Buffered cyclodextrin extraction of ^{14}C-phenanthrene from black carbon amended soil

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Abstract

The presence of black carbon (BC) in soil drastically reduced the mineralization of ^{14}C-phenanthrene and its extractability by hydroxylpropyl-β-cyclodextrin (HPCD) extractions. This study also tested the effects of pH on the HPCD extraction of ^{14}C-phenanthrene in soils with BC. Extractions using 60 mM HPCD solutions prepared in deionized water (pH 5.89) and phosphate buffers (pH 7 and 8) were conducted on ^{14}C-phenanthrene-spiked soils amended with three different types of BC (1% dry weight) after 1, 25, and 50 d of ageing. Biodegradation assays using a *Pseudomonas* sp. strain were also carried out. Results showed that after 1 and 25 d, HPCD at pH 7 extracted significantly more ^{14}C-phenanthrene ($p < 0.05$) from BC-amended soils than the other two solutions (un-buffered and pH 8), while HPCD at pH 8 extracted statistically similar ($p > 0.05$) amounts of phenanthrene compared to the un-buffered solution. At 50 d, HPCD at pH 8 generally extracted more ^{14}C-phenanthrene from all treatments. It was proposed that higher pH promoted the dissolution of soil organic matter (SOM), leading to a greater solubility of phenanthrene in the solvent phase and enhancing the extractive capability of HPCD solutions. Although correlations between extractability and biodegradability of ^{14}C-phenanthrene in BC-amended soils were poor, increasing pH was demonstrated a viable approach to enhancing HPCD extractive capability from the ^{14}C-PAH from soil.

Keywords — black carbon, phenanthrene, hydroxylpropyl-β-cyclodextrin extraction (HPCD), mineralization, pH

1. Introduction

Massive consumption on fossil fuels and combustion of biomass in modern world has dramatically increased the input of black carbon (BC) into the environment [1]. BC is a group of heterogeneous carbon possessing strong sorptive capabilities and recalcitrance to chemical and biological transformation [4]. It is mainly produced by incomplete combustion of fossil fuels or biomass [1-4]. BC is ubiquitously distributed across the environmental compartments including soil, where it impacts the fate and behaviour of a range of contaminants such as hydrophobic organic contaminants (HOCs) [5, 6]. Moreover, commercially produced BC (e.g. activated carbon, AC) has also been proposed and piloted as a tool for contaminated land remediation [7]. Nevertheless, there is still a lack of understanding regarding the implications of BC on the bioaccessibility of soil organic contaminants and risk assessment of contaminated land [4].

In the presence of BC, fastest rates and extents of biodegradation of polycyclic aromatic hydrocarbons (PAHs) can be dramatically reduced [2, 3, 8]. Furthermore, extractability of PAHs from contaminated soils by hydroxylpropyl-β-cyclodextrin (HPCD) has been shown to be influenced by the presence of BC [2, 9]. Importantly, HPCD is acknowledged as a well-established mimetic method to assess the bioaccessibility of organic contaminants in soils [10-12]. However, the HPCD extraction has been shown to underestimate the mineralization of phenanthrene in soils amended with 0.1% or more of AC [2], thereby interfering with the reliability of this technique. [2]. Rhodes *et al.* [2] and Xia *et al.* [3] attributed such incompatibility between HPCD extractability and biodegradability to the direct mineralisation of BC-associated phenanthrene by

75 microorganisms, which HPCD extraction was not able to account for. Although
76 the mechanism involved in direct microbial uptake of sorbed substances has
77 been reported by Alexander [13], this explanation is still questionable
78 considering that the uptake of organic substances by soil microorganisms
79 predominantly takes place in the aqueous phase [14, 15]. It is also possible that
80 other microbial processes (e.g. biosurfactant production) could promote the
81 desorption of the BC-associated target chemical, while water as the solvent of
82 HPCD solution used in these researches was not capable of displacing target
83 compounds from sorption sites on BC particles [16].
84 As it has been previously suggested [4], it is important to find a reliable chemical
85 method to estimate the bioaccessibility of HOCs in soils with BC given the
86 growing input of BC to soil from anthropogenic sources and the application of
87 commercially produced BC as a strategy for the remediation of contaminated
88 systems. For this purpose, a potential approach is to modify HPCD extraction
89 methodology by integrating a buffer of higher pH into the solvent to achieve a
90 greater displacement capacity for target compounds, as increasing pH promotes
91 the dissolution of SOM [17] which contributes to greater aqueous solubility of
92 organic pollutants [18]. This was also demonstrated by Reid *et al.* [10] who
93 observed enhanced extractive capability of HPCD solution prepared in
94 phosphate buffer of pH 8 for phenanthrene [10]. Therefore, this study aims to
95 investigate the effects of phosphate buffers of higher pH values on the extractive
96 capability of HPCD solutions for phenanthrene (a) in soils amended with
97 different types of commercially produced BC, (b) after different periods of soil-
98 contaminant interactions. Parallel biodegradation assays with a phenanthrene-

degrading inoculum (*Pseudomonas* sp.) to measure the microbially accessible fraction of the PAHs in the soil.

2. Materials and methods

2.1 Chemicals

Unlabelled phenanthrene was obtained from Sigma Aldrich Co, Ltd. UK. [9-^{14}C] Phenanthrene was purchased from American Radiolabelled Chemicals, Inc., USA. Liquid scintillation cocktail (Goldstar) and sample oxidation cocktails (Carbotrap and Carbocount) were obtained from Meridian Biotechnologies Ltd, UK. Hydroxylpropyl-β-cyclodextrin (HPCD) was purchased from Acros Organics, Belgium. General purpose grade agar (GPA) was obtained from Fisher Scientific, UK. Activated carbon (Colorsorb P3-1, Aquasorb CP2 and Aquasorb BP2) was obtained from Jacobi Carbons, UK.

2.2 Soil collection and characterization

Pristine soil was collected (A horizon; 5 – 20 cm) from Myerscough Agricultural College in Lancashire, UK, and passed through a 2 mm sieve to remove stones and roots. General soil properties are presented in Table 1. Particle size was analysed through laser diffraction (Hydro 2000MU, Malvern Instruments Ltd., UK). Soil organic matter content (dry weight basis) was determined by mass loss on ignition (450 °C for 24 h). Total carbon and nitrogen content (%) were assessed using an Elementar Vario EL III elemental analyser (Hanau, Germany).

2.3 BC amendment and soil spiking

Prior to BC amendment, the soil was rehydrated with deionized water to field moisture content (30 – 35% dry weight basis). Subsequently, soil treatments with 1% (dry weight basis) of three different types of BC (designated as P3-1, CP 2 and BP 2, properties presented in Table 2) were prepared by blending specific

quantities of BC with each treatment using a stainless spoon [2]. A treatment without BC was also prepared as a control. Immediately after BC amendment, soils were spiked with ^{12}C-/^{14}C-phenanthrene using acetone as carrier (3.75 ml per 300 g dry soil at 0.8 mg/ml for ^{12}C- and 6666.67 Bq/ml for ^{14}C-phenanthrene) as described by Doick *et al*. [19], to achieve a ^{12}C-phenanthrene concentration of 10 mg kg^{-1} and ^{14}C-phenenthrene-associated radioactivity of 64 – 78 kBq kg^{-1} dry soil. Unspiked control soils were also prepared for each BC treatment. As mineralisation of phenanthrene by both indigenous and inoculated microorganism has been shown to be equally efficient and dependent solely on the available amount of phenanthrene [20, 21], the soil samples were not sterilised after spiking and were incubated in sealed amber glass jars at room temperature (21 ± 1 °C) for 1, 25, and 50 d.

2.4 Preparation of phenanthrene-degrading inoculum

Prior to the mineralization assay, a phenanthrene-degrading inoculum of *Pseudomonas* sp. was cultured in a mixture of minimal basal salts solution (MBS) containing phenanthrene solution (0.1 ml l^{-1}) as the sole C-source [22] on an IKA Labortechnik KS501 digital orbital shaker at 100 rpm at room temperature (21 ± 1 °C). On the fourth day of incubation (late exponential phase of growth), the inoculum was concentrated by centrifugation at 10,000 x g for 30 minutes (Hettich Zentrifugen, Rotanta 460, UK). The supernatant was then discarded and the cell pellet washed and re-suspended with fresh MBS. A second centrifugation was subsequently carried out to ensure the removal of any residual phenanthrene, obtaining a final cell density of approximately 10^8 cells ml^{-1}.

2.5 Mineralization of ^{14}C-phenanthrene

Mineralization assays were conducted in 'respirometers', which were modified 250 ml Schott bottles as described by Reid *et al*. [22]. After 1, 25 and 50 d of soil incubation, the respirometers (*n* = 3) were set up with 10 ± 0.2 g soil wet weight (~7.5 g dry soil), 25 ml of MBS, and 5 ml of concentrated inoculum (10^5 – 10^6 cells per g soil) [23]. Uninoculated respirometers (*n* = 3) and soil incubations with no ^{14}C-activity (n=3) were also set up for each treatment. The respirometers were then incubated on an IKA Labortechnik KS501 digital orbital shaker at 100 rpm for 14 days at room temperature (21 ± 1 °C). During this period of time, $^{14}CO_2$ generated from microbial degradation of ^{14}C-phenanthrene was trapped in 7 ml glass scintillation vials suspended from the Teflon lined-lid containing 1 ml of NaOH (1 M). The vials were replaced every 24 h, after which 5 ml Goldstar scintillation cocktail was subsequently added to each of the sampled vials and the ^{14}C-associated activity was quantified by liquid scintillation counting (LSC, Canberra Packard Tri-Carb2250CA) after a >12h storage in the dark to avoid chemo-luminescence.

2.6 Extraction of ^{14}C-phenanthrene with hydroxylpropyl-β-cyclodextrin (HPCD) solutions

Three different HPCD solutions (60mM) were prepared in deionized water (pH 5.89), and phosphate buffers of pH 7 and 8 respectively. The buffers of pH 7 and 8 were prepared by combining K_2HPO_4 (0.2 M) and KH_2PO_4 (0.2 M) solutions at ratios of 1.6:1 and17.9:1 respectively. The extraction assays were carried out after 1, 25 and 50 days of ageing, following the methodology described by Reid *et al*. [10]. In brief, soil (1.25 ± 0.1 g wet weight) from each treatment was weighed into 35 ml Teflon centrifuge tubes with 25 ml of each HPCD solution (*n* = 3). The tubes were then placed onto an orbital shaker (IKA Labortechnik KS501 digital)

at 100 rpm for 22 h in darkness at room temperature (21 ± 1 °C). Subsequently, the tubes were centrifuged at 3000 x g for 1 h (Hettich Zentrifugen, Rotanta 460, UK) and 5 ml of supernatant was then mixed with 15 ml Goldstar scintillation cocktail. The samples were assessed by LSC as described previously.

2.7 Statistical analysis

Following blank-correction, statistical analysis of the results was carried out with the Statistical Package for the Social Sciences (SPSS Version 22 for Mac). The statistical significance of BC addition, BC type and ageing period to phenanthrene biodegradability and phenanthrene extractability by HPCD solutions, as well as the statistical significance of pH to HPCD extractive capability, was determined using a linear model (ANOVA, Tukey Test) and/or Student t-test at 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1 Mineralization of ^{14}C-phenanthrene in soils

^{14}C-Phenanthrene catabolism was drastically reduced in all BC-treated soils at all time points. Compared to soil without BC, the fastest rates (the highest yield of $^{14}CO_2$ per day during mineralisation assays) and extents and of ^{14}C-phenanthrene mineralization decreased by more than 99% at 1 and 25 d, and more than 93% after 50 d of soil incubation (Table 3, 4). The fastest rates of phenanthrene mineralisation did not exceed 0.10% per d at 1 and 25 d and were less than 0.3% per d at 50 d in BC-amended soils (Table 3). At 1 d, only 0.15%, 0.07%, and 0.11% of ^{14}C-PAH was mineralized in soils amended with P3-1, CP 2, and BP 2 respectively, while 63.20% of the ^{14}C-phenanthrene was mineralised in soil without BC. Furthermore, influences of BC type on biodegradation were observed. At 25 d contact time, soil amended with CP 2 yielded significantly less

198 ($p < 0.05$) $^{14}CO_2$ than the other two BC-amended soils, while significantly more
199 ^{14}C-phenanthrene ($p < 0.05$) was mineralized in soil with P3-1 at 50 d than the
200 other two BC-treated soils. Overall, these results were in agreement with
201 previous studies by Rhodes *et al.* [2, 8]. These trends have been attributed to the
202 strong sorptive capacity of BC [24, 25]. Consequently, the aqueous concentration
203 and biodegradation of target compound was reduced, as the microbial uptake of
204 organic substances mainly takes place in soil aqueous phase [14, 15]. Moreover,
205 a fraction of ^{14}C-phenanthrene may have become inaccessible to microorganisms
206 due to entrapment in collapsed pores on BC particles [4, 26]. However, the extent
207 to which ^{14}C-phenanthrene mineralization was inhibited in BC-amended soils
208 was much greater than those observed by Rhodes *et al.* [2, 8]. At least 6% of
209 spiked ^{14}C-phenanthrene was mineralised in each soil treatment in research by
210 Rhodes *et al.* (2008) all treatments (0 – 5% AC dry weight) in the study by
211 Rhodes et al. [2], while Rhodes *et al.* [8] only obtained biodegradation extents
212 lower than 1% in soils treated with 5% AC. It appears that the types of BC used
213 in this study possessed greater sorptive capacity than those used in studies by
214 Rhodes *et al*. [2, 8]. The BC type also influenced the rates and extents of
215 mineralisation of ^{14}C-phenanthrene in the present study (Table 3, 4). These
216 variations may be attributed to the specific properties of each BC such as surface
217 heterogeneity and functional groups, pore volume, activation and production
218 methods, source material, as well as processing temperature [3, 4, 27-29].
219 After 50 d ageing, all BC-amended soils yielded greater ^{14}C-phenanthrene
220 mineralization compared to 1 and 25 d. Unlike BC-amended soils, biodegradation
221 of ^{14}C-phenanthrene in soil without BC decreased significantly ($p < 0.05$) over
222 time (Table 4). Apparently, ageing effect, where biodegradability of HOCs

223 diminishes over time [30], was absent in BC-amended soils. Similar results were
224 also obtained by Rhodes *et al.*, who attributed such findings to sorptive
225 attenuation, where soil organic matter (SOM) competes for limited sorption sites
226 on BC particles and blocks them from the spiked chemical, thus lowering
227 sorptive capacity of BC for target substances [2, 31]. Such competitive sorption
228 has also been observed by other researchers; for example, Wang *et al.* [28] found
229 that organic chemicals with larger molecular sizes covered the binding sites on
230 BC particles and blocked them from smaller molecules.
231 *3.2 HPCD extraction of ^{14}C-phenanthrene in soils*
232 Addition of BC also led to drastic reduction in the extractability of the ^{14}C-PAH by
233 HPCD solutions in each soil treatment at all soil-contaminant contact times
234 (Table 5). Compared to the soil without BC, ^{14}C-activity extracted by unbuffered
235 aqueous HPCD solution decreased by more than 99% at 1 and 25 d, while 93 –
236 98% less ^{14}C-phenanthrene was extracted by the unbuffered solution after 50 d
237 of ageing. Extractions with buffered HPCD solutions were also strongly
238 influenced by the presence of BC, but the phosphate buffer also resulted in
239 changes in amounts of ^{14}C-phenanthrene extracted by HPCD. At 1 and 25 d,
240 HPCD at pH 7 extracted 1.03 – 1.56% of spiked phenanthrene from all BC-treated
241 soils (Table 5), which was statistically higher ($p < 0.05$) than the amounts
242 extracted by the other two solutions. Further increase in pH of HPCD solution to
243 8 led to statistically similar ($p > 0.05$) yield of extracted ^{14}C-activity from all BC-
244 amended soils compared to its aqueous counterpart at 1 and 25 d (Table 5).
245 However, after 50 days of soil incubation, HPCD at pH 8 extracted significantly
246 more ($p < 0.05$) ^{14}C-activity than the other two solutions in soils amended with
247 CP 2 and BP 2, while the amounts of ^{14}C-phenanthrene extracted by HPCD at pH

248 7 were statistically similar ($p > 0.05$) to the values from extractions using
249 aqueous HPCD solution for all BC-amended soils (Table 5).
250 HPCD is a well-established non-exhaustive extraction technique to measure
251 microbial bioaccessibility of numerous HOCs in soils under different conditions
252 [11, 20, 32-38]. The HPCD molecules are able to separate organic compounds
253 from water solution, thus mimicking microbial uptake of organic substances and
254 driving mass-transfer of target compound from soil matrix to dissolved phase
255 [10, 39-42]. However, in presence of BC, sorption of the ^{14}C-PAH resulted in
256 reduction of dissolved phenanthrene for HPCD molecules to separate. Moreover,
257 Jonker and Koelmans [16] suggested that water, as the solvent of aqueous HPCD
258 solution, was not capable of displacing BC-associated phenanthrene molecules
259 from binding sites on BC particles. In the present study, buffered solutions of pH
260 7, at 1 and 25 d, as well as that of pH 8, at 50 d, enhanced the extractive
261 capability of HPCD solutions in soils amended with BC. Additionally, the buffered
262 extracts from each soil treatment at each time point were highly coloured. This
263 was consistent with the observations made by Reid *et al.* [10] and was indicative
264 of the existence of dissolved organic matter in the extracts [10, 43]. The
265 promotion of dissolution of SOM by phosphate buffers at higher pH has been
266 reported in previous studies [44-46]. It was suggested that the deprotonation
267 under basic conditions brought by phosphate buffers attenuated the association
268 between SOM and soil minerals, thus increasing the amount of dissolved organic
269 matter (DOM) [44]. Although other researchers also demonstrated the ability of
270 phosphate to inhibit the sorption of phenanthrene in soils [47], the effects of
271 phosphate itself in this research on the release of phenanthrene from soil are
272 considered minimal given the amount of phosphate used and its contact time

273 with spiked soils. DOM subsequently contribute to greater solubility of
274 phenanthrene [18], so that there were more PAH molecules in the aqueous phase
275 for HPCD molecules to separate.
276 Interestingly, increases in pH did not always result in increases in extraction
277 using HPCD solutions, as a biphasic feature of increasing pH was identified in BC
278 amended soils at 1 and 25 d, and was absent after 50 days of ageing. This
279 observation reflects the complex interactions between soil, BC, phenanthrene
280 and HPCD solutions. It is therefore postulated that extensive sorption of both
281 SOM and ^{14}C-phenanthrene to BC particles was achieved shortly after BC
282 amendment and PAH spiking, while at 1 and 25 d, HPCD at pH 8 dissolved so
283 much BC-associated SOM that sorption sites on BC particles were exposed to
284 phenanthrene. Consequently, greater sorption of phenanthrene to BC was
285 facilitated. At 50 d, however, greater amount of SOM was attached to BC particles
286 and phenanthrene molecules partitioned deeper into BC. As a result, buffer of pH
287 8, which was able to dissolve more SOM, released more BC- and SOM-bound
288 phenanthrene than buffer of pH 7 (Fig. 1).
289 A simple comparison between HPCD extraction assays and mineralisation assays
290 conducted in BC-amended soils was carried out by calculating the ratios of
291 extraction to mineralisation. The results indicated that in most cases aqueous
292 HPCD extracted underestimated mineralisation of ^{14}C-phenanthrene (Table 6).
293 However, the extents to which biodegradation was underestimated were not as
294 great as those reported by Rhodes et al. [2] except for few cases (Table. 6). Such
295 findings suggest that the differences between HPCD extractive capability and
296 biodegradability of phenanthrene in soils with BC may not be as great as they
297 were previously observed. The amounts of phenanthrene extracted by HPCD in

298 pH 7 were 3 to 15 times greater those degraded by microorganisms at 1 and 25 d,
299 and mildly deviated from the degraded amounts at 50 d (Table 6). HPCD in pH 8
300 provided mixed results in ratios of extraction to mineralisation in all BC-treated
301 soils, but the deviations of extractability from biodegradability were not as great
302 as those demonstrated by aqueous HPCD and HPCD in pH 7. These findings have
303 two implications. Firstly, the mechanism which was direct degradation of BC-
304 associated phenanthrene proposed in previous studies may not be actually
305 involved in mineralisation assays. Secondly, increasing pH enhances the
306 extractive capability of HPCD and could improve this method in predicting
307 microbial accessibility of phenanthrene in soils with BC after substantial ageing
308 period, as the ratios of HPCD extraction in buffers were approach 1 compared to
309 those of aqueous HPCD (Table 6). However, due to the size of the data acquired
310 in the current study, further verification of these findings are required to
311 optimise this modification of HPCD extraction under various conditions
312 including different pH values, and soil and BC types. Besides, the order of BC
313 amendment and PAH spiking should also be considered as the faster and greater
314 binding of PAH with BC particles in pre-amended soils may occur, thus bringing
315 differences to the results obtained.

316 **4. Conclusion**

317 Addition of BC significantly reduced mineralisation and extraction of ^{14}C-
318 phenanthrene after different periods of soil-contaminant interactions, where
319 variations brought by BC type were identified among soil treatments.
320 Introduction of phosphate buffers produced varying effects to the extractive
321 capability of HPCD solutions, as HPCD at pH 7 extracted significantly more

322 phenanthrene at 1 and 25 d, and HPCD at pH 8 yielded more extracted ^{14}C-
323 activity at 50 d.
324 A biphasic feature of increasing pH on HPCD extractive capability was observed
325 at 1 and 25 d but not at 50 d. overall, these findings reflected the complex
326 interactions between SOM, BC, HPCD, and phenanthrene. Aqueous HPCD
327 extractions did not always underestimate biodegradation of phenanthrene in BC-
328 amended soil, rejecting previously proposed mechanism for the incompatibility
329 between mineralisation and HPCD extraction. More studies should be carried out
330 to find out whether presence of BC indeed leads to underestimation of
331 biodegradation by HPCD extraction. If yes, increasing pH of HPCD solution, as it
332 has been demonstrated in this study, is a viable approach to modifying this
333 technique for better prediction of the bioaccessibility of organic contaminants in
334 soils with BC.

335 Table 1. Physical-chemical properties of soil used in this study. Errors are shown as 1
336 SEM (n = 3).

Soil Properties		**Parameter Value**
pH (in dH_2O)		5.36 ± 0.01
Organic matter (%)		9.15 ± 0.06
Nitrogen (%)		0.20 ± 0.02
Carbon (%)		2.24 ± 0.01
Particle size*	Clay	23.42%
	Silt	75.26%
	Sand	1.27%
	Soil texture	Silt loam

337 * Analysis of particle size by laser diffraction reflected the distribution of particles with
338 diameter < 1 mm, using total surface area as baseline.

339 Table 2. Properties of black carbon used for soil amendments.

Activated Carbon	Source	Activation method	Processing Temperature	Surface area (m^2 g^{-1})	Pore volume (cm^3 g^{-1})	Mean particle diameter (µm)
P3-1	Wood	Chemical activation	700°C	1150	Not provided	Not applicable [a]
BP 2	Coal	Steam activation	850-950°C	1000	1.56	21
CP 2	Coconut shell	Steam activation	850-950°C	950	0.55	21

340 [a] Particle size of this grade was expressed as distribution of powder size: <150 µm = 95 -100%, <75 µm = 85 – 95%, <45µm = 65 – 85%.

Table 3. Fastest rates of ^{14}C-phenanthrene mineralization in soils amended with 0% black carbon and 1% P3-1, CP 2, and BP 2 at 1, 25 and 50 days of soil-phenanthrene interactions. Values are the % $^{14}CO_2$ per d mean (n = 3) ± standard error of the mean (SEM). Values in the same column followed by the same letter, or row followed by the same number are statistically similar (student t-test and ANOVA Tukey test, n = 3, $p < 0.05$).

Ageing period (days)	Black carbon treatment			
	0% BC	1% P3-1	1% CP2	1% BP2
1 day	26.13 ± 1.73^{a1}	0.03 ± 0.01^{a2}	0.02 ± 0.00^{a2}	0.03 ± 0.00^{a2}
25 day	13.78 ± 1.43^{b1}	0.05 ± 0.01^{a2}	0.02 ± 0.01^{a2}	0.10 ± 0.01^{b3}
50 day	3.85 ± 0.16^{c1}	0.25 ± 0.00^{b2}	0.04 ± 0.01^{a3}	0.06 ± 0.01^{c3}

345 Table 4. Total extents of ^{14}C-phenanthrene mineralised by microorganisms in soils amended with 0% black carbon and 1% P3-1, CP 2,
346 and BP 2 after 1, 25, and 50 days of soil-phenanthrene interactions. Values are the % mean (n = 3) ± standard error of the mean (SEM).
347 Values in the same column followed by the same letter, or row followed by the same number are statistically similar (student t-test and
348 ANOVA Tukey test, n = 3, $p < 0.05$).

Ageing period (days)	Black carbon treatment			
	0% BC	1% P3-1	1% CP2	1% BP2
1 day	63.20 ± 0.52^{a1}	0.15 ± 0.03^{a2}	0.07 ± 0.01^{a2}	0.11 ± 0.02^{a2}
25 day	38.79 ± 1.01^{b1}	0.24 ± 0.04^{a2}	0.09 ± 0.04^{ab3}	0.29 ± 0.02^{b2}
50 day	21.29 ± 0.98^{c1}	1.46 ± 0.00^{b2}	0.21 ± 0.03^{b3}	0.38 ± 0.06^{b3}

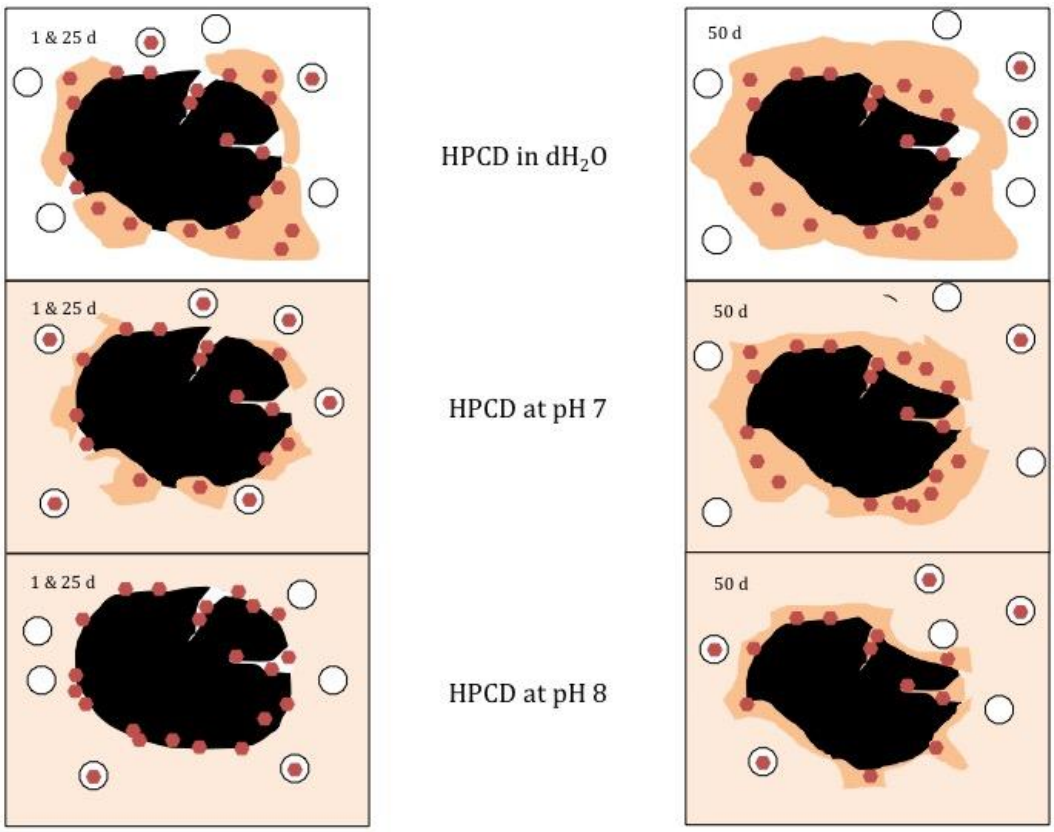
Table 5. ^{14}C-Phenanthrene extracted by HPCD solutions from soils amended with 0% black carbon and 1% P3-1, CP2, and BP2 after 1, 25 and 50 days of soil-phenanthrene interactions. Values are the % mean (n = 3) ± standard error of the mean (SEM). At each time point, values in the same column followed by the same letter are statistically similar; values in the same column generated from the extraction assays with the same HPCD solution followed by the same Greek letter are statistically similar; values in the same row followed by the same number are statistically similar (student t-test and ANOVA Tukey test, n = 3, $p < 0.05$).

Ageing period (days)	HPCD solution	Black carbon treatment			
		0% BC	1% P3-1	1% CP2	1% BP2
1 day	dH_2O	$74.16 \pm 0.39^{a\alpha1}$	$0.08 \pm 0.08^{a\alpha2}$	$0.08 \pm 0.02^{a\alpha2}$	$0.10 \pm 0.10^{a\alpha2}$
	pH 7	$74.96 \pm 0.80^{a\alpha1}$	$1.37 \pm 0.07^{b\alpha2}$	$1.03 \pm 0.21^{b\alpha3}$	$1.10 \pm 0.14^{b\alpha3}$
	pH 8	$72.70 \pm 1.46^{a\alpha1}$	$0.05 \pm 0.05^{a\alpha2}$	$0.11 \pm 0.08^{a\alpha2}$	$0.11 \pm 0.11^{a\alpha2}$
25 day	dH_2O	$14.29 \pm 1.05^{a\beta1}$	$0.01 \pm 0.01^{a\alpha2}$	$0.06 \pm 0.06^{a\alpha2}$	$0.02 \pm 0.02^{a\alpha2}$
	pH 7	$24.71 \pm 1.35^{b\beta1}$	$1.56 \pm 0.17^{b\alpha2}$	$1.11 \pm 0.09^{b\alpha3}$	$1.01 \pm 0.17^{b\alpha3}$
	pH 8	$31.69 \pm 0.06^{c\beta1}$	$0.20 \pm 0.12^{a\alpha2}$	$0.20 \pm 0.13^{a\alpha\beta2}$	$0.13 \pm 0.10^{a\alpha2}$
50 day	dH_2O	$3.76 \pm 0.35^{a\gamma1}$	$0.24 \pm 0.15^{a\alpha2}$	$0.05 \pm 0.01^{a\alpha2}$	$0.08 \pm 0.06^{a\alpha2}$
	pH 7	$8.90 \pm 0.89^{b\gamma1}$	$0.52 \pm 0.07^{a\beta2}$	$0.09 \pm 0.07^{a\beta3}$	$0.05 \pm 0.04^{a\beta3}$
	pH 8	$12.55 \pm 0.28^{c\gamma1}$	$0.64 \pm 0.33^{a\alpha2}$	$0.61 \pm 0.12^{b\beta2}$	$0.44 \pm 0.06^{b\alpha2}$

354 Table 6. The ratios of the amounts of extracted ^{14}C-activity to that of mineralised

355 ^{14}C-activity in BC-amended soils.

HPCD solution	Ageing period	BC treatment		
		1% P3-1	1% CP2	1% BP2
dH2O	1 day	0.55	1.14	0.85
	25 day	0.05	0.68	0.07
	50 day	0.26	0.06	0.42
pH 7	1 day	9.30	15.18	9.73
	25 day	6.50	12.36	3.49
	50 day	0.48	0.75	1.15
pH 8	1 day	0.31	1.61	1.01
	25 day	0.83	2.22	0.45
	50 day	0.67	2.73	1.78



356

357 Fig. 1. Proposed mechanism for the biphasic feature of increasing pH on
358 extractive capability of HPCD solutions at 1 and 25 d, and the absence of this
359 feature at 50 d. At 1 and 25 d, HPCD at pH 8 dissolved large quantity of BC-
360 associated SOM and exposed sorption sites on BC to phenanthrene, leading to
361 greater sorption of phenanthrene to BC particles. At 50 d, more SOM and
362 phenanthrene was attached to BC, HPCD at pH was more capable of dissolving
363 SOM and therefore released more BC- and SOM- bound phenanthrene.

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