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Effects of biochar and the earthworm *Eisenia fetida* on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements

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Biochar decreased PAH biovailability but was less effective at reducing PTE mobility, whilst E. fetida increased both PAH and PTE bioavailability.

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and potentially toxic elements (PTEs) were monitored over 56 days in calcareous contaminated-soil amended with either or both biochar and *Eisenia fetida*. Biochar reduced total (449 to 306 mg kg^{-1}) and bioavailable (cyclodextrin extractable) (276 to 182 mg kg^{-1}) PAHs, PAH concentrations in *E. fetida* (up to 45%) but also earthworm weight. Earthworms increased PAH bioavailability by >40%. Combined treatment results were similar to the biochar-only treatment. Earthworms increased water soluble Co (3.4 to 29.2 mg kg^{-1}), Cu (60.0 to 120.1 mg kg^{-1}) and Ni (31.7 to 83.0 mg kg^{-1}) but not As, Cd, Pb or Zn; biochar reduced water soluble Cu (60 to 37 mg kg^{-1}). Combined treatment results were similar to the biochar-only treatment but gave a greater reduction in As and Cd mobility. Biochar has contaminated land remediation potential, but its long-term impact on contaminants and soil biota needs to be assessed.

1. Introduction

Organic and inorganic contaminants usually co-exist in soils located at sites with a previous history of industrial land-use. Therefore, when attempting to remediate these kinds of soils it is important to consider the effect of remediation strategies on both of these types of contaminants. For this purpose the use of biochar as a soil amendment is of particular interest, as apart from benefits in terms of carbon sequestration (Lehmann, 2007), it has the potential to reduce the bioavailability of both organic and inorganic contaminants in soil (Beesley et al., 2010). Carbonaceous sorbents like biochar have a large surface area, several thousand fold greater than un-charred material (Thies and Rillig, 2009), giving them a large affinity for, and capacity to sorb, organic compounds (Cornelissen and Gustafsson, 2005; Lohmann et al., 2005; Oen et al., 2006; Brandli et al., 2008). As a result biochar and other carbonaceous sorbents have been found to reduce the bioavailability of a number of organic contaminants in soils and sediments (Cornelissen et al., 2006; Rhodes et al., 2008). The addition of biochar to soil can also decrease dissolved organic carbon in the soil solution (Pietikäinen et al., 2000; Beesley et al., 2010) and increase soil pH and cation exchange capacity (Liang et al., 2006; Uchimiya et al., 2010). Associated increases in the availability of some key soil macro-elements and decreases in the mobility of a number of potentially toxic elements have been reported previously (Novak et al., 2009; Beesley et al., 2010).

The importance of earthworms in ecosystem functioning has been widely documented; they are considered as an essential part of the soil fauna in most soils, and their presence is regarded as a useful indicator of soil health (Edwards, 2004). As a result earthworms have been widely used to give a measure of both organic (Bergknut et al., 2007; Gomez-Eyles et al., 2010) and inorganic (Spurgeon et al., 1994; Hobbelen et al., 2006) contaminant bioavailability. On top of giving a measure of soil toxicity it has also been suggested that earthworms could be inoculated into soils contaminated with organic pollutants (Contreras-Ramos et al., 2008) and metals (Wong et al., 2008) during remediation. However, a recent review suggests that earthworms generally increase the mobility and bioavailability of metals (Sizmur and Hodson, 2009). Also carbonaceous amendments may have an adverse effect on the habitat quality of the soils to the earthworms as found in previous studies with aquatic oligochaetes (Jonker et al., 2004). This suggests there is a need to examine the interactions between biochar and earthworms in soils contaminated with organic and inorganic contaminants.

This study aims to assess the impact of biochar on the bioavailability of polycyclic aromatic hydrocarbons (PAHs), a group of widely distributed organic contaminants, and a number of

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potentially toxic elements (PTEs) in a field contaminated soil, and how this is influenced by the presence of the earthworm *Eisenia fetida*.

2. Materials and methods

2.1. Experimental setup

Soil was obtained from a former gasworks site in Brighton, UK. Properties are given in Table 1. Soil was air dried at 20–25 °C before being sieved to <2 mm. Soil was then homogenised and half of it was separated and thoroughly hand mixed with deciduous, hardwood-derived biochar obtained from Southern Woodland Products (Salisbury, UK) produced at a pyrolysis temperature of 600 °C (Properties given in Table 1) on a 10% by dry weight basis. The biochar was ground and sieved to 2 mm before mixing. The homogenised soil and the soil and biochar mixture were then re-wetted with ultra pure (>18 MΩ cm) water to 60% of their respective water holding capacities to provide optimum moisture conditions for earthworms. Adult earthworms (*E. fetida*, Sav.) obtained from Blades Biological (Cowden, UK) were then placed in amber glass jars in the following treatments:

S: 200 g of soil (d.w.) per jar.
SB: 200 g of soil and biochar mixture (d.w.) per jar.
SE: 200 g of soil (d.w.) and 10 *E. fetida* per jar.
SBE: 200 g of soil and biochar mixture (d.w.) and 10 *E. fetida* per jar.

Each treatment was replicated 8 times, 4 to be destructively sampled after 28 days and 4 to be destructively sampled after 56 days. The initial combined weight of the 10 earthworms (average = 5.58 ± 0.34 g, ±standard deviation, $n = 16$) added to every jar was recorded to monitor weight changes throughout the exposure. No manure or other food source was added to the jars to ensure the effects observed were due to the presence of the earthworms or the biochar rather than the mixing of the food with the soil. The jars were kept in a dark room at 20 °C for the duration of the experiment. After 28 days four replicates of each treatment were sampled. The number of surviving earthworms was recorded. Earthworms were rinsed with deionised water and depurated for 24 hours (Arnold and Hodson, 2007) after which they were cleaned, weighed and frozen at −20 °C. Half of the earthworms from each replicate were analysed for PAHs, the other half for PTEs. Similarly the soil from each jar was divided in half; one half was air dried before being analysed for water soluble PTEs and the other used immediately for PAH analysis. The above procedure was repeated with the remaining jars after a further 28 days.

2.2. PAH analysis

Total PAH concentrations in the soil were determined on days 0, 28 and 56 by extracting 4 g samples of soil in 8 mL of acetone-hexane extractant (1:1 v/v) containing an internal standard on a roller mixer for 1 hour. Partitioning of PAHs into the hexane phase was encouraged by addition of 4 mL distilled water and a further 10 min of mixing, according to the principles of extracting lipids from soil using Bligh and Dyer solvent (Bligh and Dyer, 1959) in Frostegård and Bååth (1996). The extractions were then left to settle for 10 min and the hexane phases were transferred to GC vials, capped and stored at −20 °C until analysis by GC-MS (internal standard recovery = 89.6 ± 3.6%, method detection limit (MDL) = 0.05 mg kg^{-1}).

Bioavailable PAH concentrations were measured using cyclodextrin extractions which have been shown to be good surrogates of bioavailability in a number of studies (Reid et al., 2000; Cuypers et al., 2002; Swindell and Reid, 2006), including a study on the impact of a carbonaceous sorbent on PAH bioavailability (Rhodes et al., 2008). The cyclodextrin extraction was performed using a 60-mM HPCD (Sigma Aldrich, Poole, UK) solution as described in Stokes et al. (2005). The method detection limit was 0.07 mg kg^{-1}.

PAH concentrations in earthworm tissues were determined by grinding them with 7 times their weight of dry sodium sulphate using a pestle and mortar immediately after thawing them at room temperature (Van der Wal et al., 2004). A sub-sample was used to determine the lipid content of the earthworms gravimetrically by Soxhlet extraction in a 1:1 acetone/hexane solvent mixture and evaporation to dryness. The remaining sample was then extracted following a saponification method to remove fat from the earthworms (Contreras-Ramos et al., 2008). This consisted of adding 10 ml of 0.5 M KOH and 10 ml of a 1:1 acetone/hexane solvent mixture to the ground earthworm and ultrasonicating the mixture at 45 °C for 1 h. Partitioning of PAHs into the hexane phase was encouraged by adding 5 mL of distilled water and mixing for 10 min in a roller mixer. The hexane phase was then concentrated down to 1 ml under a stream of nitrogen prior to analysis by GC-MS. Extraction efficiencies were calculated using spiked recoveries and ranged between 78.5–102.9% for all PAHs.

GC-MS analysis was performed using a Thermo Trace GC Ultra system equipped with a Thermo TR-5MS capillary column (dimensions: 30 m × 250 μm × 0.25 μm; Thermo Scientific, Runcorn, UK) operating with helium as a carrier gas, coupled to a Thermo ITQ 1100 mass spectrometer (MS) through a heated transfer line (300 °C). The GC injector (220 °C) was operated in a pulsed splitless mode, 1 μl aliquots were

Table 1
Properties of the test media.

	pH	TOC (%)	CEC[c] (cmol$_c$kg^{-1})	PAHs[a] (mg kg^{-1})					Pseudo-total elements in soil[b]/Exchangeable cations in biochar[c] (mg kg^{-1})						
				2-ring	3-ring	4-ring	5/6-ring	ΣPAHs	As	Cd	Co	Cu	Ni	Pb	Zn
Soil	7.63 ± 0.1	10.6 ± 0.37	9.9 ± 0.4	5.40 ± 0.25	42.5 ± 3.1	256 ± 8.4	469 ± 9.8	773 ± 18	26.1 ± 5.3	10.7 ± 2.4	6.22 ± 0.5	83.8 ± 2.1	51.2 ± 2.6	244 ± 14	182 ± 7.3
Biochar	–	88.71 ± 11.9	11.0 ± 0.1	0.99 ± 0.02	0.20 ± 0.02	nd	nd	1.21 ± 0.04	–	<0.30	<0.23	<0.24	0.34 ± 0.03	<0.71	4.0 ± 0.16

$n = 3$, ±standard errors of the mean.
nd = below detection limits.
[a] Acetone hexane extractable concentrations based on Song et al., 2002.
[b] Aqua regia extractable concentrations based on BS7755-3.9, 1995.
[c] Based on Rowell, 1994.