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ARTICLE

Impact of Biochar on the Bioaccessibility of ^{14}C-phenanthrene in Aged Soil

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Biochar is a carbon rich product from the incomplete combustion of biomass and it has been shown to reduce bioavailability of organic contaminants through adsorption. This study investigated the influence of 0%, 1%, 5% and 10% of two different particle sized wood biochars (≤2 mm and 3-7 mm) on the bioaccessibility of ^{14}C-phenanthrene (10 mg kg^{-1}) in aged soil. The extents of ^{14}C-phenanthrene mineralisation by phenanthrene-degrading *Pseudomonas* sp inoculum was monitored over a 14 day period in respirometric assays and compared to hydroxypropyl-β-cyclodextrin (HPCD) aqueous extraction. Notably, biochar amendments showed significant reduction in extents of mineralisation and HPCD extractions. Linear correlations between HPCD extractability and total amount mineralised revealed good correlations, with 2 mm biochar showing best fit (r^2 = 0.97, slope = 1.11, intercept = 1.72). Biochar reduced HPCD extractability and bioaccessibility of ^{14}C-phenanthrene to microorganisms in similar manner. Biochar can aid risk reduction to phenanthrene exposure to biota in soil and HPCD can serve as a useful to assess the extent of exposure in biochar-amended soils

Keywords: Biochar, desorption, mineralisation and HPCD

Introduction

The exploitation of petroleum products has led to a significant deterioration of the environment and resulted in hazards to the human health.[1] The restoration and management of such contaminated sites is a very important component of the regeneration strategy for urban areas where undeveloped land is scarce.[2] Petroleum products can contain polycyclic aromatic hydrocarbons (PAHs) which may undergo photolysis, chemical oxidation, volatilisation, leaching, bioaccumulation and/or adsorption in soil.[3-4] Although the fate of PAHs in soil is controlled by its physicochemical properties and interaction with soil organic matter (SOM) and mineral fractions,[5] an important factor affecting PAH biodegradation is the degree to which they are available to microorganisms.[4,6-8] Following intra-organic matter diffusion of contaminants, PAHs become chemically adsorbed or physically entrapped within 'rubbery' and 'glassy' regions of soil,[9-10] hence, as soil-contaminant time increases, biodegradation ability decreases[11] and the contaminant can become inextractable.[12] However, contaminant adsorption processes become reversible when maximum capacity of adsorption in soil is reached[13] and either the parent contaminant or metabolite can be released through SOM biodegradation or photodegradation.[12,14] In order to overcome this, recalcitrant geosorbents can be utilised. Geosorbents such as black carbon consists of a wide range of thermally altered products which includes: charcoal, biochar and graphite.[15] Biochar has recently gained significant attention due to its ease of production, long term stability in soil,[16] potential recalcitrance,[17-18] availability and corresponding economic value. Biochar is a carbon-rich product obtained from pyrolysis of biomass such as wood,[19] in which its properties can be determined by nature of feedstock, pyrolytic temperature and duration of heating.[20] Biochar can also be obtained cheaply from energy companies that utilize wood waste to generate electricity, where biochar is a waste product from wood gasification, hence the energy companies may potentially sell biochar at a relatively low cost or possibly pay to get rid of biochar. Biochar amended in soil increase surface area and cation exchange capacity (CEC) of the receiving soil.[21] The sorptive capacity of biochar is controlled by carbonised and non-carbonised fractions as well as the surface and bulk properties.[22-23] The adsorption of aromatics, such as PAHs, to wood chars is also assisted by π -electron interaction and pore-filling mechanism,[20] multilayer adsorption, surface coverage, condensation in capillary pores, and adsorption into the polymetric matrix.[24] Although, a proportion of PAHs may be sorbed to the exterior surfaces of biochar; other portions may become trapped within internal nanopores, thereby limiting mass transfer to microorganisms.[25-26] The implication for biochar sorption capability is that it reduces the rapidly desorbable (bioaccessible) fraction of the contaminants, therefore attenuating potential accumulation or risk to biota.[10,27-29] Bioaccessibility has previously been referred to as the endpoint of biodegradation.[30]

Hydroxypropyl-β-cyclodextrin (HPCD) extraction has been effective in predicting biodegradation endpoints in single and co-contaminated soils,[5,31-33] field contaminated soils[34-35] and sediments.[36]

More recently, Rhodes *et al*.[37-39] showed decline in bioaccessibility of phenanthrene to degraders (*Pseudomonas* sp) due to soil amended with activated carbon (AC). However, to the authors' knowledge, HPCD sequential extraction has not been tested in soils containing two different particle-sized wood biochars. Also, little report but no research has shown impact of biochar on the mineralisation of phenanthrene by phenanthrene degraders and the corresponding impact on bioaccessibility of the PAHs in soil.[27,39,40] Hence, this research has the following aims: (i) to compare the impact of biochar particle size on the biodegradation of ^{14}C-labelled phenanthrene in soil aged for 365 d. This was achieved by adding two particle sized biochar (≤2 mm and 3-7 mm) to the soil at varying concentrations (0%, 1%, 5%, and 10%), and assessing mineralisation after further aging over 1 and 40 days, (ii) the bioaccessibility of aged ^{14}C-phenanthrene in soil amended with the different concentrations of biochar using HPCD extraction, (iii) It also expands on determining the desorption characteristics of the ^{14}C-phenanthrene using sequential HPCD extractions.

Materials and Methods

Chemicals

Non-labelled and [9-^{14}C]-phenanthrene (98% radioactive purity) were obtained from Sigma Aldrich Co. Ltd. UK. Goldstar multipurpose liquid scintillation fluid was obtained from Meridian, UK. HPCD was obtained from Fischer Scientific, UK. Sample oxidizer cocktails (Carbotrap and Carbocount) were from Meridian UK, and combustaid from Perkin Elmer, USA. Biochar was obtained from Yorkshire Charcoal Co., UK and was formed by the slow pyrolysis (16 - 18 hours duration at 450 – 500 ^{o}C) of a feedstock containing approximately 90% *Acer*, and the remaining 10% a mixture of *Quercus* and *Fraxinus* spp. Plate count agar and agar-agar were supplied by Oxoid, UK.

Soils spiking and amendment

An uncontaminated pristine agricultural soil (Dystric Cambisol) was collected from a depth of 5-20 cm, from Myerscough College, Preston, UK classified as surface texture of sandy loam was used in this study. The soil contained 19.5% clay, 20% silt, 60.3% sand and 2.7% organic matter and the soil pH in dH_2O and $CaCl_2$ was 6.5 and 5.2, respectively. The soil was air-dried for 24 h and passed through a 2 mm sieve to remove stones and plant roots. The moisture content of the soil was determined by drying 1.25 g sample of the soil in 3 porcelain crucibles at 105 ^{o}C for 24 h. After drying, the samples were then cooled in a dessicator for 1 h and weighed again.

The soil was rehydrated with de-ionised water to original moisture content of 21%. A third of whole soil was first spiked with ^{12}C-phenanthrene and ^{14}C-phenanthrene (43.75 Bq g^{-1}) standards prepared in toluene as a solvent carrier to achieve a concentration of 10 mg kg^{-1}, then blended for 1 min followed by a period of venting (1 - 2 h); after which, the amended soil was mixed with the remaining unspiked soil fraction Doick *et al*.[31] The mixing was done using a stainless steel spatula in glass beakers. Then toluene was allowed to evaporate within the fume hood, the soils were then stored in sterile amber glass jars. The soils later were sterilised by gamma (γ) irradiation (32.2 KGy; Isotron Plc, Bradford, U.K.) and were then contained within the sealed amber jars in dark at room temperature (21 ± 2 ^{o}C) for 365 d. Microbial sterility was assessed in soils using standard microbiological techniques through colony forming units (CFUs) counting. The soils were then amended by independently mixing the ≤2 mm (BioC1) and 3-7 mm (BioC2) particle sized biochars in 0%, 1%, 5% and 10% levels to the soil and further stored in separate dark sterile amber jars at room temperature for 1 and 40 days. The results of the biochar analysis[41,42,43] are shown in Table 1.

Table 1 Properties of the biochar used in this study

Temperature (^{o}C)	Particle size (mm)	pH	Ash content (%)	Pore volume (ml g^{-1})	Liquid quantity (µl g^{-1})
450-500	≤ 2	9.6	13.7	1.39	44
450-500	3-7	9.6	14.4	2.20	60

Determination of total ^{14}C-phenanthrene-associated activity in soil

The ^{14}C-phenanthrene associated activity was determined by combustion using a Packard 307 sample oxidizer at each sampling point of aging. Soil samples (1 g; n = 3) were weighed into cellulose combustion cones with an addition of 200 µl Combustaid and combusted (3 min). Carbotrap (10 ml) and Carbocount (10 ml) were used to trap $^{14}CO_2$. The trapping efficiency was >90%. ^{14}C-Activity was quantified by liquid scintillation counting (LSC) (Canberra Packard TriCarb 2300 TR, UK.) using standard calibration and quench correction techniques.[5,38]

Mineralisation of ^{14}C-phenanthrene in soil

A bacterial inoculum (*Pseudomonas* sp), which was previously isolated from a petroleum contaminated soil able to utilise phenanthrene as sole carbon source for growth was cultured at 0.1 g l^{-1} phenanthrene in 300 ml minimal basal salt (MBS) solution at 20 ^{o}C and at 100 rpm on an orbital shaker. After the 4th day of late-exponential phase growth during the incubation period, the culture was centrifuged at 4,500 rpm for 30 min (Rotanta 460 Centrifuge, Hettich, Germany). The supernatant was discarded and cells resuspended in fresh MBS solution.[37] This procedure was repeated twice to achieve a thorough washing of cells and removal of residual phenanthrene.

The mineralisation was performed in modified 250 ml Schott bottles using the method described by Reid *et al*.[44] The respirometer assays incorporate a Teflon lined screw cap and a CO_2 trap containing 1 M NaOH (1 ml) within a 7 ml glass scintillation vial. After the initial aging of 365 d, respirometers were prepared in triplicate with 10 ± 0.2 g soil containing 0%, 1%, 5%, and 10% biochar, 25 ml MBS solution, and 5 ml phenanthrene-degrading inoculum bacteria (10^{7} cells g^{-1} soil). Cells were enumerated by measurement of CFUss on plate count

agar following standard microbiological techniques. Sterile MBS solution was prepared as per Reid *et al*.[44] in deionised water. The respirometers were placed on an orbital shaker and set at 100 rpm and 21 °C ± 1 °C over a period of 14 d. Evolved $^{14}CO_2$ as a result of ^{14}C-phenanthrene catabolism was trapped in 1 M NaOH. The ^{14}C-activity was assessed every 24 h for 14 d by LSC during the sampling period (1 and 40 d).

Extraction of ^{14}C-phenanthrene-associated activity by hydroxypropyl-β-cyclodextrin (HPCD)

Determination of ^{14}C-phenanthrene extractability using HPCD was carried out at each sampling point (1 and 40 d) as described by Reid *et al*.[5] It was assessed sequentially at 24 h intervals over a period of 6 d. HPCD solutions (50 mM) were prepared using deionised water. Soils (1.25 g) were weighed into 30 ml Teflon centrifuge tubes (n = 3) and 25 ml HPCD solution was added to each. The tubes were placed onto an orbital shaker at 100 rpm for 24 h. The tubes were then centrifuged at 3,500 rpm for 1 h (Rotanta 460 Centrifuge, Hettich, Germany) and 6 ml supernatant was pipetted into 20 ml glass scintillation vials containing Goldstar scintillation cocktail (14 ml). The ^{14}C-activity in the resultant solution was then quantified using the LSC, as described previously. After the sequential extraction, the remaining soil pellet was air dried, weighed into combust cones and then oxidized using the method of determination of ^{14}C-phenanthrene-associated activity in soil. This was to ensure a mass balance of ^{14}C-phenanthrene activity before and after desorption.[5,33]

Statistical analysis

Statistical analysis of results was conducted using one-way ANOVA ($P < 0.05$) using SigmaStat software (Ver 2.0; Systat, Richmond, CA, USA). Statistical tests were done to compare biochar amendment, particle sizes and soil-PAH contact times.

Results

Determination of ^{14}C-phenanthrene mineralisation in soil

The mineralisation of ^{14}C-phenanthrene was monitored over a period of 14 d incubation in soil amended with 0%, 1%, 5% and 10% biochar after 1 and 40 d soil-phenanthrene-biochar interaction period (Figure 1). Following 365 d aging of the ^{14}C-phenanthrene (10 mg kg^{-1}) spiked soils, there was approximately 58-65% loss due to volatilisation as background ^{14}C-phenanthrene-associated activity decreased. Statistical analysis of the generated data confirmed that the addition of biochar had a significant impact on the total extent of ^{14}C-phenanthrene mineralisation during the aging period. After 1 d aging, increasing biochar concentration resulted in consistent decrease ($P < 0.05$) in extent of ^{14}C-phenanthrene mineralisation (Figure 1; Table 2).

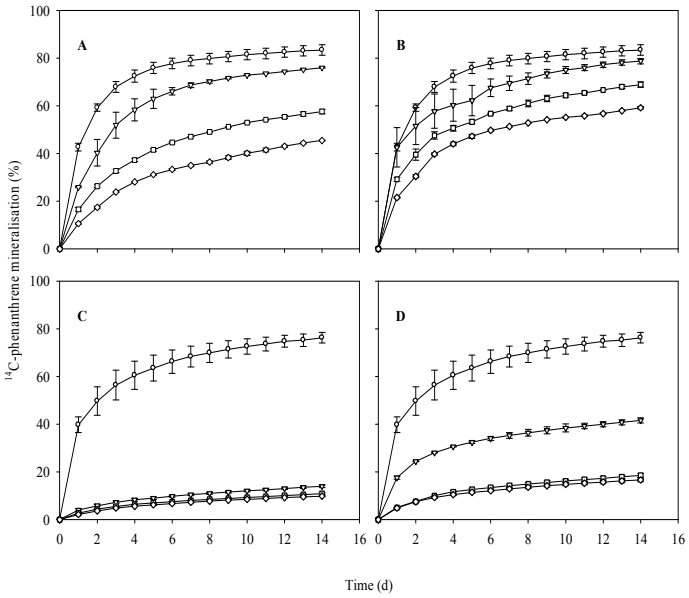

Figure 1 Amount of ^{14}C-phenanthrene mineralised (%) in Myerscough soil amended with 0% (○), 1% (▽), 5% (□) and 10% (◊) of biochar. Error bars represent standard error of mineralisation (SEM) (n = 3). A and C = ≤ 2 mm particle size biochar amendment in soil at 1 d and 40 d, respectively. B and D = 3-7 mm particle size biochar amendment in soil at day 1 and 40 respectively.

Table 2 Total amount of ^{14}C-phenanthrene extracted by HPCD after first 24 h and mineralised by phenanthrene degraders

Aging (d)	BioC	BioC1 (≤2 mm) Extracted	BioC1 (≤2 mm) Mineralised	BioC2 (3-7 mm) Extracted	BioC2 (3-7 mm) Mineralised
1	0	73.5 ± 0.6	83.4 ± 2.2	73.5 ± 0.6	83.4 ± 2.2
	1	61.1 ± 3.2	76.1 ± 0.2	54.2 ± 3.7	78.8 ± 1.1
	5	43.3 ± 2.3	57.6 ± 0.9	40.9 ± 2.3	68.9 ± 1.1
	10	35.7 ± 4.9	45.5 ± 0.2	24.4 ± 2.9	59.2 ± 0.5
40	0	75.7 ± 0.8	76.3 ± 2.2	75.7 ± 0.8	76.3 ± 2.2
	1	13.6 ± 1.7	14.0 ± 0.5	22.0 ± 0.2	41.6 ± 1.1
	5	11.7 ± 1.7	10.9 ± 0.3	23.2 ± 1.3	18.5 ± 0.8
	10	8.2 ± 1.1	9.9 ± 0.1	12.9 ± 1.2	16.6 ± 0.8

Values are in (%)

Following 40 d aging period, all three concentrations (1%, 5% and 10%) of biochar in soil showed more significant reduction in extent of ^{14}C-phenanthrene mineralisation ($P < 0.001$). The extent of mineralisation with 1%, 5% and 10% biochar amendments (BioC1) were 14.0%, 10.9% and 9.9%, respectively, whilst BioC2 amendments had 41.6%, 18.5% and 16.6% extent of mineralisation (Table 2; Figure 1). In comparing 1 and 40 d extent of mineralisation, there was at least a 50% reduction in the extent of mineralisation in 1%, 5% and 10% biochar amended soils following subsequent aging (40 d) (Table 2). However, there was no significant difference in the extent of mineralisation with the 0% biochar amendment when 1 and 40 d aging periods were compared ($P > 0.05$). When comparing impact of both BioC1 and BioC2 on the extent of mineralisation, the results showed that there was a significant difference between both particle sizes at both

time points. At each time point, 5% and 10% (1 d), as well as 1%, 5% and 10% (40 d) concentrations of BioC1 significantly reduced ($P < 0.05$) the extent of ^{14}C-phenanthrene mineralisation compared to BioC2 in soil.

Despite biochar amendment conditions, the time taken for the extent of ^{14}C-phenanthrene mineralization to exceed 5% threshold (lag phase) was observed not to exceed 1.4 d for the first time point. Following increase in soil-phenanthrene-biochar aging period (40 d), increasing biochar concentration resulted to increasing lag phase in both particle size-amended soil (Table 3).

Table 3 Lag phases (d) and maximum rates of ^{14}C-phenanthrene mineralisation (% d^{-1}) in biochar amended soils.

Aging (d)	BioC	Lag Phase (d)		Maximum rate (% d^{-1})	
1	0	1.1 ± 0.0	1.1 ± 0.0	41.2 ± 1.6	41.2 ± 1.6
	1	1.2 ± 0.0	1.1 ± 0.0	25.2 ± 1.7	27.4 ± 1.6
	5	1.3 ± 0.2	1.2 ± 0.0	16.5 ± 1.2	25.5 ± 1.0
	10	1.3 ± 0.0	1.2 ± 0.0	10.0 ± 0.7	22.8 ± 2.6
40	0	1.1 ± 0.0	1.1 ± 0.0	33.5 ± 2.2	33.5 ± 2.2
	1	2.5 ± 0.3	1.3 ± 0.1	4.1 ± 0.5	20.0 ±2.4
	5	3.6 ± 0.1	2.0 ± 0.1	2.7 ± 0.1	5.1 ± 0.1
	10	4.2 ± 0.0	2.1 ± 0.1	2.2 ± 0.0	4.8 ± 0.2

The maximum rate of ^{14}C-phenanthrene mineralisation was assessed per day and results showed that after 1 d aging, increasing concentration of biochar resulted in consistent statistical reduction ($P < 0.05$) in maximum rate of mineralisation. This was much more obvious in BioC1 amended soil. Interestingly, all concentrations of BioC2 indifferently reduced maximum rate of ^{14}C-phenanthrene mineralisation, where there was insignificant difference ($P > 0.05$) amongst BioC2 amended soils (1%, 5% and 10%). However, the 5% and 10% BioC1 amendments reduced maximum rate of ^{14}C-phenanthrene mineralisation to a greater extent when compared to 5% and 10% BioC2 amendments, respectively. Following increase in soil-phenanthrene-biochar aging period (40 d), there was also significant decrease ($P < 0.05$) in maximum rate of mineralisation with increasing concentrations of biochar (Table 3). Increasing the aging period to 40 d showed that biochar amendment reduced rate of mineralisation to a greater extent ($P < 0.01$) compared to 1 d aging.

HPCD-extractable ^{14}C-phenanthrene in soil

The extraction values generated were analysed statistically and identified that addition of biochar led to significant reduction in the amount of ^{14}C-phenanthrene extraction ($P < 0.01$). After the first extraction, increasing biochar amendments resulted in decreasing extent of HPCD extractability, 5% and 10% concentrations of both particle size biochars led to significant reduction ($P < 0.05$) in extractability of ^{14}C-phenanthrene. For example, 5% of BioC1 and BioC2 amendments resulted to $\geq$28% reduction in amounts extracted by HPCD (Table 2). Similarly, >50% reduction in ^{14}C-phenanthrene extractions were observed from 73.5% to 35.7% and 73.5% to 24.4% in 10% BioC1 and BioC2 amended soils, respectively (Table 2). Noticeably, the reduction was more pronounced when soil-phenanthrene-biochar aging period increased (40 d), where all biochar concentrations showed significant reductions ($P < 0.01$) in the amount of ^{14}C-phenanthrene extracted, compared to control (Table 2). Differences between both biochars was only noticed after 40 d aging, HPCD extraction of ^{14}C-phenanthrene in 1% and 5% BioC1 amended soil was lower than BioC2 amended soil.

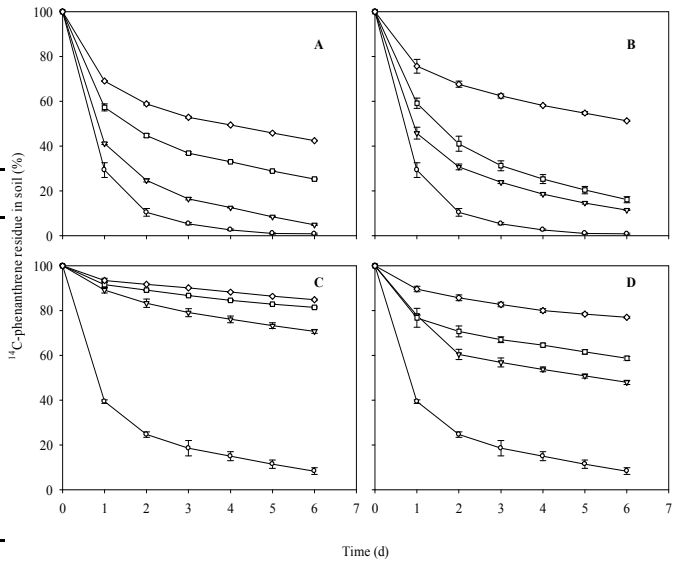

Figure 2 Amount of ^{14}C-phenanthrene sequentially extracted by HPCD from soil amended with 0% (○), 1% (▽), 5% (□) and 10% (◊) of biochar. Error bars represent standard error of extraction ($n = 3$). A and C = $\leq$ 2 mm (BioC1) biochar amendment in soil at 1 d and 40 d, respectively. B and D = 3-7 mm (BioC2) biochar amendment in soil at 1 d and 40 d, respectively.

Desorption behaviour was observed through six sequential extractions, which were seen to be triphasic in nature, where there was an initial rapid drop in amount of phenanthrene sorbed and a subsequent slow and very slow decrease. In the 0% and 1% biochar amendments, there was statistical difference amongst the first, second and third extractions ($P < 0.001$), whilst the third extraction was insignificant ($P > 0.05$) to the subsequent extractions. Noticeably, increase in biochar concentration aging period consistently led to remarkable reduction ($P < 0.01$) in extent and trend of desorption as shown in desorption curves (Figure 2). For example, the addition of 5% and 10% biochar amendments to the soil resulted in minimum of 40% decrease in total amounts of ^{14}C-phenanthrene desorbed. Although the desorption trend of ^{14}C-phenanthrene between 2 and 6 d were similar for all biochars amendments, subsequent aging (40 d) resulted in more significant decrease ($P < 0.01$) in total amount of ^{14}C-phenanthrene extracted due to aging. No greater than 30% of ^{14}C-phenanthrene-associated activity was recovered from biochar amended soils, whilst increasing concentrations of biochar caused significant ($P < 0.01$) reduction in desorption (Figure 2).

During desorption, there was significant difference in total amount of ^{14}C-phenanthrene sequentially extracted ($P < 0.05$) between the BioC1 and BioC2 in soil. In this regard, HPCD extracted less ^{14}C-

phenanthrene from 5 and 10% BioC1 amended-soils compared to 5 and 10% BioC2 amended-soils, respectively after 1 d aging. Similarly, HPCD extracted less ^{14}C-phenanthrene from 1 and 5% BioC1 amended-soils compared to 1 and 5% BioC2 amended-soils after 40 d aging.

Correlation between mineralisation and HPCD extractability

The correlations between the extent and rate of mineralisation to HPCD extractability of ^{14}C-phenanthrene were analysed using linear regression (Figure 3 and 4). This was done to test the ability of HPCD extraction to predict the extent of microbial degradation or rate of microbial degradation of ^{14}C-phenanthrene in biochar amended soils. Results showed that there was a very good relationship between the extent of ^{14}C-phenanthrene mineralised to amount extracted in BioC1 amended soils (r^2 = 0.97; slope = 1.11; intercept = 1.72) (Figure 3). Additionally, the BioC2 amended soils also showed a good agreement, between the extent of ^{14}C-phenanthrene mineralisation and the extent of HPCD extraction (r^2 = 0.72, slope = 0.93, intercept = 17.42) (Figure 3). There was insignificant difference (P > 0.05) between the extent of mineralisation and the amount extracted (24 h), except in 10% (BioC2) and 1% (BioC2) amended soils at 1 and 40 d time points, respectively (Table 2). However, results showed that HPCD extraction was significantly greater than the rate of ^{14}C-phenanthrene mineralisation. The linear relationship between the rate of ^{14}C-phenanthrene mineralisation and HPCD extraction in BioC1 and BioC2 amended soils (r^2 = 0.94; slope = -4.25; intercept = 0.52), (r^2 = 0.77; slope = 0.46; intercept = 3.91) (Figures 4), respectively, showed that linear correlation was not as good as in the extents of mineralisation. This indicates that HPCD extraction is a better predictor for extent of phenanthrene mineralisation.

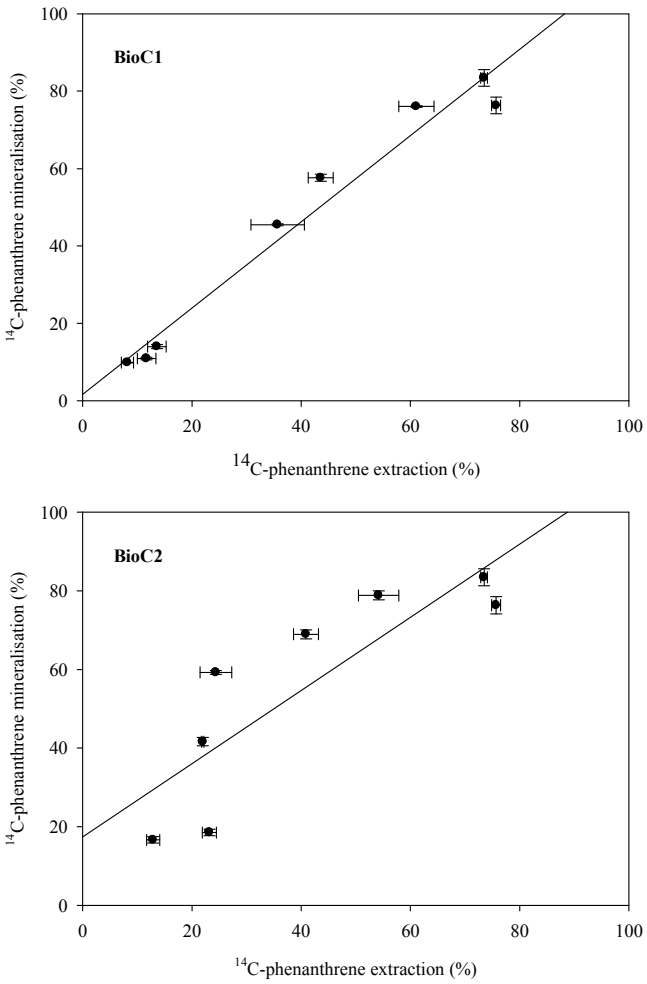

Figure 3 Correlation between total the extent of ^{14}C-phenanthrene mineralised by enriched phenanthrene-degraders and ^{14}C-phenanthrene extracted with HPCD after first 24 h in BioC1 and BioC2 amended-soils, respectively, after 1 and 40 d aging.

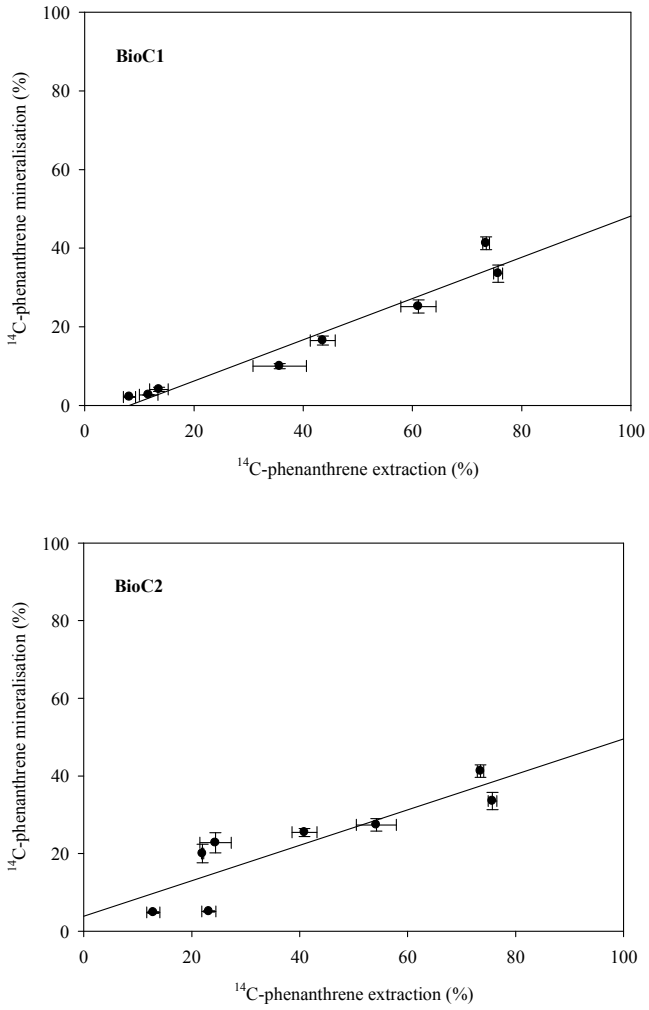

Figure 4 Correlation between the rate of ^{14}C-phenanthrene mineralised by enriched phenanthrene-degraders and ^{14}C-phenanthrene extracted with HPCD after first 24 h in BioC1 and BioC2 amended soil, respectively, after 1 and 40 d aging.

Discussions

Determination of ^{14}C-phenanthrene mineralisation in soil

Organic contaminants are most readily biodegradable when dissolved in the aqueous phase[45] or rapidly desorbable fraction from soil.[33] In this study, biochar reduced the amount of ^{14}C-phenanthrene that could be desorbed into the aqueous phase or made bioavailable, which is supported by Rhodes *et al*.[37] Several studies and reports have shown that biochar can reduce the degradation of PAHs, pesticides and PCBs through sorption to soil.[29,45-49] A portion of phenanthrene is sorbed to the exterior surface of biochar which can be bioaccessible for biodegradation.[37] Another fraction of the phenanthrene molecule maybe trapped within nanopores that is inaccessible to microbial degradation.[50-51] Indeed the aromatic structure formation and molecular disorder for nanopore formation is temperature dependent.[52-53] Although the mechanism of sorption was not investigated, the reduction in the extent of mineralisation was due to the sorption (partitioning and adsorption) of phenanthrene onto the biochar surface and inner pores.[20,23,53,56] Nam and Alexander[57] showed that neither chemical hydrophobicity nor adsorbate surface area alone renders phenanthrene less bioaccessible to bacteria, but rather size and distribution of nanopores of less than 6 nm within adsorbents materials.

In this current study, the addition of biochar to such sterile soils reduced the bioaccessibilty of ^{14}C-phenanthrene through particle size and nanopore sorption properties. Mass transfer kinetics of the contaminant is often slower in larger particle size biochar and it takes longer time for larger particle sized biochar to attain equilibrium.[27] Also, chemical sorption is governed by the micropore (2 nm diameter) region of biochar, more time will be required for organic contaminants to reach micropore regions of larger particle size biochars.[27,49] Hale *et al*.[49] showed that difference in particle size did not affect pyrene sorption onto biochar particles. The inconsistent effect of particle size in this study does not leave out the possibility of biochar hot spots within the soil-biochar matrix due to lower mass volume of larger particle sizes.

HPCD-extractable ^{14}C-phenanthrene in soil

The HPCD extraction is a biomimetic technique, which crudely mimics the mass transfer mechanism that governs microbial interaction with hydrophobic organic compounds.[5,34] Although HPCD extractability is particularly influenced by the SOM of soils,[8] addition of activated carbon (AC) has been shown to further reduce extractability.[37] The amendment of soil with increasing concentrations of biochar reduced the amounts of ^{14}C-phenanthrene extracted using HPCD at each time point, which is supported by Rhodes *et al*.[37,39]

The first single day extraction supports previous studies in that it represents the rapidly desorbing fraction of phenanthrene which is labile,[8,33,39] while the subsequent 5 x 24 h extractions represent the non-labile fraction that fractions may be desorbed subsequently. Two and three-compartment models have described phenanthrene desorption kinetics using HPCD solution and described the trend to consist of rapidly desorbing (bioaccessible), slowly desorbing and very slowly desorbing fractions.[3,33,39,59-62] However, this was not assessed in this study as sequential extractions were done on a daily basis rather than in hours. The decline in desorption of ^{14}C-phenanthrene was attributed to entrapment and sorption within biochar nanopores that can accommodate phenanthrene. In addition, the presence of liquid hydrocarbons within biochar pores sites (Table 1) can enhance the sorption of phenanthrene in soil.[63] The movement of chemicals from accessible soil-biochar compartments (macropores) into less accessible (mesopores) and inaccessible compartments (micropores), results in reduction in bioaccessibility.[54,64-65] This implies that the entrapped phenanthrene within higher concentrations of biochar will not be bioaccessible over a long period of time due to occlusion within biochar structure,[12] thereby increasing the non-extractable or non-bioaccessible fractions. Although biochar was shown to limit extractability of phenanthrene after 1 d aging, HPCD still has the capacity to desorb significant pool of the slowly and very slowly desorbing fractions. This implies that aging is also a vital factor for sorption and desorption of contaminants within biochar. Rhodes *et al*.[8] demonstrated that higher OM and clay in soil led to a 40%

reduction in phenanthrene extraction following 100 d soil-PAH aging period. Similarly, Swindell and Reid[66] showed large decreases in the labile fraction of PAHs in low organic matter soil following 40 d residence time. This current study demonstrated that the addition of increasing concentrations of biochar to low OM soils led to decreasing concentrations of the labile and rapidly desorbing fractions which could have probably been accomplished over longer aging periods in high OM and TOC soils, as per Rhodes *et al*.[37-39] Also, increasing aging time led to decreasing concentration of the slowly and very slowly desorbing fractions of ^{14}C-phenanthrene. When such sequestered residues become inaccessible within biochar, it can mitigate the exposure of the contaminants to biota.

Correlation between mineralisation and HPCD extractability

In previous studies, Rhodes *et al*.[8,33] showed good correlation between amounts of ^{14}C-phenanthrene mineralised to amounts of ^{14}C-phenanthrene extracted by HPCD in soils. Reid *et al*.[5] Stokes *et al*.[34] and Allan *et al*.[67] showed that HPCD extractability of organic contaminants in soils represented the labile and potentially labile fractions of the contaminant that can undergo biodegradation to CO_2. However, amendment with black carbon materials can alter this prediction. For instance, Rhodes *et al*.[37] showed good linear correlations between amounts of ^{14}C-phenanthrene mineralised to amount extracted in control soils (0%) (r^2 = 0.864; slope = 0.89; intercept = 5.74) and 0.1% (r^2 = 0.67; slope = 0.95; intercept = 20.1) AC amended soils. But the authors reported a significant impact on this relationship when amended with 0.5%, 1%, 2.5%, and 5% AC. The authors suggested that HPCD extractability was reduced to a greater extent in presence of AC and microbes had the capability to attach to black carbon surfaces to mineralise ^{14}C-phenanthrene. In contrast to this current study, biochar reduced the HPCD extractability in the same manner as it reduced mineralisation, which implies that HPCD extraction is a good predictor for phenanthrene bioaccessibility in biochar-amended soil.[30,68] Although the biochar used in this study contains similar total pore volume as that of Rhodes *et al*.[37] the biochars are however >80% macroporous (>50 nm), rendering higher ^{14}C-phenanthrene molecules accessible for HPCD displacement and microbial degradation than in AC amended soils. On the other hand, AC has been shown to be dominantly microporous (≤2 nm diameter) in nature with pore sizes similar in size to HOCs,[69-71] whilst HPCD molecule has a similar dimensional size with micropore structure.[69-70,72] Prior extraction, ^{14}C-phenanthrene molecules would have occupied majority of the micropore sites of the AC, rendering limited or no access for displacement by HPCD extraction. In contrast, since the biochars are dominantly macroporous with less internal surface area as compared to AC, ^{14}C-phenanthrene molecules most likely would have accumulated on edges of the macropore sites.[51] Phenanthrene degraders (*Pseudomonas* sp.) have the potential to desorb sorbed contaminants through secretion of rhamnolipid biosurfactants, that most likely would have reduced surface tension, facilitated transport and enhanced solubility of the phenanthrene.[73-74] Although not investigated, these biopolymers such as biosurfactants are produced by phenanthrene degraders in liquid cultures.[75] Another mechanism is the competitive inhibitive effect of biosurfactants on phenanthrene adsorption in black loamy soils.[76] and rhamnolipid biosurfactants desorb PAHs more effectively than HPCD.[77] However, nanopore distribution alone may not control contaminant adsorption, but surface chemistry will also have effects on desorption of phenanthrene from such biochar.[70] Although a mass balance on ^{14}C-phenanthrene mineralised and adsorbed was not done, it is expected that would be composed of biogenic, SOM and biochar bound ^{14}C-residues,[78,79,80] with biochar having the larger fraction of bound residues.

Conclusions

The biochar used in this study was not subject to any form of activation by potassium hydroxide (KOH), steam or CO_2 and it was shown to be a viable tool in adsorbing phenanthrene in soil, thus reducing phenanthrene mobility and bioaccessibility. This was shown by longer lag phase, lower rate and extent of ^{14}C-phenanthrene mineralisation by phenanthrene degraders in increasing concentration of biochar in soil. Similarly, HPCD extractability decreased remarkably with increasing concentration of biochar. HPCD extraction was also shown to be good predictor of extent of phenanthrene mineralisation (bioaccessibility) in biochar-amended soil. However, the duration of soil-phenanthrene-biochar interaction posed as a determining factor for bioaccessibility, as well as slowly and very slowly desorbing fractions of ^{14}C-phenanthrene. Hence, with constructive investigation and planning, biochar can be utilised as a cheap tool to limit exposure of such contaminants to biota and other remediation purposes, depending on the concentration, particle size and aging period. However, further research is required to investigate the stability in locking up contaminants.

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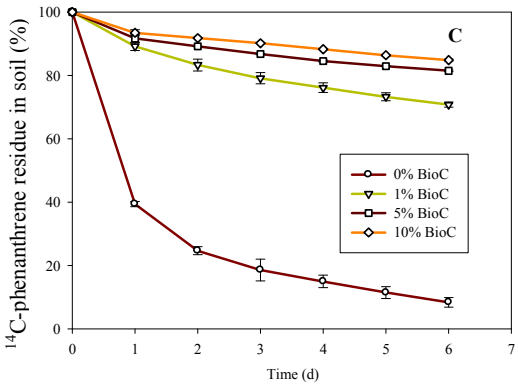

Biochar as a potential remedial tool to mitigate risk of phenanthrene exposure to biota

Environmental Impact Statement

Polycyclic aromatic hydrocarbons (PAHs) are constituents of petroleum hydrocarbons that have become ubiquitous in the environment due to the persistent exploration and exploitation of crude oil and its derivatives. Some of the PAHs have been shown to be highly toxic to biota and have disturbing properties such as; persistence, leaching and bioaccumulation. There are numerous brownfield sites in the UK contaminated with PAHs, as well as the persistent spillage, leakage and persistent exploitation in the Niger Delta region of Nigeria are currently endangering the lives of individuals in pre-exposed communities. Significant concentrations of PAHs have already been found in vegetation, aquatic organisms and in soils. Several approaches have been adopted to remediate such sites such as; chemical washing, use of surfactants, thermal remediation and composting but most of these methods have shown to be expensive, low aesthetic acceptance and destructive to ecosystems. Bioremediation which involves the use of organisms to neutralise contaminants in the environment to less toxic substances, has also been shown to have promising potential as it is cost effective, requires less manual supervision, naturally acceptable and requires less energy input to conduct. However, it does not remove all the contaminants, takes a long to time fully remediate and requires major environmental conditions to be met. Also, in order to know if bioremediation is applicable, rigorous tests are often required which makes the selection of the method challenging. We propose and validate the use of respirometry and HPCD extraction techniques to tests the applicability of bioremediation. Also, the amendment of contaminated soils with cheap super adsorbent to sorb the non-biodegradable fractions of the contaminants in a shorter time frame and mitigate the risk of exposure of PAHs to biota is proposed. However, the type and appropriate properties of the adsorbent need to be tested as well which is done in this study.