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Article

Impact of Different Types of Activated Carbon on the Bioaccessibility of ^{14}C-phenanthrene in Sterile and Non-Sterile Soils

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Abstract: In this study, the impact of three different types of activated carbon (AC) on the bioaccessibility of ^{14}C-phenanthrene in non-sterile and sterile soils was investigated. A single dose (1%) of each of the different AC (CB4, CP1 and AQ5000) was blended with soil spiked with 50 mg·kg^{-1} of ^{12}C/^{14}C-phenanthrene. The mineralisation of the ^{14}C-phenanthrene was monitored over a 14 day incubation period by indigenous soil microflora and an enriched inoculum of *Pseudomonas* sp., while uptake in earthworms, *Eisenia fetida,* was measured after incubation for 10 days at 1, 25, 50 and 100 d. Bioaccessibility was assessed using hydroxypropyl-β-cyclodextrin (HPCD) solution. Results showed that the presence of AC had a significant effect upon the extents of mineralisation, earthworm uptake and HPCD extraction, when compared to the control. Aquasorb CB4 was the least effective amongst the different AC used. The characteristics of the different AC used was also seen to have a major influence on how each AC would have an effect on its use in soil remediation in reducing bioaccessibility, mobility and risk.

Keywords: activated carbon; earthworm; HPCD extraction; phenanthrene; soil

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrophobic organic contaminants (HOCs) widely found in the environment from many natural and anthropogenic sources [1,2]. Due to their carcinogenic and mutagenic characteristics, as well as their persistence, there is significant interest in their fate and behaviour in soils and sediments [3]. As a result of their recalcitrance, strategies for reducing their mobility and bioaccessibility may be appropriate for reducing the risks of these contaminants in soil.

Black carbon (BC) is a collective term for the natural forms of carbonaceous sorbents, such as soot and charcoal [4]. They are widely present in soil and may account for about 1%–15% of total organic carbon [5,6]. BC is believed to enhance sorption of PAHs in soils and sediments [7,8]; leading to reductions in desorption [9], thus causing reductions in extractability and bioaccessibility of PAHs to biota, including microorganisms [10].

Activated carbon (AC) is a modified form of BC produced under particular conditions; AC is an effective sorbent removing organic pollutants from soils due to its high adsorption capacity, high surface area and surface activation, which is why it is used in water purification, contaminated soils and sediments remediation [11,12]. AC has a strong tendency to adsorb PAHs and, as a result, has the potential to reduce bioaccessibility of organic contaminants in soil [5,10,13]. Previous studies have demonstrated that PAHs pose a reduced environmental risk in the presence of BC as a result of its strong sorption, subsequently leading to inaccurate estimations or prediction of ecological target values and remediation end points [10]. Several studies demonstrated that addition of 3%–4% of AC resulted in a reduction of pore water concentration and a reduced bioavailability of HOCs [14,15]. The addition of AC to soil is known to reduce freely dissolved HOC concentrations, thereby reducing ecotoxicological risk [15,16].

Hydroxypropyl-β-cyclodextrin (HPCD) consists of α-1,4-linked glucose molecules, and is classified based on the number of glucose molecules in the ring. HPCD has a hydrophilic exterior (due to the hydroxyl groups of the glucose molecule) but also contains a hydrophobic organic cavity within their molecular structure, which allows the formation of a water soluble inclusion complex between the HPCD molecule and an organic compound [17,18]. As a result, the HPCD extraction technique relies on the mass transfer from the solid phase to the aqueous phase, thereby mimicking the processes governing microbial bioavailability. It has been demonstrated that the ability of HPCD to predict bioavailability is dependent on the properties of the soil and the nature of the contaminants [19]. Thus, it is important to take into account both the composition of the soil and the amount of time that the soil has been contaminated when selecting an extraction method for predicting bioavailability [19]. The HPCD extraction used in this study has been described elsewhere [17].

Earthworms are considered an important part of the soil biota, and their presence is regarded as a useful indicator of soil health. This is due to their sensitivity to relatively low concentrations of environmental pollutants, which makes them one of the most suitable bio-indicator organisms for risk assessment in the soil, therefore they have been used to give a measure of organic contaminant bioavailability [20,21]. Thus, in terrestrial ecosystems, earthworms serve as an important link in the transport of PAHs and other HOCs from the soil to organisms higher up in the terrestrial food web. However, AC amendments have been found to have an adverse effect on the habitat quality of the soil

to the earthworm, as found in previous studies [22]. On the other hand, no adverse effect was observed in oligochaetes when AC was added to sediments in several studies [23,24]

The aims of this study were to study the capacity of different types of activated carbon on bioaccessibility so as to: (i) compare the mineralisation of ^{14}C-phenanthrene in soil, by a phenanthrene degrading bacterium, *Pseudomonas* sp. and by indigenous soil microflora in the presence and absence of AC; and (ii) assess the bioaccessibility of ^{14}C-phenanthrene uptake in earthworms, *Eisenia fetida*.

2. Materials and Methods

2.1. Materials

Non-labelled phenanthrene (>96%) was obtained from Sigma Aldrich (Poole, UK) and 9-^{14}C-phenanthrene (radio-chemical purity > 96%, specific activity 55 mCi·mmol^{-1}) was obtained from American Radiolabeled Chemical Inc. (ARC, St. Louis, MO, USA). Goldstar multipurpose liquid scintillation fluid, Carbontrap and Carbon Count were obtained from Meridian, UK, and Combustaid from Canberra Packard, UK. Hydroxypropyl-β-cyclodextrin (HPCD) (97% purity) was obtained from Arcos Organics, UK. Acetone used to clean equipment before and during experimental procedures was obtained from Fisher Scientific, UK. Sodium hydroxide (NaOH) and chemicals for minimal basal salts (MBS) were purchased from Fisher-Scientific, UK. Activated carbon (produced from coconut shell); Aquasorb CP1 PAC-F (hereinafter referred to as CP1), Aquasorb CB4 PAC-S (hereinafter referred to as CB4) and Aquasorb 5000 PAC-S (hereinafter referred to as AQ5000) were purchased from Jacobi carbons, Sri Lanka. The properties are summarised in Table 1. Earthworms, *Eisenia fetida*, were purchased from Blades Biologicals Ltd. (Kent, UK).

Table 1. Characteristics of the different activated carbon (AC) types.

Specification	CB4	CP1	AQ5000
Surface Area ($m^2 \cdot g^{-1}$)	653	1106	1249
Moisture content (%)	3.1	4.8	4.7
Ash content (%)	9.8	2.8	12.9
3–25 mesh	74.8 (65–85)	95 (90–100)	84.6 (65–85)
Iodine number	603	1056	1199
Pore volume/unit dry mass ($mL \cdot g^{-1}$)	0.29	2.5	0.80
Liquid quantity/unit dry mass ($\mu L \cdot g^{-1}$)	151	422	253

2.2. Soil and Soil Spiking

A pristine agricultural (Dystric Cambisol) soil was collected (from the A horizon; depth of 5–20 cm) from Myerscough college, Lancashire, UK. Soil physico-chemical properties are as follows: pH 6.5, organic matter 2.7%, sand 60.4%, silt 20%, and clay 19.5%. The air-dried soil was sieved with a 2 mm sieve to remove roots and stones, and then stored at 4 °C until ready for use. When ready for use, soil was rehydrated with deionised water back to original water holding capacity (WHC). Aliquots of soil were then amended with 1% AC: CB4, AQ5000 or CP1, respectively. The soils were then spiked with ^{12}C/^{14}C-phenanthrene standards prepared in toluene to achieve a concentration of 50 mg·kg^{-1} (42 Bq·g^{-1} soil). Soil-AC aliquots were blended following the method of Doick, Lee [25]. Soil-AC

aliquots were then sealed in amber glass jars (in triplicate per treatment), left to age in the dark at 20 ± 2 °C and analysed at 1, 25, 50 and 100 d. Blank soils with neither phenanthrene nor AC were also prepared. A corresponding experiment with sterilised soils was also conducted, where soils were sterilised by γ-irradiation (32.2 kGy; Isotron, Bradford, UK) within 1 d after amendment with ^{14}C-phenanthrene. Soil-AC aliquots were then sealed in amber glass jars (in triplicate per treatment) and left to age in the dark at 20 ± 2 °C and analysed at 1, 25, 50 and 100 d. At each time-point, soils were tested to confirm sterility following standard microbiological plating methods. Experiments were performed using both non-sterile and sterile soil microcosms, in order to evaluate the fate of phenanthrene in the presence and absence of microbial degradation.

2.3. Determination of Total ^{14}C-phenanthrene Activity in Soil

At each time point, aliquots of soil treatments (1 g, n = 3) were analysed for total ^{14}C-phenanthrene-associated activity by combustion for 3 min using a sample oxidiser (Packard 307). Carbon trap was used to trap $^{14}CO_2$, and Carbon count was used as a scintillation cocktail, the trapping efficiency was found to be >90%. The resultant solutions were counted by liquid scintillation counting (Tri-Carb 2250TR liquid scintillation counter, Canberra Packard, London, UK) using standard calibration and quench techniques.

2.4. Preparing Phenanthrene-Degrading Inoculum

A phenanthrene-degrading bacterial inoculum identified as *Pseudomonas* sp. was cultured on phenanthrene at 0.1 g·L^{-1} in 600 mL of minimal basal salts (MBS) solution at 20 °C at 100 rpm on an orbital shaker (IKA Labortechnik KS501 digital) following the method of [26]. After incubation for 4 d (late exponential phase of growth), the culture was centrifuged at 4000× g for 60 min (Beckman J-6M, Beckman Coulter, Atlanta, GA, USA). The resultant supernatant was discarded to remove residual phenanthrene; the cells were then re-suspended in MBS. This procedure was repeated to ensure thorough washing of the cells. The settled bacterial cells were then re-suspended in fresh MBS to give 10^7 cells·mL^{-1}.

2.5. Extraction of ^{14}C-phenanthrene Associated Activity by HPCD

At each time point, the HPCD extraction of ^{14}C-phenanthrene from soil was determined in both non-sterile and sterile soils, following the method of [17]. Soil samples (1.25 g; n = 3) were weighed into 30 mL Teflon centrifuge tubes and 25 mL of 50 mM HPCD solution was added into each of the tubes. The sealed centrifuge tubes were placed horizontally onto an orbital shaker (IKA Labortechnik KS501 digital) rotating at 100 rpm for 22 h at 21 °C. The tubes were then centrifuged at 3600× g for 60 min (Beckman J-6M, Beckman Coulter, USA). After centrifugation, 6 mL of supernatant was added into 20 mL glass scintillation vials containing 14 mL scintillation cocktail, and then quantified by scintillation analyser (Tri-Carb 2250TR liquid scintillation counter, Canberra Packard, London, UK) using standard calibration and quench correction techniques. The rest of the supernatant was discarded and the soil remaining in the centrifuge tubes was allowed to dry,

dried pellet was carefully placed in combustion cones and combusted by sample oxidation in a sample oxidizer (Packard 307).

2.6. Mineralisation of ^{14}C-phenanthrene in Soil

Each soil was spiked with ^{14}C-phenanthrene (50 mg·kg^{-1}) following the method of Doick, Lee [25], and respiration rate of $^{14}CO_2$ was monitored daily over 14 days. Soil samples were incubated using either soil indigenous microflora or an enriched inoculum to assess the availability of ^{14}C-phenanthrene in soil after 1, 25, 50 and 100 d contact time. Evolution of $^{14}CO_2$ was measured by using the method of [26]. Each respirometer incorporated a Teflon-lined screw cap and a CO_2 trap containing 1 M NaOH (1 mL) within a suspended 7 mL glass scintillation vial. Respirometers were prepared in triplicate, with 10 ± 0.2 g soil (dry weight) and 30 mL sterile MBS according to the method of Doick and Semple [27]. The respirometric flasks were placed securely on an orbital shaker (IKA Labortechnik KS501 digital) rotating at 100 rpm to ensure adequate mixing of the slurry, and incubated at 20 °C throughout the duration of the experiment. The ^{14}C-activity in the $^{14}CO_2$ traps was assessed daily by replacing the NaOH traps and adding LSC (5 mL) to each spent $^{14}CO_2$ trap. After storage in darkness overnight, trapped ^{14}C-activity was quantified using a (Tri-Carb 2250TR liquid scintillation counter, Canberra Packard, London, UK), using standard protocols for counting and automatic quench correction. An analytical blank (containing no ^{14}C-phenanthrene) determined the level of background activity. The length of the lag phase (defined as the time taken for mineralisation to reach 5%), maximum rates and overall extents of ^{14}C-phenanthrene mineralisation were determined [10].

2.7. Uptake of ^{14}C-phenanthrene by Eisenia Fetida

The earthworms were maintained on a soil with high organic matter (OM) content (5%) at 11–13 °C, and were fed on a diet of compost once a week. Fully clitellated adult earthworms weighing between 0.3 g and 0.56 g live weights were selected for the experiment. Prior to weighing, earthworms were carefully rinsed with deionised water and placed on a moist filter paper for 24 h for earthworm depuration. At each time-point, one mature earthworm was added to the surface of the soil, the earthworms were not fed during the exposure period and all measurements were done using five replicates [28]. All jars were covered with aluminium foil perforated for ventilation and maintained for 10 d at 20 °C. At the end of incubation, the earthworms were removed from the soil, depurated on moistened filter paper in Petri dishes for 24 h to allow purging of gut contents, rinsed with deionised water, dried with filter paper and weighed [28]. Earthworm survival was noted and the survived earthworms were snap-frozen in liquid nitrogen until ready for use. Earthworms were combusted in a sample oxidiser (Packard 307) to know the amount of ^{14}C-phenanthrene associated activity ingested.

2.8. Statistical Analysis

To investigate the impact of the different types of AC amendment in the soils, the total extents of ^{14}C-phenanthrene mineralisation, HPCD extraction of ^{14}C-phenanthrene and uptake by *E. fetida*, were compared at each time point. Following blank correction, statistical analysis of the results was performed using ANOVA (Tukey's test) after normality testing by Sigma Stat for Windows (Version

3.5, SPSS Inc., London, UK). All graphs were presented using SigmaPlot for Windows (Version 10.0, SPSS Inc., London, UK).

3. Results

3.1. Total ^{14}C-phenanthrene Associated Activity in Soil

At each time point, aliquots of AC-amended non-sterile and sterile soils were analysed for total ^{14}C-activity through combustion. No statistically significant difference ($p > 0.05$) was observed in the associated ^{14}C-activity for the sterile soil samples over the 100 d ageing period. In contrast, there was a reduction in associated ^{14}C-activity for the non-sterile soils; however, these reductions were not statistically significant ($p > 0.05$) over 100 d.

3.2. Mineralisation of ^{14}C-phenanthrene in Soil

The impact of CB4, AQ5000 and CP1 on the mineralisation of ^{14}C-phenanthrene was measured in non-sterile (indigenous activity) and sterile soils (inoculum activity), respectively. At 1 d, the shortest lag phase was observed in the control soil (5.16 d), and the longest in CP1-amended soil (12.3 d) in the non-sterile soils. A statistical significant difference ($p < 0.05$) was observed, when CB4-, AQ5000-, and CP1-amended soils were compared to the control in the non-sterile soils. With an increase in contact time, there were significant reductions ($p < 0.05$) in the lag phases; the longest observed at 1 d (5.16 d), and the shortest observed at 100 d (1.02 d), in control soils. However, AC-amended soils, at 1 d, revealed significant differences ($p < 0.05$) in the lag phases, when the CB4-amended soils were compared AQ5000 and CP1-amended soils, respectively. For example, CB4-, AQ5000- and CP1-amended soils, took 5.49 d, 9.61d and 12.3 d, respectively, to reach 5% mineralisation in non-sterile soils; however, at the other time-points, AC-amended soils did not reach 5% mineralisation for the lag phase to be measured. Overall, the highest maximum rates of ^{14}C-phenanthrene mineralisation were observed in the sterile control soils. In addition, the control soils consistently had higher ($p < 0.05$) maximum rates of mineralisation, in comparison to the CB4-, AQ5000- or CP1-amended soils (Table 2) in non-sterile and sterile soils, respectively.

For the control soils, ^{14}C-phenanthrene mineralisation rates differed significantly ($p < 0.05$) in non-sterile soils, in comparison to sterile soils; the rates of mineralisation declined as contact time increased in the non-sterile soils. However, in the AC-amended soils, maximum rates of ^{14}C-phenanthrene mineralisation were observed to decrease significantly ($p < 0.05$), as contact time increased in both non-sterile and sterile soils, and this was observed at subsequent time points (Table 2).

A comparison between CB4, AQ5000 and CP1-amended soils revealed that, maximum rates of ^{14}C-phenanthrene generally reduced after 1 d for all AC-amended soils in non-sterile and sterile soils, respectively. This was significant ($p < 0.05$) in CB4-amended soils, but not in AQ5000- and CP1-amended soils, respectively. The different AC showed a similar pattern in the maximum rates of ^{14}C-phenanthrene mineralisation at all time-points ($p > 0.05$). However, differences were observed at 1 day in non-sterile soils and at 25 d in sterile soils. The maximum rates of ^{14}C-phenanthrene mineralisation generally increased in the order CB4 > AQ5000 > CP1 (Table 2).

The overall extents of ^{14}C-phenanthrene mineralisation ranged from 1.38%–47.2% in non-sterile soils, and 1.97%–52.9% in sterile soils (Figures 1 and 2; Table 3). Generally, the greatest extents of ^{14}C-phenanthrene mineralisation were seen in control soils, and the lowest extents in CP1-amended soils. Controls soils exhibited a significant difference ($p < 0.001$) from the AC-amended soils in the overall extents of ^{14}C-phenanthrene mineralisation, and a similar pattern was observed in non-sterile and sterile soils, respectively (Figures 1 and 2). The effect of increases in contact time on control and AC-amended soils revealed that overall extents of ^{14}C-phenanthrene mineralisation declined at subsequent time points, in both non-sterile and sterile soils. However, results suggested that control soils showed statistical significant differences ($p < 0.001$) at 25 d, 50 d and 100 d in the non-sterile condition but no statistical significant difference ($p > 0.05$) in sterile soils. At 1 d, it was seen that AQ5000- and CP1- amended soils showed a similar pattern ($p > 0.05$) in the extents of ^{14}C-phenanthrene mineralisation to each other, but the non-sterile CB4-amended soils behaved differently ($p < 0.05$). However, CB4-, AQ5000- and CP1-amended soils showed a statistical significant difference ($p < 0.05$) from one another in sterile soils. A comparison of the ^{14}C-phenanthrene mineralised by indigenous microflora and the microbial inoculum after 100 d was made by linear regression. The amount of ^{14}C-phenanthrene mineralised by the indigenous microflora and that mineralised by the microbial inoculum revealed that there was a strong and significant ($p < 0.001$) relationship, as indicated by the regression modelling parameters were significantly correlated; the slope of regression was 1.02, an intercept of 5.04 and $r^2 = 0.91$ (Figure 3).

3.3. HPCD Extraction of ^{14}C-phenanthrene from Soil

The presence of AC had a considerable impact upon HPCD extraction of ^{14}C-phenanthrene. Generally, the addition of AC resulted in significant reductions ($p < 0.05$) in the extraction of ^{14}C-phenanthrene, when compared to the control in non-sterile and sterile soils, respectively (Table 3).

Furthermore, with an increase in contact time, the amount of ^{14}C-phenanthrene extracted from soils declined considerably. However, this was only observed to be significant ($p < 0.05$) after 50 d. A comparison between CB4-, AQ5000- and CP1- amended soils, indicated that AC type significantly ($p < 0.05$) affected the amount of extractable 1^4C-phenanthrene. From the data, it was observed that CB4-amended soils consistently extracted a higher amount of ^{14}C-phenanthrene, in comparison to AQ5000- and CP1-amended soils, respectively. However, only CP1-amended soil showed a statistically significant difference ($p < 0.05$) to the soil amended with CB4 (Table 3).

Table 2. Maximum rates of ^{14}C-phenanthrene mineralisation (% h^{-1}) in control and 1% AC-amended soils for both non-sterile and sterile soils. Values are mean ± standard error (n = 3).

Time (d)	Non Sterile				Sterile			
	Control (% h^{-1})	CB4 (% h^{-1})	AQ5000 (% h^{-1})	CP1 (% h^{-1})	Control (% h^{-1})	CB4 (% h^{-1})	AQ 5000 (% h^{-1})	CP1 (% h^{-1})
1	0.70 ± 0.02	0.14 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.72 ± 0.04	0.12 ± 0.01	0.07 ± 0.01	0.03 ± 0.01
25	0.46 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.81 ± 0.05	0.14 ± 0.02	0.06 ± 0.01	0.03 ± 0.01
50	0.41 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	1.01 ± 0.05	0.04 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
100	0.24 ± 0.06	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	1.39 ± 0.09	0.06 ± 0.01	0.03 ± 0.01	0.01 ± 0.01

Table 3. ^{14}C-Phenanthrene extracted by hydroxypropyl-β-cyclodextrin (HPCD) (after 22 h) and ^{14}C-phenanthrene mineralised in non-sterile and sterile soils. Values are mean ± standard error (n = 3).

Ageing (d)	AC	Non-Sterile		Sterile	
		Mineralised (%)	Extracted (%)	Mineralised (%)	Extracted (%)
1	Control	61.1 ± 2.11	74.1 ± 5.11	63.4 ± 0.66	74.1 ± 3.43
	CB4	18.9 ± 1.34	26.5 ± 3.23	18.4 ± 0.72	20.1 ± 1.25
	AQ5000	8.41 ± 0.63	7.21 ± 0.56	11.7 ± 0.48	10.8 ± 0.18
	CP1	5.93 ± 0.94	5.42 ± 0.87	5.84 ± 0.56	5.34 ± 0.24
25	Control	54.3 ± 0.61	76.1 ± 0.69	60.8 ± 1.86	71.5 ± 2.38
	CB4	4.93 ± 0.94	8.24 ± 0.66	12.8 ± 0.74	9.81 ± 0.33
	AQ5000	1.22 ± 0.33	7.13 ± 1.74	10.4 ± 1.02	5.84 ± 0.46
	CP1	0.71 ± 0.08	3.22 ± 0.49	5.77 ± 1.64	3.22 ± 0.91
50	Control	51.5 ± 2.71	67.5 ± 5.30	57.3 ± 3.91	66.8 ± 3.80
	CB4	2.35 ± 0.24	4.77 ± 1.21	7.07 ± 0.88	7.54 ± 0.81
	AQ5000	1.19 ± 0.38	5.82 ± 0.94	3.84 ± 1.42	3.68 ± 0.84
	CP1	0.54 ± 0.09	0.88 ± 0.33	3.13 ± 0.81	4.32 ± 0.11
100	Control	13.1 ± 1.81	15.4 ± 3.61	41.8 ± 0.22	42.1 ± 2.94
	CB4	1.94 ± 0.09	2.93 ± 0.08	3.99 ± 0.48	3.21 ± 0.12
	AQ5000	1.41 ± 0.11	0.92 ± 0.06	3.41 ± 0.21	2.81 ± 0.28
	CP1	0.43 ± 0.06	0.88 ± 0.09	2.05 ± 0.92	1.24 ± 0.09

Figure 1. ^{14}C-Phenanthrene mineralisation by indigenous microorganisms in non-sterile Myerscough soil after AC addition at contact time: (**A**) 1 d (**B**) 25 d (**C**) 50 d (**D**) 100 d. Error bars are SEM (n = 3). Legend key: 0% (●), 1% CP1 (○), 1% CB4 (▼) and 1% AQ5000 (Δ).

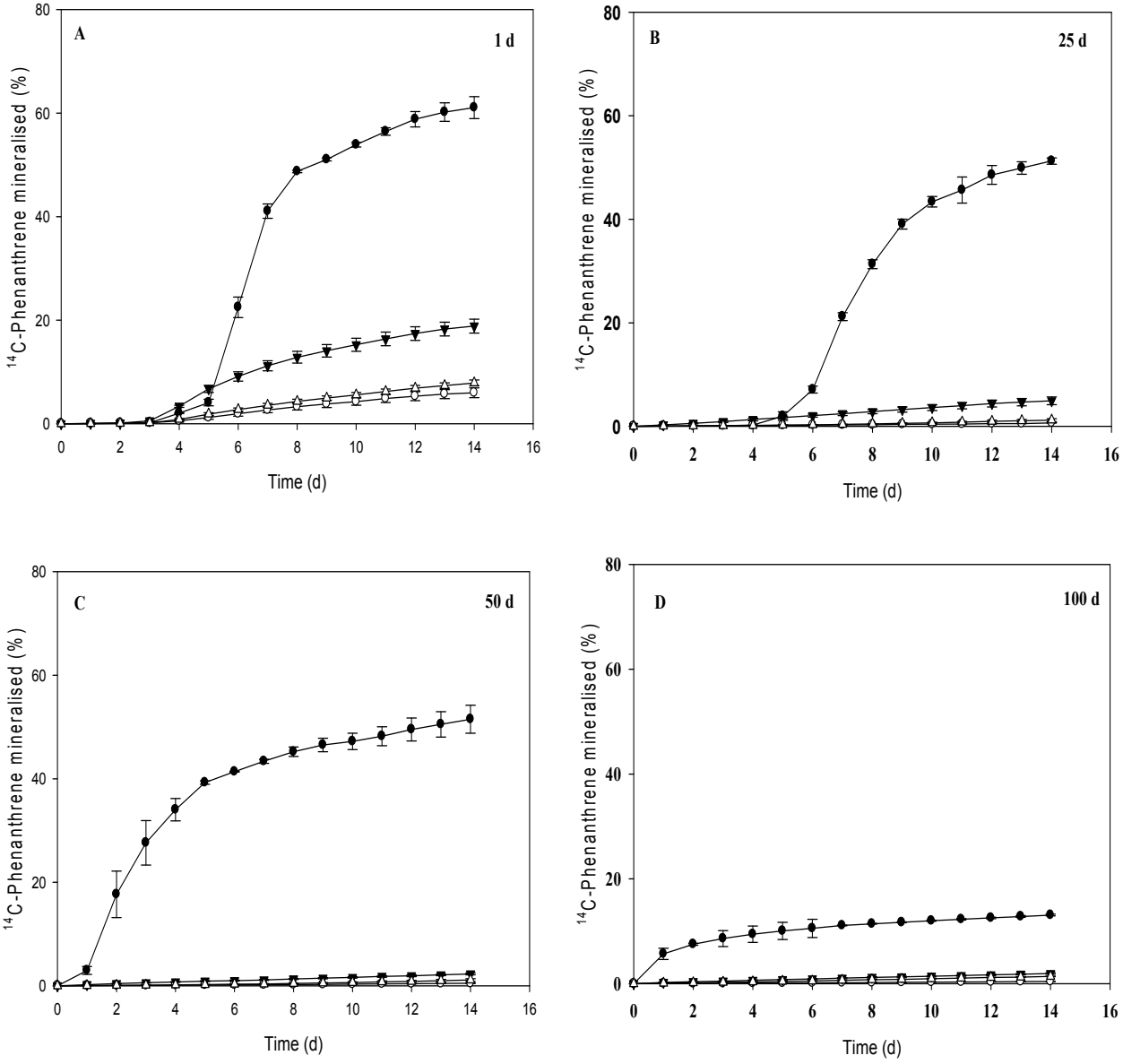


Figure 2. ^{14}C-Phenanthrene mineralisation by enriched inoculum in sterile Myerscough soil after AC addition at contact time: (**A**) 1 d (**B**) 25 d (**C**) 50 d (**D**) 100 d. Error bars are SEM (n = 3). Legend key: 0% (●), 1% CP1 (○), 1% CB4 (▼) and 1% AQ5000 (Δ).

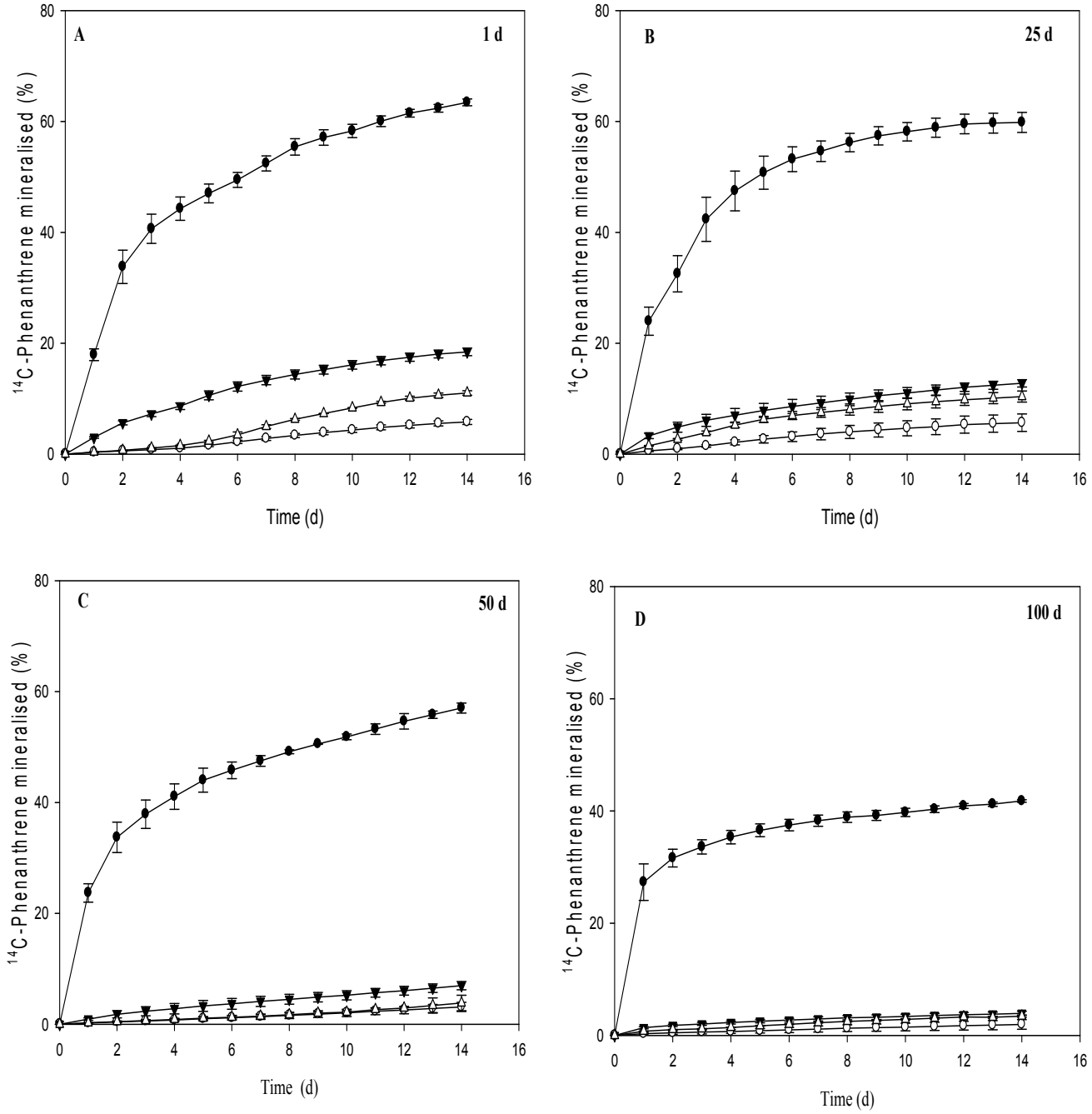
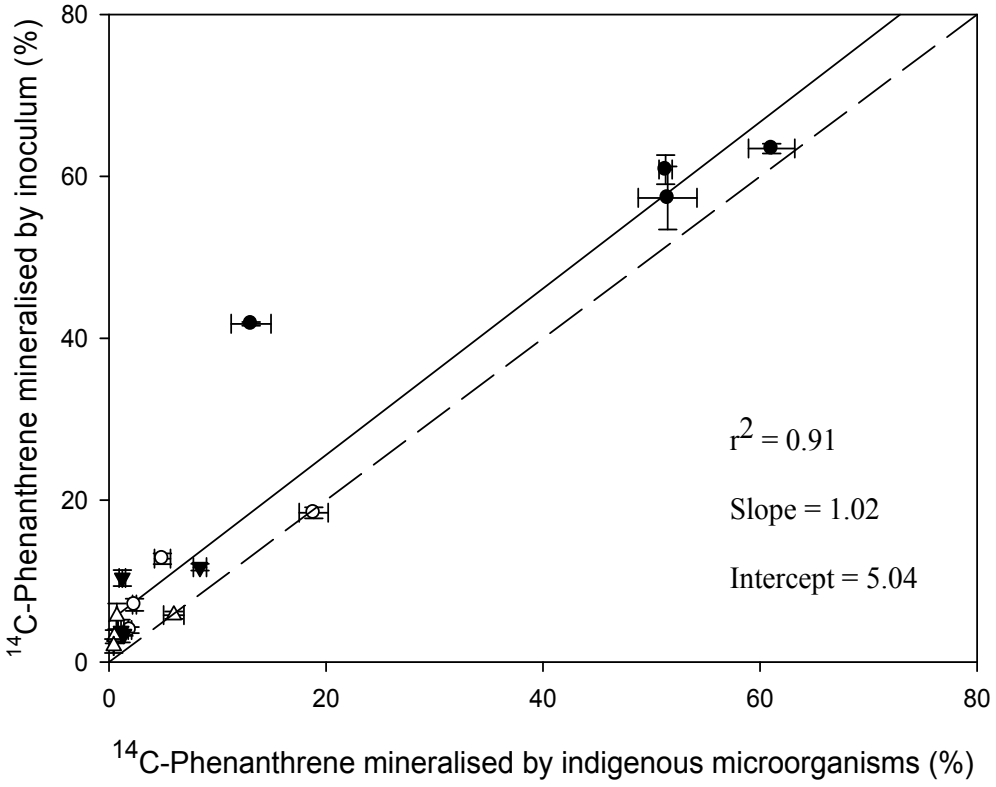


Figure 3. Linear regression showing the correlation between total extents of ^{14}C-phenanthrene mineralised by an enriched inoculum (*Pseudomonas* sp.) and indigenous soil microorganisms. Data points represent both AC-amended and controls soils. Dashed line represents y = x correlation between the two variables. Error bars are SEM (n = 3). Legend key: Control (●), 1% CB4 (○), 1% AQ5000 (▼) and 1% CP1 (Δ).



3.4. Earthworm Uptake of ^{14}C-phenanthrene

The amount of ^{14}C-phenanthrene taken up by the earthworms is presented in Table 4. No mortality was recorded throughout the study, and the earthworms remained active throughout. The average weight of earthworms decreased, but no significant weight differences ($p > 0.05$) were found, in the presence or absence of AC (data not shown). Control soils consistently had the highest uptake of ^{14}C-phenanthrene at all time-points, and the addition of AC to soils significantly reduced ($p < 0.05$) the uptake of ^{14}C-phenanthrene in *E. fetida,* in comparison to the control non-sterile and sterile soils, respectively (Table 4). An increase in contact time led to significant reductions ($p < 0.05$) in the uptake of ^{14}C-phenanthrene in *E. fetida* after 100 d. For example, the addition of 1% AC to soil reduced the uptake of ^{14}C-phenanthrene in the earthworms by up to 85% after 100 d in both non-sterile and sterile soils. Uptake declined significantly ($p < 0.001$) at 25 d for both non-sterile and sterile soils; however, with further contact time, the effect of AC on earthworm uptake of ^{14}C-phenanthrene seemed to diminish, and no significant difference ($p > 0.05$) was found at consecutive time points between 25 d and 100 day. A comparison of earthworm uptake of ^{14}C-phenanthrene in the presence of CB4, AQ5000 and CP1, revealed that earthworms in CB4-amended soils had the greatest uptake of ^{14}C-phenanthrene, while earthworms in CP1-amended soils had the lowest uptake of ^{14}C-phenanthrene, although this was not statistically significant ($p > 0.05$) (Table 4).

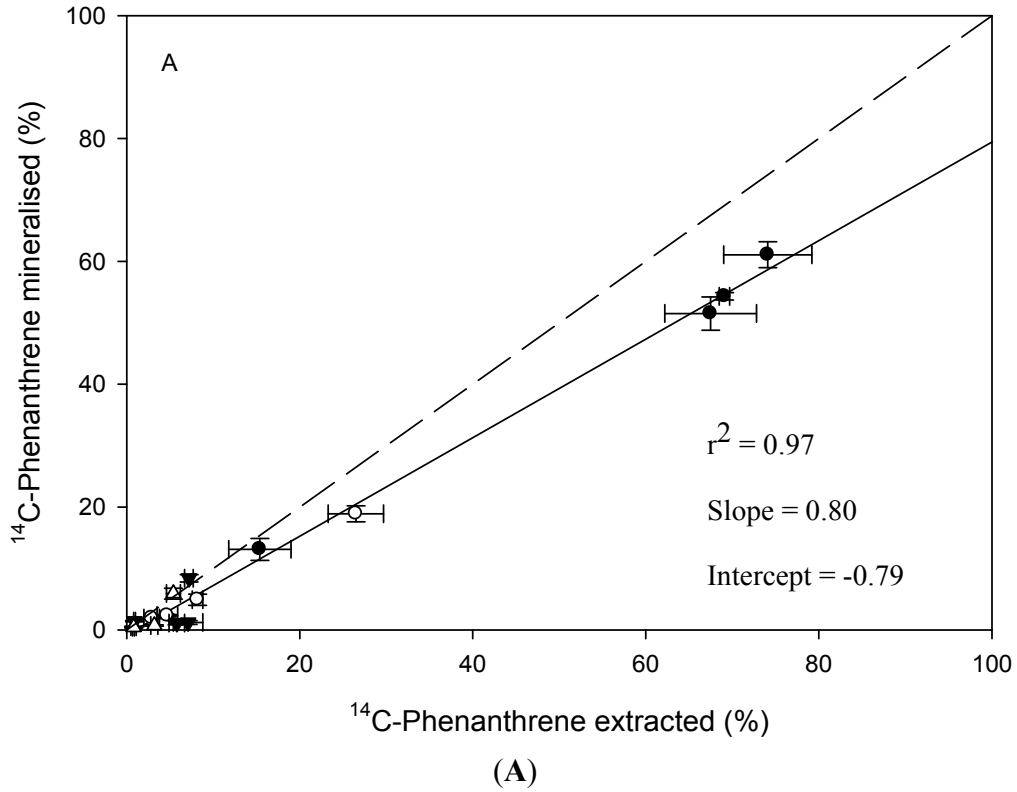
Table 4. ^{14}C-Phenanthrene uptake (%) by *E. fetida* in control and 1% AC-amended soils for both non-sterile and sterile soils. Values are mean ± standard error (n = 3).

Time (d)	Non Sterile				Sterile			
	Control (%)	CB4 (%)	AQ5000 (%)	CP1 (%)	Control (%)	CB4 (%)	AQ5000 (%)	CP1 (%)
1	6.75 ± 0.21	0.63 ± 0.02	0.59 ± 0.11	0.61 ± 0.08	6.75 ± 0.21	0.63 ± 0.02	0.59 ± 0.11	0.61 ± 0.08
25	8.30 ± 0.02	0.11 ± 0.05	0.06 ± 0.02	0.11 ± 0.05	5.85 ± 0.78	0.24 ± 0.05	0.21 ± 0.04	0.13 ± 0.02
50	5.01 ± 0.07	0.09 ± 0.03	0.04 ± 0.04	0.09 ± 0.07	4.02 ± 0.07	0.11 ± 0.01	0.17 ± 0.03	0.09 ± 0.01
100	3.73 ± 0.04	0.10 ± 0.05	0.08 ± 0.03	0.10 ± 0.04	2.18 ± 0.04	0.14 ± 0.05	0.10 ± 0.07	0.11 ± 0.09

3.5. Relationship between Microbial Mineralisation and HPCD Extractability of ^{14}C-phenanthrene

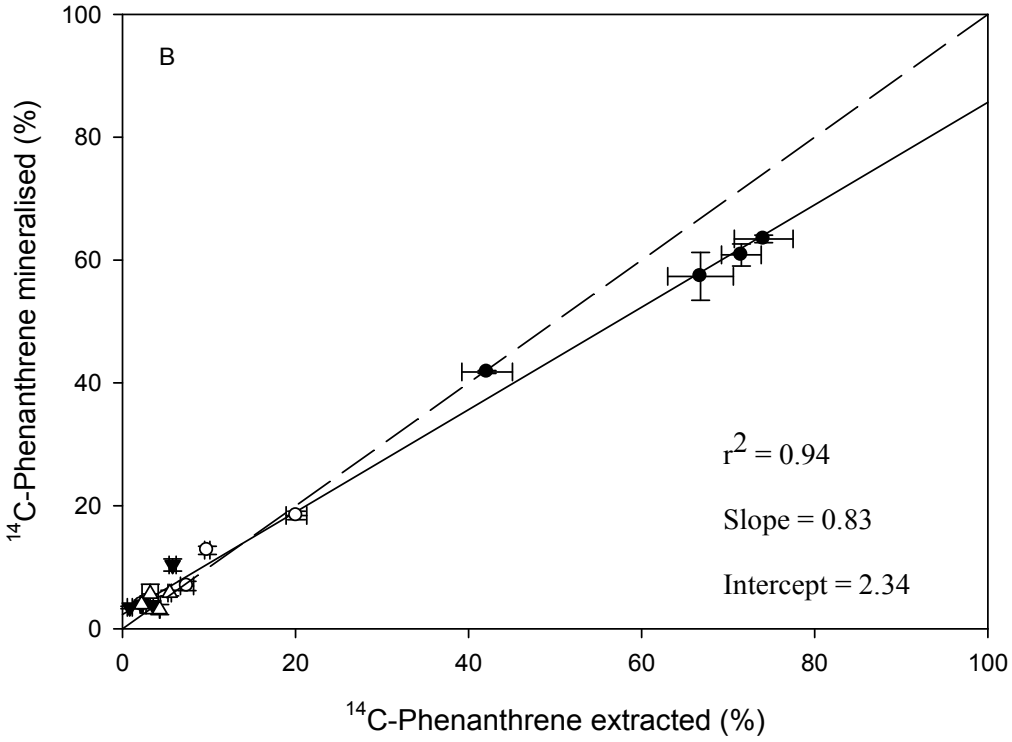
Figure 4 illustrates the relationship between the HPCD extraction and microbial mineralisation of ^{14}C-phenanthrene, in non-sterile and sterile soils, respectively. A correlation between the two variables was investigated using linear regressions. The data showed that the correlations were highly significant ($p < 0.05$) and indicated a strong relationship between the HPCD extraction and extents of mineralisation, in the presence of AC. The relationship between the amounts of ^{14}C-phenanthrene mineralised and the amounts extracted by HPCD gave the following values: non-sterile soils (r^2 = 0.97; slope = 0.80; intercept = −0.79) and sterile soils (r^2 = 0.94; slope = 0.83; intercept = 2.34), respectively (Figure 4).

Figure 4. Linear regressions showing the correlation between total extents of ^{14}C-phenanthrene mineralised and total ^{14}C-phenanthrene extracted by HPCD in (**A**) non-sterile soil (**B**) sterile soil. Data points represent both AC-amended and controls soils. Dashed lines represent y = x correlation between the two variables. Error bars are SEM (n = 3). Legend key: Control (●), 1% CB4 (○), 1% AQ5000 (▼) and 1% CP1 (Δ).



(**A**)

Figure 4. *Cont.*



(**B**)

3.6. Relationship between ^{14}C-phenanthrene Uptake in Earthworms and HPCD Extractability

Figure 5 shows the relationship between earthworm uptake and HPCD extraction of ^{14}C-phenanthrene, in non-sterile and sterile soils, respectively. There were statistically significant differences ($p < 0.05$) in the amount of ^{14}C-phenanthrene taken up by earthworms and that extracted by HPCD, for both non-sterile and sterile soils, suggesting little or no relationship. A correlation between ^{14}C-phenanthrene uptake in earthworms and HPCD extraction of ^{14}C-phenanthrene revealed that HPCD extraction greatly overestimated the fraction available to earthworms, by a factor of at least 10. For the non-sterile soils, linear regressions displayed a slope of 0.09, an intercept of −0.16 and $r^2 = 0.88$, while in sterile soils, linear regressions displayed a slope of 0.08, an intercept of −0.19 and $r^2 = 0.93$.

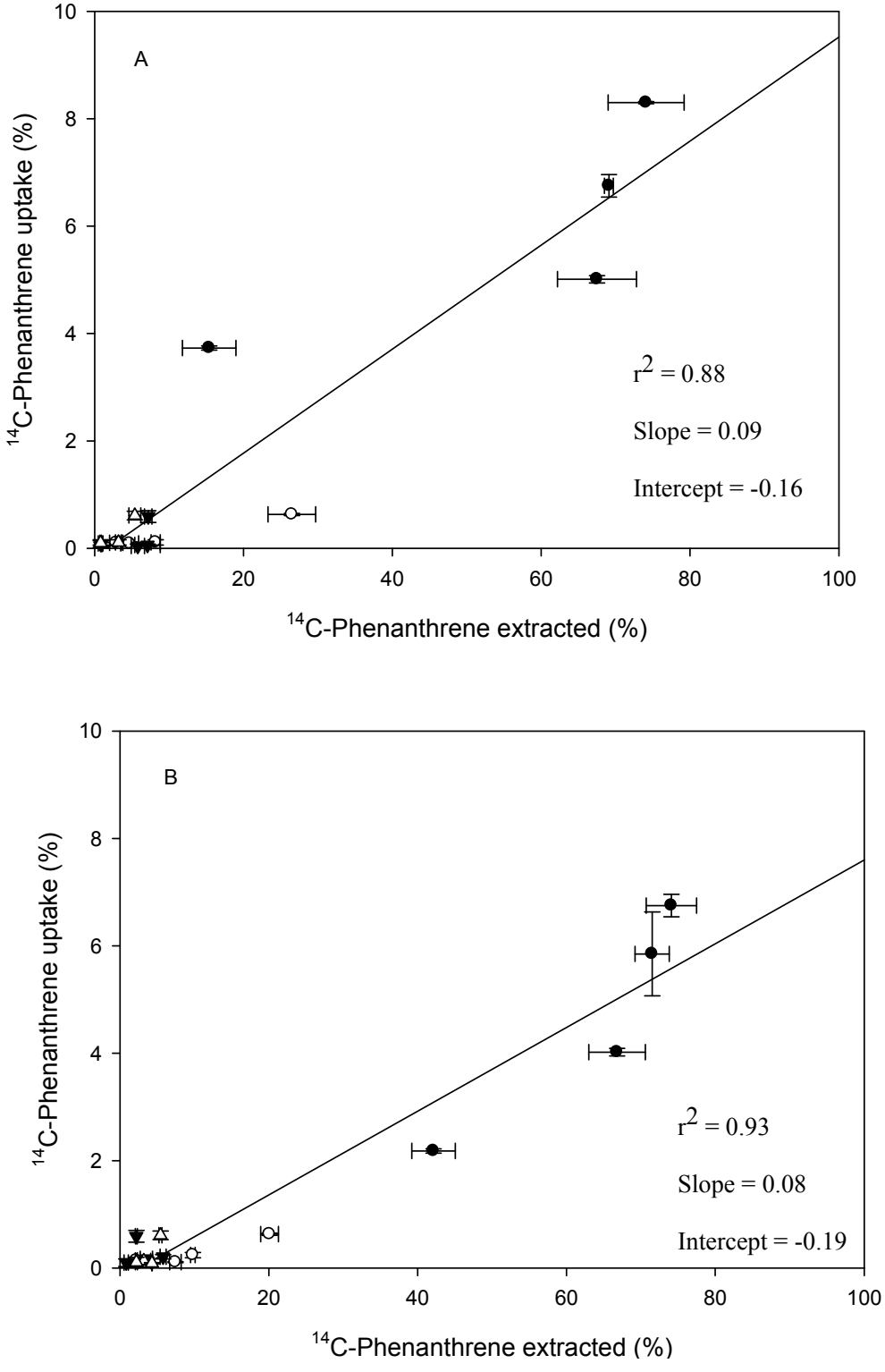
4. Discussion

4.1. Measurement of ^{14}C-phenanthrene Bioaccessibility by HPCD in AC-Amended Soil

HPCD extraction has been proved to be a useful tool in predicting the bioaccessibility of PAHs to microorganisms [29–31]. This study demonstrated that the addition of 1% AC to soil affected the bioaccessibility of ^{14}C-phenanthrene in comparison to control soils. The reduced extractability may be due to enhanced sorption of AC to ^{14}C-phenanthrene. This indicates that HPCD may be unable to displace phenanthrene from the energetically favourable AC sites, therefore limiting extractability at high AC concentrations [10,32,33]. Overall, the extractability of ^{14}C-phenanthrene from non-sterile and sterile soils, respectively, decreased with increased soil-phenanthrene contact time. A more

pronounced reduction in extractability was observed in CB4-, AQ5000- and CP1-amended soils after 100 d, compared to the control soils. This is consistent with results from previous studies [10,33].

Figure 5. Linear regression showing the correlation between ^{14}C-phenanthrene uptake by *E. fetida* and ^{14}C-phenanthrene extracted by HPCD in (**A**) non-sterile soil (**B**) sterile soil. Data points represent both AC-amended and controls soils Error bars are SEM (n = 3). Legend key: Control (●), 1% CB4 (○), 1% AQ5000 (▼) and 1% CP1 (Δ).



A correlation between the extent of ^{14}C-phenanthrene mineralised and HPCD extraction showed a good relationship for non-sterile soils (r^2 = 0.98) and sterile soils (r^2 = 0.96) in the presence of AC, which indicates a significant relationship between HPCD extractability and mineralisation. The slopes observed in this study were 0.80 in the non-sterile soils and 0.83 in the sterile soils, indicating a direct prediction of phenanthrene bioaccessibility by HPCD. These results suggest that both indigenous microflora and microbial inoculum degraded ^{14}C-phenanthrene to a lesser extent than estimated by HPCD extraction. The results obtained in this study have demonstrated that ^{14}C-phenanthrene bioaccessibility by HPCD may be used to predict the amount of ^{14}C-phenanthrene mineralised by indigenous microflora or microbial inoculum, even in the presence of high concentrations of AC [34].

4.2. Biota-^{14}C-phenanthrene Interactions in AC-Amended Soils

In this study, the catabolic potential of indigenous microflora and an enriched microbial inoculum were used to assess the mineralisation of ^{14}C-phenanthrene in soil using a well-established respirometric assay [17,35]. The reduction in the overall extents of ^{14}C-phenanthrene after 100 d may be accounted for by sequestration processes, e.g., sorption to OM and AC [10,36]. For the non-sterile soils, the loss of phenanthrene may be attributed to phenanthrene degradation by an active indigenous capability [27,29]. The microbial inoculum used in the experiment has been demonstrated as very efficient in previous studies [10,35,37], and its efficiency manifested in the sterile soils, as evidently no lag phase was observed at the start of the experiment. In this study, the maximum rates (bioavailability) and overall extents (bioaccessibility) of ^{14}C-phenanthrene declined in soils amended with 1% AC, in comparison to control soils, in non-sterile and sterile soils. This reduction may be due to an enhanced phenanthrene sorption of ^{14}C-phenanthrene to AC. Previous studies have demonstrated that AC reduces contaminant bioaccessibility in AC-amended soils [10,38]. With an increase in contact time, there was a reduction in maximum rates and overall extents of ^{14}C-phenanthrene mineralisation, and this was apparent for all soils. This is consistent with previous studies that have shown a reduction in overall extent of mineralisation as contact time increased, in the presence of BC [10,32]. The mineralisation of ^{14}C-phenanthrene by indigenous microorganisms correlated well with that of the enriched inoculum in the presence of AC. However, data showed that the overall extents mineralised by the enriched inoculum were marginally greater than that observed for the indigenous microflora. The data presented in this study highlights the similarity between the extents of ^{14}C-phenanthrene mineralised by indigenous microflora and an enriched microbial inoculum, as reported by Doick, K.J. *et al.* [29].

As with microbial degradation, the amount of ^{14}C-phenanthrene taken up by earthworms reduced in the presence of AC-amended soils, in comparison to the control in non-sterile and sterile soils, respectively. This indicates that there was a reduced concentration of ^{14}C-phenanthrene available in soil, as a result of strong sorption by AC [39,40]. This demonstrates that the addition of AC to soils may reduce the risk of uptake and perhaps bioaccumulation of HOCs in the food chain. For example, Paul and Ghosh [41] found that AC > 1% reduced the bioaccumulation of PCBs in *E. fetida* after 19 months in soil. In this study, it was observed that the uptake of ^{14}C-phenanthrene decreased as contact time increased. This is in agreement with previous studies with PAHs and other HOCs, where results have shown that earthworm uptake decreased with increased ageing time, as more of the

chemical became sequestered within the soil matrix and less available for uptake [39,42]. The data from the correlation showed that HPCD extraction generally appeared to overestimate the amount of ^{14}C-phenanthrene taken up by *E. fetida* by a factor of greater than 10. This is supported by results from previous studies [34,43,44]. For example, Gomez-Eyles, Jonker [43] found that HPCD extractions consistently over-predicted biouptake by a factor of 10–10,000 and therefore seem inappropriate for predicting PAH bioaccumulation in field contaminated soils. In addition, Hickman and Reid [34] found out that earthworm biouptake was overestimated by HPCD, suggesting little or no relationship. The data revealed that both non-sterile and sterile soils showed large deviations from the 1:1 relationship, and this may be due to the large difference between uptake in earthworms and HPCD extraction. The slopes observed in this were 0.08 in the non-sterile soils and 0.09 in the sterile soils, indicating that HPCD cannot be used as a direct prediction of earthworm biouptake. This is due to the fact that earthworms have complex uptake mechanisms, and that they can access compounds from both the aqueous and the solid phase, which may suggest that the simple aqueous to hydrophobic sink model provided by cyclodextrin may not account for the complexity of the uptake mechanisms [20,45].

A comparison of the effects of AC type showed that CB4-amended soil consistently had higher values for maximum rates, overall extents of mineralisation, earthworm uptake and HPCD extraction ^{14}C-phenanthrene, when compared to AQ5000- and CP1-amended soils in non-sterile and sterile soils, respectively. A probable reason may be attributed to the differences in the properties of each AC. Pore size, pore volume and specific surface area (SSA) are important properties of AC, with respect to its use as an adsorbent, and these characteristics may differ among ACs (Table 1). The higher values observed for maximum rates, overall extents of mineralisation, earthworm uptake and HPCD extraction ^{14}C-phenanthrene in CB4-amended soils, in comparison to AQ5000- and CP1-amended soils, respectively, indicates that the adsorption capacity of CB4 towards ^{14}C-phenanthrene is lower than that of the AQ5000 and CP1, as observed from the values of the SSA in Table 1. The large surface area of fine particles positively correlates with sorption capacities of the sorbent [3], which corroborates findings in this study. AQ5000 and CP1 exhibited similarity in the overall extent of mineralisation, uptake and HPCD extraction, even though there were differences in the pore volume and porosity of these two adsorbents. The differences between the AC would be investigated in further studies.

5. Conclusions

This study has demonstrated that the use of AC reduces bioavailability and bioaccessibility of ^{14}C-phenanthrene to microorganisms and earthworms in soil. The use of a specific dose (1%) may be sufficient to reduce the risk posed by contaminants over an extensive period of time, thereby reducing toxicity to organisms in the different trophic levels. This study further demonstrated that the use of HPCD to predict uptake in earthworm might be out of place, as observed in this study. The benefits of AC in reducing organic contaminant bioavailability was seen across the three different AC used. CP1 seems to be the most promising AC as seen from studies in the respirometry, earthworm assay and extraction studies. The results imply that bioaccessibility of phenanthrene could be dependent on the characteristic and type of AC used for remediation studies. It is recommended that further investigations should be carried out on the factors controlling sorption and desorption of different types of AC, so as to understand which AC would be effective in complex ecosystems.

Author Contributions

Ayodeji Oyelami developed the initial idea of the research and designed the whole experiment. He conducted the experimental sampling stage, data collection and presentation of data. Babajide Elegbede conducted parts of the experiment, and analysed part of the data. Kirk Semple provided funds for the purchase of AC and provided technical advice on the manuscript. Ayodeji Oyelami drafted the manuscript and Kirk Semple approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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