) (Cho et al., 2007), a 50 to 75% decrease in estuarine amphipod (*Leptocheirus plumulosus*) (Cho et al., 2007; Millward et al., 2005), and an 87% decrease in marine polychaete (*Neanthes arenaceodentata*) (Millward et al., 2005). Optimal AC particulate size, dose, and mode of application have also been tested in aquatic sediments (McLeod et al., 2008; Sun and Ghosh, 2007; Zimmerman et al., 2005). Studies have demonstrated that AC favors PCB sequestration in aquatic sediments when the concentration is around 3.4% (McLeod et al., 2008; Zimmerman et al., 2005), AC particulates are finer (i.e., 45–180 μm), and AC is mixed for a 2-min regime into the sediments (Sun and Ghosh, 2007). Therefore, AC amendment to PCB-contaminated sediments is a promising tool to immobilize remaining PCBs after maximal PCB remediation has been achieved.

Terrestrial contaminated sites could potentially use a similar technology to trap residual POPs. In a preliminary study, Lunney and colleagues demonstrated that high concentrations of AC had the potential to inhibit DDT bioavailability in soils (Lunney et al., 2010). The addition of 50% AC to highly contaminated soils (v/v) prevented DDT bioaccumulation in pumpkins and zucchinis (*Cucurbita pepo* ssp *pepo* cv. Howden) (Lunney et al., 2010).

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Abbreviations: AC, activated carbon; BAF, bioaccumulation factor; DDT, dichlorodiphenyltrichloroethane; PCBs, polychlorinated biphenyls.

The objectives of the current research are to determine if AC has the potential to reduce PCB-bioaccumulation in terrestrial plants and invertebrates and to assess the optimal concentration of AC needed to reduce bioavailability of PCBs in contaminated soils.

Materials and Methods

Soil Preparation

Soil contaminated with weathered commercial Aroclors 1254 and 1260 (mean concentrations of 37.6 ± 5.8 μg g^{-1} and 32.2 ± 3.4 μg g^{-1}, respectively) was collected from an industrial site located in Ontario (Canada). The soil is mainly clay with a total organic carbon content of 3.5 to 4.3% (Whitfield-Åslund et al., 2007). Soil was dried, sieved at 16 mm, and homogenized by the Japanese pie-slab mixing method (Pitard, 1993). The soil was then treated with 0, 0.2, 0.8, 3.1, or 12.5% (w/w) of AC made from bituminous coal (ρ = 0.384 g mL^{-1}, size ranging from 420 to 1680 μm; pH 8) (BC1240; A.C. Carbone Canada, Inc., St. Jean sur Richelieu, QC). Each treatment was tumbled for 24 h in a leachate soil tumbler, and 0.5-kg of the soil mixture was placed in 5-in-diameter planting pots. Each treatment was represented in triplicate or quadruplicate. To provide more aeration to the soil, 1:1 (v/v) vermiculite (Schultz; Sure-Gro IP, Brantford, ON) was added to each soil pot and manually mixed.

Experimental Design

Exposures were performed under greenhouse conditions at a temperature maintained at 27°C (±3°C) and at a fluorescent photoperiod of 14:10 h (day/night). Three *C. pepo* ssp *pepo* seeds (Ontario Seed Company, Waterloo, ON) were planted halfway down each planter. Soil surface was watered and kept moist until seeds germinated. If more than one seed germinated, extra seedlings were removed. Worms were acclimatized to greenhouse conditions for 17 d in fresh soil (Miracle-Gro, 0.14–0.14–0.14; Scotts Canada, Mississauga, ON) and were fed ad libitum with oat flakes prior the beginning of exposure. Then, 5.5 ± 0.5 g (n = 15–18) of mature *E. fetida* with full clitellum were added to each planter. Plant and worm planters were monitored daily, watered ad libitum, and harvested after 2 mo of exposure.

Tissue Collection and Preparation

At the end of the exposure, plants were harvested, rinsed with water, separated into root and shoot tissues, measured, weighed, and cut into small pieces. Worms were extracted from the soil, counted, rinsed with water, weighed, and depurated. The depuration process consisted of leaving worms on a piece of moist paper until all gut content was removed (moist papers were changed daily). All samples were dried at 25°C for 24 h, placed in Whirlpak bags (Nasco Canada, Newmarket, ON), and stored at −20°C until analysis.

PCB Analysis

Each sample was ground and mixed with approximately 40 g of anhydrous sodium sulfate (Na_2SO_4) and 20 g Ottawa sand and spiked with 100 μL of decachlorobiphenyl. Samples were extracted using soxhlet with 250 mL of dichloromethane (4–6 cycles h^{-1}; 4–6 h), and extracts were concentrated by rotoevaporation where dichloromethane was exchanged for hexane. All concentrates were applied to Florisil extraction columns for cleanup, and hexane was added up to 10 mL before quantitation. Determination of total PCB concentrations was performed using an Agilent 6890 Plus gas chromatograph (Agilent, Mississauga, ON) equipped with a micro-63Ni electron capture detector (GC/μECD) and a SPB-1 fused silica capillary column (30 m, 0.25 mm ID × 0.25-μm film thickness). Detection limits were 0.1 μg g^{-1}, and all values were reported as μg g^{-1} dry weight.

Quality Assurance/Quality Control

During extraction, an analytical blank (Ottawa sand), a control sample (a blank sample spiked with a known amount of Aroclor 1254 or 1260), and an analytical duplicate were run for every nine samples. None of the analytical blanks contained PCB congeners at concentrations above detection limits, and all control samples were within 88% of the expected value. Relative standard deviations of the analytical duplicates were below 30% for all results.

Data Analyses

Quantification of Aroclor concentrations was performed using the HPChem station software (Agilent). Statistical analyses were conducted using S-Plus (version 8.0; Insightful Corp., Seattle, WA). Shoot, worm, and soil data were tested for normality and homoscedasticity using the Kolmogorov-Smirnov and the Levene's tests, respectively, before being analyzed by one-way ANOVA. When data failed to meet the assumptions, data were $\log_{10}$ transformed. Analyses were followed by the Bonferroni multiple comparisons test. Given the sample size of root tissues, data were analyzed using a t test. Significance was set at $p < 0.05$.

Results

Soil PCB concentrations did not significantly vary from the beginning and the end of the experiment for Aroclor 1254 and 1260 (mean, 36.6 and 31.1 μg g^{-1}, respectively). The different AC treatments mixed with PCB-contaminated soil did not significantly affect length and weight of *C. pepo* ssp *pepo* plants (Table 1). Shoots were on average 30 ± 10 cm (p = 0.45) and 6.95 ± 2.74 g (p = 0.06). Similarly, exposure to AC did not affect *E. fetida* wet weights (mean of 0.18 ± 0.01 g per worm; p = 0.68) (Table 1). Gut content weights (wet weight of worms

Table 1. Morphological characteristics of *Cucurbita pepo* ssp. *pepo* shoots and *Eisenia fetida* after being exposed to polychlorinated biphenyl–contaminated soil amended with activated carbon.

Treatment of AC†	*C. pepo* ssp. *pepo*		*E. fetida*
	Length	Wet weight	Wet weight
%	cm	g	
0	25 ± 8‡	5.14 ± 2.45	0.19 ± 0.03
0.2	35 ± 12	7.94 ± 3.63	0.18 ± 0.02
0.8	32 ± 10	8.21 ± 2.77	0.19 ± 0.02
3.1	34 ± 11	6.80 ± 1.48	0.18 ± 0.01
12.5	24 ± 9	6.20 ± 2.38	0.17 ± 0.02

† Activated carbon.

‡ Mean ± SD. No significant changes were reported among treatments.

before depuration − wet weight of worms after depuration) were not different between treatments (data not shown).

Plants and worms bioaccumulated PCBs in the control treatments (Table 2). In *C. pepo* ssp *pepo*, bioaccumulation factors (BAFs = [PCB]tissue/[PCB]soil) of Aroclor 1254 and 1260 were on average 0.19 and 0.04 in shoots, respectively, and 11.5 and 3.8 in root, respectively. In *E. fetida*, BAFs were 87.7 and 33.0 for Aroclor 1254 and 1260, respectively. The translocation factors ([PCB]shoot/[PCB]root) were also calculated in plants for both PCB mixtures and were 0.02 (Aroclor 1254) and 0.01 (Aroclor 1260).

Most of the concentrations of PCBs in plant and worm tissues were significantly decreased as the concentration of AC increased in the soil mixture. In *C. pepo* ssp *pepo*, PCB concentrations in roots were significantly reduced from 431 to 15 μg g^{-1} for Aroclor 1254 (reduction of 97%; $p = 0.01$) and from 121 to 13 μg g^{-1} (reduction of 89%; $p = 0.03$) for Aroclor 1260 (Fig. 1a). Similarly, in *C. pepo* ssp *pepo* shoots, significant reductions from 7.1 to 2.6 μg g^{-1} for Aroclor 1254 (reduction of 63%; $p = 0.006$) and from 1.2 to 1.0 (reduction of 17%; $p = 0.50$) for Aroclor 1260 were obtained (Fig. 1b). The same patterns were observed for *E. fetida* (Fig. 2), where Aroclor 1254 levels were reduced from 3297 to 26 μg g^{-1} ($p < 0.001$) and Aroclor 1260 concentrations decreased from 1064 to 20 μg g^{-1} ($p < 0.001$), which correspond to reductions of 99 and 98%, respectively.

Discussion

This study provides evidence that application of AC is a promising technology for PCB immobilization in PCB-contaminated terrestrial soil. We tested a range of AC concentrations in a naturally contaminated PCB soil mixture and our data show that increasing AC concentrations resulted in a diminution of PCB levels in plant and invertebrate tissues. Other groups have investigated the potential use of AC to sequester POPs in aquatic sediments and demonstrated similar reductions (Cho et al., 2007; Janssen et al., 2010; McLeod et al., 2008; Millward et al., 2005; Sun and Ghosh, 2007); however, this is one of the few studies to assess the use of AC for terrestrial sites contaminated with PCBs.

Table 2. Bioaccumulation for Aroclors 1254 and 1260 in polychlorinated biphenyl–contaminated soil amended with activated carbon.

Aroclor	AC†	Bioaccumulation factor		
		Cucurbita pepo ssp *pepo*		*Eisenia fetida*
		Root‡	Shoot	
	%			
	0	11.5	0.19	87.7
	0.2	6.0§	0.14	68.4§
1254	0.8	4.4§	0.12§	19.8§
	3.1	0.8§	0.09§	2.9§
	12.5	0.2§	0.07§	0.6§
	0	3.8	0.04	33.0
	0.2	1.7§	0.03	28.7
1260	0.8	2.1	0.03	9.4§
	3.1	0.5§	0.03	1.8§
	12.5	0.4§	0.03	0.6§

† Activated carbon.

‡ Shoot and worm data were analyzed using one-way ANOVA ($n = 3$–4 per treatment; $p < 0.05$). Root data were analyzed using *t* test ($n = 2$ per treatment; $p < 0.05$).

§ Difference between a treatment and its respective control.

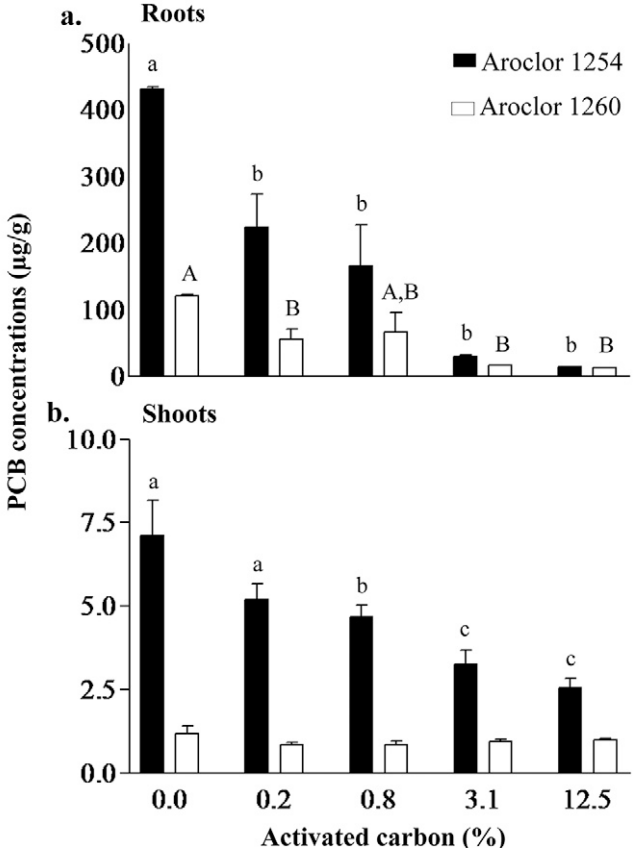

Fig. 1. Polychlorinated biphenyl (PCB) concentrations in roots (a) and shoots (b) of *Cucurbita pepo* ssp *pepo* untreated and treated with a range of activated carbon rates. Bars represent the mean + SD. Root data using *t* test ($n = 2$ per treatment; $p < 0.05$) and shoot data were analyzed using one-way ANOVA ($n = 4$ per treatment; $p < 0.05$). Lowercase letters (Aroclor 1254; black bars) and uppercase letters (Aroclor 1260; white bars) indicate statistically significant differences between treatments. No significant changes were reported for Aroclor 1260 in shoots.

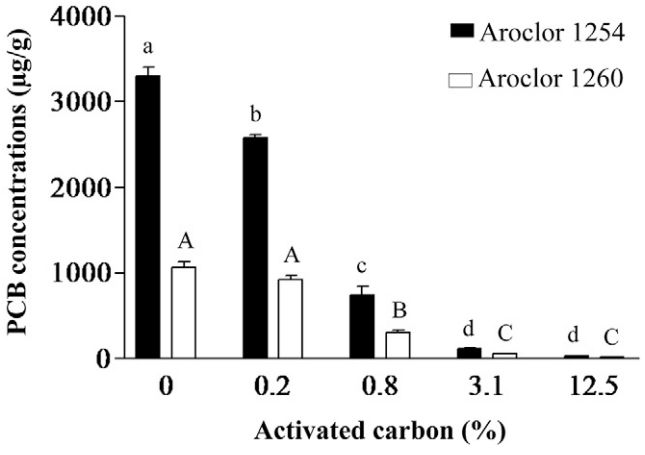

Fig. 2. Polychlorinated biphenyl (PCB) concentration in *Eisenia fetida* untreated and treated with a range of activated carbon rates. Bars represent the mean + SD. Data were analyzed using one-way ANOVA ($n = 3$ per treatment; $p < 0.05$). Lowercase letters (Aroclor 1254; black bars) and uppercase letters (Aroclor 1260; white bars) indicate statistically significant differences between treatments.

In Millward et al. (2005), a rate of 3.4% AC significantly decreased PCB bioaccumulation in *L. plumulosus* and *N. arenaceodentata* by 70 and 82%, respectively. These reductions were even higher when the contact time between contaminated sediments and AC was increased from 1 to 3 mo (Millward et al., 2005). At a similar AC rate, the present study showed significant decreases in PCB concentrations in invertebrate tissues (>95% for *E. fetida*). In addition, our data demonstrated that AC diminishes PCB extraction in plants. Pumpkins are known to be excellent phytoextractors of PCBs at contaminated sites (Whitfield-Åslund et al., 2007); however, the presence of AC in soil significantly reduces PCB uptake of plants. Similarly, amendments with AC (0.1 and 0.23%; w/w) to agricultural soil contaminated with an organic insecticide (heptachlor epoxide) prevented 73 to 98% of the uptake of the pesticide in the shoot of winter squash (*Cucurbita maxima*) (Murano et al., 2009), and the addition of 0.02 to 0.08% AC reduced dieldrin uptake up to 66% in cucumbers (*Cucumis sativus L.*) (Hilber et al., 2009). Furthermore, weathered PCB-contaminated soil amended with AC (0.5–7%) showed greater germination rate than untreated soil in white clover (*Trifolium repens L.*) (Vasilyeva et al., 2010). These results support the use of AC as an effective adsorbent of PCBs in contaminated terrestrial environments. In plants, the strong sorption of PCBs on AC appears to prevent PCB molecules from being adsorbed to the root and from being taken up into the root. Activated carbon predominantly consisting of micropores (<2 nm in size) might adsorb the solute molecules on the inner pore wall surfaces, which may lead to low bioavailability. This in turn reduces the amount of PCB available for translocation from the root to the shoot tissue. In terrestrial worms, the mechanism that controls the effect of AC on reduced biouptake remains unclear. One can speculate that worms ingest both contaminated soil and AC and that the affinity of the organic contaminant–AC complex is so strong that it cannot be broken down by digestive enzymes and microbial flora as it passes through the intestines. Therefore, the contaminant remains sorbed on AC from the ingestion-to-egestion process, preventing the pollutant from being taken up by the worm. It also remains unclear whether dermal contact makes noticeable contribution to the overall uptake of PCBs by earthworms, as compared with direct digestion. Future studies should measure the fate of AC during worm digestion.

Because there was no significant difference between the two higher AC concentrations tested for plants and invertebrates, we suggest that the optimal AC concentration to use on PCB-contaminated fields is 3% (w/w). This optimal concentration is comparable to concentrations tested in aquatic sediments for POP immobilization (McLeod et al., 2008). McLeod and colleagues also examined a range of AC concentrations (0.7, 1.3, and 2.5%) and found that 2.5% AC was the most efficient concentration to reduce PCB bioavailability in water and clam tissues. Furthermore, a 2 to 3% AC range is a realistic concentration to use for larger-scale applications at contaminated sites.

In this study, granulated AC was tumbled with contaminated soil for 24 h to ensure complete mixing; however, this method might have altered the physical condition of the tested AC to a finer consistency, which could increase AC effectiveness (Tomaszewski et al., 2007). A previous study has demonstrated that the mass transfer of PCBs to AC particulates was more rapid with smaller diameter AC, although with time, grain sizes would not affect the mass transfer (Sun and Ghosh, 2007). Future studies should focus on evaluating the efficiency of AC to decrease organism bioaccumulation using practical spreading field methods.

There is evidence that AC could also induce negative biological effects on living organisms (Jonker et al., 2004; Jonker et al., 2009; McLeod et al., 2008; Millward et al., 2005). Exposures of benthic animals to low concentrations of AC have led to a concentration-response decrease in lipid content in *L. variegatus* (Jonker et al., 2004; Jonker et al., 2009) and in growth in Asian clam (*Corbicula fluminea*) (McLeod et al., 2008) and in *N. arenaceodentata* (Millward et al., 2005). In the present study, the measured morphological endpoints (i.e., length and weight) in both species did not vary between treatments. Jonker and colleagues suggested that AC would decrease feeding behavior (egestion) in worms; however, if we estimate the soil intake of the worms in the present study by subtracting wet weight before and after depuration, no differences in weight are obtained between treatments. Future studies should assess other physiological and genetic endpoints to evaluate potential health deficiencies induced by AC exposure.

In the present study, *C. pepo* ssp *pepo* and *E. fetida* had the ability to bioaccumulate PCBs, and BAFs were calculated after 2 mo of exposure. In *C. pepo* ssp *pepo*, the BAF obtained in the control treatment of 0.19 is comparable to previous reports of BAFs in *C. pepo* ssp *pepo* studies, which range from 0.06 to 0.53 (Low et al., 2010; Whitfield-Åslund et al., 2008; Whitfield-Åslund et al., 2007). There is a difference in BAFs between the Aroclor 1254 and 1260 for plants and invertebrates. In control worms, tissue concentrations of Aroclor 1254 are three time higher than 1260, which could be explained by their difference in their ratio of lower-chlorinated to higher-chlorinated congeners. Modeling studies of PCB homologs have established that lower-chlorinated congeners tend to be the first assimilated (e.g., in polychaetes) (Janssen et al., 2010) and are more readily mobilized along the length of an organism (e.g., in pumpkins) (Whitfield-Åslund et al., 2007), whereas higher-chlorinated PCBs accumulate overtime and are the most persistent. This suggests that the chemical properties of Aroclor 1254 (i.e., fewer chlorines and less hydrophobic) could explain its higher BAFs compared with that of Aroclor 1260.

The addition of AC to contaminated soils virtually eliminated the bioavailability of PCBs to plant and invertebrate species, which resulted in an important diminution of BAFs in both organisms. This study showed that the addition of AC to soil could become part of a comprehensive site closure strategy. For example, a possible application could be to apply AC after maximal phytoextraction at PCB-contaminated sites to reduce the risks of possible PCB trophic transfer. Further studies should continue to assess other health endpoints of plants and animals to ascertain that AC does not interfere with ecosystem health.

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