

23 **Abstract**

24 This study investigated the impact of two different wood biochars (BioC1 and
25 BioC2) on the extractability and biodegradation of ¹⁴C-naphthalene in soil. Both
26 biochars had contrasting properties due to difference in feedstocks and pyrolytic
27 conditions (450 – 500 °C and 900 – 1000 °C, designated as BioC1 and BioC2,
28 respectively). This study investigated effects of biochar on the relationship
29 between ¹⁴C-naphthalene mineralisation and calcium chloride (CaCl₂),
30 hydroxypropyl-β-cyclodextrin (HPCD) or methanol extraction in soil amended
31 with 0%, 0.1%, 0.5% and 1% BioC1 and BioC2 after 1, 18, 36 and 72 d contact
32 times. Total extents of ¹⁴C-naphthalene mineralisation and extraction were reduced
33 with increasing concentrations of biochar; however, BioC2 showed greater
34 sorptive capacity. Good linear correlation existed between total extents of ¹⁴C-
35 naphthalene mineralisation and HPCD extractions in BioC1 (slope = 0.86, r² =
36 0.92) and BioC2 (slope = 0.86, r² = 0.94) amended soils. However CaCl₂ and
37 methanol extractions underestimated and overestimated extents of mineralisation,
38 respectively. These results indicate that biochar can reduce the bioaccessibility of
39 PAHs and the corresponding risk of exposure to biota, whilst HPCD extraction
40 estimated the bioaccessible fraction of PAHs in soil. Bioaccessibility assessment is
41 vital in evaluation of biodegradation potential and suitability of bioremediation as
42 a remediation option.

43

44

45

46 **Keywords:** Biochar; mineralisation; HPCD; naphthalene; bioaccessibility; macroporous

47 **1. Introduction**

48 Black carbon (BC) encompasses naturally occurring soot and char in the environment
49 as well as **some others** produced as a by-product of natural and anthropogenic
50 activities [1,2]. Previous studies have investigated the ability of biochar to sequester
51 atmospheric CO₂ in soil to aid climate change mitigation [3,4]. Additionally, biochar
52 has been shown to increase soil nutrients to encourage plant growth [5], improve soil
53 characteristics [6] and stimulate other biological functions [7]. Furthermore, biochar
54 has an intrinsic ability to effectively sequester organic contaminants, such as
55 polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-*p*-dioxins, and
56 bisphenol A [8-12]. The organic contaminant sorption characteristics of biochar have
57 been attributed to large surface area [13] and high porosity [14], which results in
58 decreased mobility and bioaccessibility of the contaminants [15,16]. Some factors
59 exist which affect biochar properties and consequently the capacity to influence the
60 contaminant bioavailability in soils. These factors include (a) the source biomass
61 (feedstock) and (b) the production method (pyrolysis) [12,17]. Therefore, the biomass
62 feedstock for the pyrolysis process is important in determining the resulting biochar
63 properties. Varying biochar characteristics occur as feedstock biomass materials
64 differ; wood chip, tree bark and crop residues, others can be sourced from poultry
65 litter, dairy manure and sewage sludge [18,19].

66

67 Contaminated land practitioners also require reliable and robust techniques to
68 determine the applicability of biodegradation and reduce the exposure of contaminants
69 to receptors. Hydroxypropyl- β -cyclodextrin (HPCD) extraction has been shown to
70 predict extents of microbial mineralisation of spiked PAHs at varying concentrations,
71 time and in different soils [20-25]. Semple et al. [26] referred the endpoint of

72 biodegradation as the bioaccessible fraction. HPCD extraction has further been
73 effective in predicting biodegradation of co-contaminated soils [27,28], field
74 contaminated soils [29,30] and sediments [31]. HPCD extraction clearly represents the
75 fraction of PAHs loosely partitioned to soil matrix and fraction of PAH in the aqueous
76 phase available for biodegradation [32].

77

78 Moreover, Rhodes et al. [2] investigated the potential of HPCD extractability to
79 predict ^{14}C -phenanthrene mineralisation in activated carbon (AC) amended soils. The
80 authors showed that HPCD extraction underestimated extent of ^{14}C -phenanthrene
81 mineralisation in $>0.1\%$ AC amended soils. In addition, Rhodes and collaborators
82 suggested that such concentrations of AC in soils affect bioaccessibility of PAHs and
83 would affect regulatory procedures. Consequently, the presence of such BC
84 substances can influence the exposure of contaminants to receptors. Therefore the aim
85 of this study was to test investigate (i) the effect of two contrasting wood biochars on
86 the mineralisation of ^{14}C -naphthalene by indigenous microflora; (ii) the extractability
87 of ^{14}C -naphthalene using calcium chloride (CaCl_2), HPCD and methanol solutions;
88 (iii) the correlation between amounts of ^{14}C -naphthalene mineralised to ^{14}C -
89 naphthalene extracted; (iv) the correlation between maximum rate of ^{14}C -naphthalene
90 mineralisation to amount of ^{14}C -naphthalene extracted.

91

92 **2. Materials and Methods**

93 **2.1. Chemicals**

94 Non-labelled (^{12}C) naphthalene was obtained from BDH laboratory supplies, UK and
95 [^{14}C] naphthalene ($>95\%$ radioactive purity) was obtained from Sigma Aldrich Co.,
96 Ltd, UK. Goldstar multipurpose liquid scintillation fluid was obtained from Meridian,

97 UK. Hydroxypropyl- β -cyclodextrin (HPCD) was obtained from Fischer Scientific,
98 UK. Calcium chloride ($\geq 99.0\%$) was obtained from Sigma Aldrich Co., Ltd, UK.
99 Methanol was obtained from Fisher scientific, UK. Sample oxidizer cocktails
100 (Carbotrap and Carbocount) were from Meridian, UK, and Combustaid from Perkin
101 Elmer, USA.

102

103 **2.2. Soil preparation**

104 An uncontaminated soil (Myerscough soil) classified as surface texture of sandy loam
105 was used in this study. The physicochemical characteristics of the soil can be found in
106 Table 1. The soil was air-dried for 24 h and passed through a 2 mm sieve to remove
107 stones and plant roots. The moisture content of the soil was determined by drying 2 g
108 samples of the soil ($n = 3$) in porcelain crucibles at 105 °C for 24 h. After drying, the
109 samples were then cooled in a dessicator (1 h) and weighed again.

110

111 **2.2. Biochars**

112 The first biochar (BioC1) was obtained from Yorkshire Charcoal Co., UK and the
113 second biochar (BioC2) was obtained from O-Gen UK. Plate count agar and agar-agar
114 were supplied by Oxoid, UK. BioC1 was produced by slow pyrolysis (16 - 18 hours
115 duration at 450 – 500 °C) of a feedstock containing approximately 90% *Acer*, and the
116 remaining 10% a mixture of *Quercus* and *Fraxinus* sp of wood. BioC2 was produced
117 by gasification (1 hour duration at 1,000 °C) of a feedstock containing demolition
118 wood waste. Both were sieved to ≤ 2 mm particle size in preparation for amendment to
119 the soil. Ash content was measured by heating biochar samples at 760 °C for 6 hours
120 [33] using Carbolite Furnace RHF 1400 and calculated using the equation:

121

122
$$\text{Ash content (\%)} = \frac{\text{Bb} - \text{Ba}}{\text{Bb} \times 100} \quad (\text{Eq. 1})$$

124 where Ba and Bb were biochar weight after and before heating, respectively [34].
126 Result showed that BioC1 and BioC2 exhibited 13.7% and 34.0% ash, respectively.
127 Biochar pH analysis was measured in triplicate at 1% (w/v) (1 g biochar to 100 ml
128 distilled water) slurry, where BioC1 and BioC2 had pH of 9.6 and 11.2, respectively.
129 The mixture was shaken for 24 hours at 100 rpm and then measured using a digital pH
130 meter. The total pore volume analysis and surface area were measured by using Lab-
131 Tools NMR Cryoporometer (Version 2) [35]. BioC2 exhibited significantly ($P < 0.05$)
132 greater total pore volume (4.10 ml g^{-1}) compared to BioC1 (1.39). BioC1 and BioC2
133 were both macroporous in nature as they possessed 87.1% and 95.7% macropores
134 ($\geq 50 \text{ nm}$), respectively. BioC2 had surface area of $209 \text{ m}^2 \text{ g}^{-1}$, whilst BioC1 had
135 significantly lower ($P < 0.01$) surface area of $79 \text{ m}^2 \text{ g}^{-1}$.

136

137 **2.4. Soil amendment and spiking**

138 The air-dried soil was rehydrated back to the original field moisture content of 21%
139 (regional average approximately $21 \text{ }^\circ\text{C}$) using deionised water. Following rehydration,
140 the soil was spiked with ^{12}C -naphthalene and labelled ^{14}C -naphthalene at 46.67 Bq g^{-1}
141 soil following the method demonstrated by Doick et al. [36], using toluene as a
142 solvent carrier. This achieved a naphthalene concentration of 50 mg kg^{-1} . The soil was
143 then separated and amended with biochar concentrations of 0%, 0.1%, 0.5%, and
144 1.0% (w/w) by blending the specific quantities into each soil through the use of a
145 stainless steel spoon. This was carried out individually for both BioC1 and BioC2.
146 Blank soils were also prepared for blank corrections. After spiking, 100 g soils were
147 sealed in amber glass jars and then incubated in darkness at room temperature for 1,

148 18, 36, and 72 days, after which, the soils were analysed as described in the following
149 sections.

150

151 **2.5. Determination of total ¹⁴C-naphthalene-associated activity in soil**

152 The ¹⁴C-naphthalene associated activity was determined by combustion using a
153 Packard 307 sample oxidiser at each sampling point of aging (1, 18, 36 and 72 d). Soil
154 samples (1 g; $n = 2$) were weighed into cellulose combustion cones with an addition of
155 200 μ l Combustaid and combusted (3 min). Carbotrap (10 ml) and Carbocount (10
156 ml) were used to trap ¹⁴CO₂. The trapping efficiency was >95%. ¹⁴C-Activity was
157 quantified by liquid scintillation counting (LSC) (Canberra Packard TriCarb 2300 TR,
158 UK) using standard calibration and quench correction techniques [37].

159

160 **2.6. Extraction of ¹⁴C-naphthalene-associated activity by calcium chloride 161 solution (CaCl₂), hydroxypropyl- β -cyclodextrin (HPCD) and methanol**

162 Determination of ¹⁴C-naphthalene extractability using CaCl₂ was carried out at each
163 sampling point (1, 18, 36 and 72 d). Calcium chloride solutions (10 mM) were
164 prepared using deionised water. Soils (2 g) were weighed into 50 ml Teflon centrifuge
165 tubes ($n = 3$) and 30 ml CaCl₂ solution added to each. Determination of ¹⁴C-
166 naphthalene extractability using HPCD was carried out at each sampling point (1, 18,
167 36 and 72 d) as described by Reid et al. [20]. HPCD solutions (50 mM) were prepared
168 using deionised water. Soils (1.25 g) were weighed into 50 ml Teflon centrifuge tubes
169 ($n = 3$) and 25 ml HPCD solution added to each. The determination of ¹⁴C-
170 naphthalene extractability using methanol solvent was done at each sampling point (1,
171 18, 36 and 72 d). Soils (1 g) were weighed into 30 ml Teflon centrifuge tubes ($n = 3$)
172 and 15 ml of methanol solvent (1:15) was added to each tube.

173

174 The tubes were placed onto an orbital shaker at 100 rpm for 22 h. The tubes were then
175 centrifuged at 3000 rpm (Rotanta 460 Centrifuge, Hettich, Germany) for 1 h and 5 ml
176 supernatant was pipetted into 20 ml glass scintillation vials containing Goldstar
177 scintillation cocktail (15 ml). The ^{14}C -labeled radioactivity in the resultant solution
178 was then quantified using the LSC. After extraction, the soil pellet remaining was air
179 dried, weighed into combust cones and then oxidised using the method of
180 determination of ^{14}C -associated activity in soil pellet. This was to establish a mass
181 balance of ^{14}C -associated activity before and after desorption.

182

183 **2.7. Mineralisation of ^{14}C -naphthalene in soil**

184 This process was used to determine the rate and extent of ^{14}C -mineralisation of
185 naphthalene by the indigenous soil microorganisms. Mineralisation assays were
186 carried out in respirometers to assess the catabolism of ^{14}C -naphthalene by the soil
187 indigenous microflora. Respirometers were modified Schott bottles as described in
188 Reid et al. [38]. These were set up in triplicates and into each was added 10 ± 0.2 g
189 soil (dry weight) containing either BioC1 or BioC2 (0%, 0.1%, 0.5%, 1.0%) as well as
190 30 ml minimum basal salts (MBS). The respirometers incorporated a CO_2 trap
191 containing 1 M NaOH (1 ml) within a suspended 7 ml glass scintillation vial. The
192 respirometers were placed on an orbital shaker set at 100 rpm and 25°C over a period
193 of 14 days. Evolved $^{14}\text{CO}_2$ as a result of ^{14}C -naphthalene catabolism was trapped in 1
194 M NaOH with ^{14}C -activity assessed daily by adding Ultima Gold (5 ml) and then
195 utilising liquid scintillation counting (LSC).

196

197

198 2.8. *Statistical Analysis*

199 Statistical analysis of data was conducted using SigmaStat software (Ver 2.0; Systat,
200 Richmond, CA, USA). One way ANOVA ($P < 0.05$) was used to demonstrate
201 differences in extent of mineralisation and extractions amongst each biochar
202 amendment at each time point. Student's *t*-test was used to compare differences in
203 extent of mineralisation and extractability by CaCl₂, HPCD and methanol. **Linear**
204 **regression was used to correlate extent of mineralisation to individual chemical**
205 **extraction.**

206

207 3. **Results**

208 3.1. *Loss of ¹⁴C-naphthalene-associated activity from biochar-amended soils*

209 At each contact time (1, 18, 36 and 72 d), the total amount of ¹⁴C-naphthalene-
210 associated activity was determined. Following an increase in soil-PAH contact time,
211 there were statistically significant ($P < 0.05$) losses of ¹⁴C-naphthalene associated
212 activity in control and 0.1% biochar amended soils. Following 18 d soil-PAH contact
213 time, >22% loss of total amount of spiked ¹⁴C-naphthalene activity in both 0% and
214 0.1% biochar amendments regardless of biochar type (Figure 1). However, ≤20% of
215 ¹⁴C-naphthalene activity was lost in 0.5% and 1% biochar amended soils. Following
216 subsequent increasing soil-PAH contact time (36 and 72 d), there were further
217 significant ($P < 0.05$) loss in ¹⁴C-naphthalene associated activity in 0%, 0.1% and
218 0.5% BioC1 and BioC2 amendments. Interestingly, despite 72 d soil-PAH contact
219 time, there was no significant ($P > 0.05$) loss in activity in 1% BioC2 amended soil as
220 no greater than 10% ¹⁴C-naphthalene activity was lost (Figure 1).

221

222 **3.2. Extraction of ¹⁴C-naphthalene-associated activity by CaCl₂, HPCD, and**
223 **methanol**

224 The extractability of ¹⁴C-naphthalene-associated activity using CaCl₂, HPCD, and
225 methanol was measured over time in unamended and biochar amended soils. CaCl₂
226 extraction removed significantly ($P < 0.05$) less ¹⁴C-naphthalene-associated activity
227 compared to HPCD or methanol across all contact times. At 1 d time point, all three
228 concentrations (0.1%, 0.5% and 1%) of BioC2 significantly reduced ($P < 0.001$)
229 HPCD extractability; whereas, only 0.5% and 1% BioC1 amendments had similar
230 effects. This trend was similar to CaCl₂ extractability. The BioC2 amendments often
231 showed stronger reduction in amounts of ¹⁴C-naphthalene removed by CaCl₂
232 extraction compared to BioC1, where 40.4%, 10.2%, 1.6% and 1.5% were removed
233 from soil amended with 0%, 0.1%, 0.5% and 1% BioC2, respectively (Table 2). In
234 HPCD extraction, 72.9%, 39.9%, 22.2% and 7.9% were extracted from soils amended
235 with 0%, 0.1%, 0.5% and 1% BioC2 amended soils, respectively (Table 2). However,
236 only the 1% of both biochars (BioC1 and BioC2) significantly reduced ($P < 0.05$) ¹⁴C-
237 naphthalene extractability by methanol.

238

239 Following increasing soil-PAH contact time (18, 36, and 72 d), the increasing
240 concentration of biochar amendments resulted in further reduction ($P < 0.05$) in
241 HPCD extractability of ¹⁴C-naphthalene (Table 2) compared to the control soil.
242 However, BioC2 often showed lower extent of CaCl₂ and HPCD extractions
243 compared to BioC1 extractions after 18 d soil-PAH contact time (Table 2).
244 Noticeably, 1% BioC2 amended soils exhibited the lowest ($P < 0.001$) extent of
245 extraction compared to other concentrations and BioC1. However, methanol and
246 CaCl₂ extraction methods resulted to greater and lower ($P < 0.05$) ¹⁴C-naphthalene

247 extractability, respectively, compared to HPCD extraction. After 36 and 72 d contact
248 times, BioC1 and BioC2 had no significant effect on CaCl₂ extractability ($P > 0.05$)
249 (Table 2). Also, CaCl₂ and HPCD could extract no greater than 10% and 20% ¹⁴C-
250 naphthalene, respectively at later contact times (36 and 72 d) (Table 2).

251

252 3.3. Mineralisation of ¹⁴C-naphthalene in soil

253 The mineralisation of ¹⁴C-phenanthrene was monitored over a period of 14 d
254 incubation in soil amended with 0%, 0.1%, 0.5% and 1.0% biochars (BioC1 and
255 BioC2) after 1, 18, 36 and 72 d soil-PAH contact times. The lag phases, rates and
256 extents of mineralisation were calculated and analysed for significant impacts of
257 biochar on mineralisation. The lag phases were measured and defined as the time
258 taken for the extent of ¹⁴C-naphthalene mineralisation to exceed 5%. Increasing
259 concentrations of biochar amendment largely served to increase the lag phase of ¹⁴C-
260 naphthalene mineralisation (Figure 2 and Table 3). Lag phases for control and BioC1
261 amendments were between 2.5 and 3 days at 1 d soil-PAH time point. Noticeably, 1%
262 BioC2 amendment caused a significant increase ($P < 0.01$) in lag phase to 8 days
263 compared to control and 1% BioC1. Following 18 d aging period, lag phases were
264 below 2 days in control and BioC1 amended soils, whilst BioC2 amendments resulted
265 in further increases ($P < 0.001$) in lag phases (Figure 2, Table 3). For example, 0.1%
266 and 0.5% BioC2 extended ($P < 0.001$) the lag phases to 9 and 14 d, respectively,
267 whilst lag phase was immeasurable in 1% BioC2 amended soils (Table 3). This trend
268 was consistent following subsequent aging (36 and 72 d), where 0.5% and 1% BioC2
269 and 1% BioC1 showed immeasurable lag phases beyond 14 days (Table 3). Despite
270 this, there was no significant difference ($P > 0.05$) in lag phase between 0.1% BioC1
271 and BioC2 after 36 and 72 day time points (Table 3).

272

273 The mean maximum rates of mineralisation per day were generally shown to be lower
274 with increasing biochar concentration and soil-PAH contact time. However, at 1 d
275 soil-PAH contact time, the highest maximum rate of ^{14}C -naphthalene mineralisation
276 was $35\% \text{ d}^{-1}$ and was achieved after 3 days of mineralisation in 0.1% BioC1 amended
277 soils. Generally, BioC1 amendments had no significant effect ($P > 0.05$) on rate of
278 mineralisation, except for 1% BioC1. In contrast, all concentrations of BioC2
279 amendments (0.1%, 0.5% and 1.0%) demonstrated significant reductions ($P < 0.001$)
280 in maximum rates of ^{14}C -naphthalene mineralisation of $5.81\% \text{ d}^{-1}$, $2.52\% \text{ d}^{-1}$ and
281 $1.32\% \text{ d}^{-1}$, respectively (Table 3) compared to control. Noticeably, the increase in soil-
282 PAH time developed consistent decreases in maximum rates of ^{14}C -naphthalene
283 mineralisation, except for 0.1% BioC1. It was also observed that BioC2 amendments
284 significantly reduced ($P < 0.05$) the rates of mineralisation compared to BioC1 at 1,
285 18 and 36 d contact time (Table 3).

286

287 The total extents of ^{14}C -naphthalene mineralisation were monitored over 14 days and
288 showed decrease with increasing biochar concentrations (Figure 2 and Table 3). This
289 occurred for both types of biochar (BioC1 and BioC2) and after each contact time (1,
290 18, 36 and 72 d). For instance, the total extents of mineralisation after 1 d contact time
291 for 0%, 0.1%, 0.5% and 1.0% BioC1 were 62.0%, 58.8%, 52.6%, 29.0%, respectively
292 (Table 3). Similarly, fractions of ^{14}C -naphthalene mineralised in 0.1%, 0.5% and 1%
293 BioC2 amended soils were 25%, 17.3% and 9.9%, respectively. The total extents of
294 ^{14}C -naphthalene mineralised in soil amended with 1% BioC1 and 0.5% and 1% BioC2
295 were often 50% less of the control soil (0%) at all contact time points. Furthermore,
296 the addition of BioC2 to the soil reduced the extents of mineralisation by $\geq 50\%$

297 compared to BioC1 (Figure 2 and Table 3). Following increases in soil-PAH contact
298 time, the mineralisation of ^{14}C -naphthalene significantly decreased ($P < 0.05$); this
299 was apparently observed irrespective of biochar amendment in the soils. It is
300 noteworthy that microbial activity was not invigorated by further spiking of ^{14}C -
301 naphthalene into the respirometry assays nor was there any addition of naphthalene
302 degrading inoculum. This was to evaluate the potential of intrinsic microbial inoculum
303 to degrade bioaccessible fraction of ^{14}C -naphthalene. In the control soil (0%), for
304 instance, the total extents of mineralisation was 62.0%, 34.1%, 17.6% and 10.1% after
305 1, 18, 36 and 72 d soil-PAH contact time (Figure 2 and Table 3). All three (0.1%,
306 0.5% and 1%) concentrations of both biochars showed significant decrease ($P <$
307 0.001) in extents of ^{14}C -naphthalene mineralisation with increase in soil-PAH contact
308 time.

309

310 **3.4. Relationship between extraction and mineralisation of ^{14}C -naphthalene**

311 The relationship between the maximum rates of ^{14}C -naphthalene mineralisation and
312 either of CaCl_2 , HPCD or methanol extractability was assessed to test the ability of
313 either extraction method to predict microbial degradation rate of the compound in
314 biochar amended soils. Equally, the total extents of ^{14}C -naphthalene mineralisation
315 were also correlated individually to CaCl_2 , HPCD or methanol extractability. Figures
316 3 and 4 (A - C) shows the relationship between rates of ^{14}C -naphthalene
317 mineralisation to CaCl_2 , HPCD, and methanol extractability, individually. There was
318 very good agreement between rate of ^{14}C -naphthalene mineralisation d^{-1} and CaCl_2 in
319 BioC1 and BioC2 amended soils (slope of 0.82, $r^2 = 0.89$, intercept = -1.63; slope of
320 0.59, $r^2 = 0.97$, intercept = -0.24), respectively (Figures 3 and 4). In support, there was
321 no significant difference ($P > 0.05$) between the amount extracted by CaCl_2 and the

322 rate of mineralisation at each contact time in biochar-amended soils. However, both
323 HPCD and methanol extractions overestimated the rates of ^{14}C -naphthalene
324 mineralisation in BioC1 and BioC2 amended soil (Figures 3 and 4). Figures 5 and 6
325 (A - C) illustrate relationship between total extents of ^{14}C -naphthalene mineralisation
326 individually to CaCl_2 , HPCD and methanol extraction.

327

328 Results showed that CaCl_2 extractability of ^{14}C -naphthalene underestimated the
329 extents of mineralisation (slope of 1.58, $r^2 = 0.93$, intercept = 5.34), (slope of 1.53, r^2
330 = 0.90, intercept = 4.15) for BioC1 and BioC2, respectively. However, HPCD
331 extraction of ^{14}C -naphthalene showed better agreement with slope of 0.86 for both
332 biochar amendments and r^2 of 0.92 (intercept = 0.74) and r^2 of 0.94 (intercept = -
333 1.23), respectively, for BioC1 and BioC2 amendments (Figures 5A and 6A). Also the
334 slope was approximated to 1 (0.86). Whereas, methanol extractability overestimated
335 the extents of mineralisation (slope of 0.74, $r^2 = 0.49$, intercept = -16.30) and (slope of
336 0.30, $r^2 = 0.12$, intercept = -4.40) of BioC1 and BioC2 amended soils, respectively.

337

338 **4. Discussions**

339 **4.1. Loss of ^{14}C -naphthalene-associated activity**

340 The overall losses of ^{14}C -naphthalene-associated activity in controls and 0.1%
341 biochars amended soils were mainly attributed to degradation and volatilisation
342 [22,39]. The inherent biodegradation of the bioaccessible fraction of ^{14}C -naphthalene
343 would have occurred during the aging period since naphthalene catabolic potential can
344 be found diversely in the environment [40,41]. Biochar is a form of recalcitrant
345 organic matter produced through pyrolysis of biomass [42,43] and reduces the
346 bioavailability of PAHs and TCDDs in soil by sorption [11,15]. This property caused

347 insignificant loss ($P > 0.05$) of ^{14}C -naphthalene-associated activity in 0.5% and 1%
348 biochar amended soils compared to control. This was also attributed to the enhanced
349 level of sequestration due to higher concentrations of biochar, which reduced any loss
350 of naphthalene in the soil

351

352 ***4.2. Extractability of ^{14}C -naphthalene-associated activity using CaCl_2 , HPCD,*** 353 ***and methanol extraction techniques***

354 This study tested the ability of different non-exhaustive extraction techniques (CaCl_2 ,
355 HPCD and methanol) to remove labile fractions of naphthalene [26]. CaCl_2 and
356 HPCD extractions showed significant decreasing extractability ($P < 0.05$) with
357 increasing biochar concentrations (Table 2). This was attributed to sequestration
358 processes, including sorption via partitioning and physical entrapment of the ^{14}C -
359 naphthalene-associated activity to biochar particles [44-46]. Sorption may occur via
360 physical adsorption through weak binding force, entrapment into nanopores and/or
361 chemical or internal adsorption through strong hydrophobic and binding force [47].
362 There were differences in the amounts of ^{14}C -naphthalene extracted from soil with
363 differing biochar particles (BioC1 and BioC2), mainly due to the difference in total
364 pore volume of individual biochars which accommodated the ^{14}C -naphthalene [48].
365 Obviously, the biochars differed in feedstock and production process. For example,
366 BioC2 exhibited greater pore volume which clearly sequestered more ^{14}C -naphthalene
367 than BioC1. This was because of the higher temperature of BioC2 production, whilst
368 BioC1 was produced at 450 °C [49]. Since the biochars contain less internal surface
369 area and micropores, PAHs tend to accumulate within the macroporous region [46],
370 which is dominantly in BioC2. This study supports Zhang et al. [50], in which biochar

371 produced at 700 °C incorporated in soil effectively sorbed phenanthrene to greater
372 extent compared to a 350 °C biochar.

373

374 Following increasing soil-PAH contact time, there was a general reduction in amounts
375 of ¹⁴C-naphthalene removed by CaCl₂ or HPCD irrespective of biochar
376 concentrations. When organic contaminants are in contact with soil, there is a rapid
377 uptake of the organic compounds via fast and slow stages (hours to days) through
378 partitioning and adsorption within pores of soil matrix [51]. The inability of CaCl₂ to
379 extract ¹⁴C-naphthalene in control and biochar amended soils was attributed to the
380 poor extractability of the solution, inability of solution to penetrate into nanopore
381 regions containing ¹⁴C-naphthalene to desorb the contaminant [23]. Despite HPCD
382 being an effective extracting solution [23,26,30,37], ¹⁴C-naphthalene was shown to be
383 irreversibly extractable due to significant adsorption and partitioning within nanopore
384 sites [8,15,52]. This was better explained as methanol solvent extraction described the
385 physical entrapment of naphthalene within soil-biochar matrix following intra-organic
386 matter diffusion [15,53].

387

388 **4.3. Mineralisation of ¹⁴C-naphthalene-associated activity from soil**

389 Although biochar affects the extent of biodegradation of organic contaminants, the
390 degree to which different biochars impact on biodegradation differs considerably
391 when incorporated into soils [9,16,25,54]. Extents of ¹⁴C-naphthalene mineralisation
392 were consistently lower as the concentration of biochar amendments increased (0% >
393 0.1% > 0.5% > 1%). Rhodes et al. [2,55], Marchal et al. [16] and Ogbonnaya et al.
394 [25] confirmed that the addition of AC and biochar to soils reduced the extents of ¹⁴C-
395 PAH mineralisation through sorption and reduction of the PAH in aqueous phase.

396 Similarly, biochar reduced extents of ^{14}C -naphthalene mineralisation and the reduction
397 was more pronounced in the BioC2 amended soils; thus, the degree of sorption differs
398 amongst biochar materials. This is often attributed to differences in physical
399 properties, owing to difference in feedstock material and production processes
400 [50,56]. Indeed, Chen and Yuan [8], Bornemann et al. [49] and Zhang et al. [50]
401 illustrated that higher temperature biochar tend to sorb organic contaminants to a
402 greater degree. Biochar strongly sequesters naphthalene molecules within its
403 micropore network [1] and resists desorption even while experiencing shaking in
404 slurry assay, thereby reducing the bioavailable/bioaccessible fractions. High pore
405 volumes were observed for both biochars, but it was greater in BioC2 and
406 accompanied with higher surface area which resulted in the higher extent of sorption
407 that governed the bioaccessibility of naphthalene. BioC1 initially sustained rate of
408 mineralisation but increasing biochar concentrations and contact time accompanied
409 increases in lag phases and reductions in the rates and extents of biodegradation [12].
410 Reduction in extents of ^{14}C -naphthalene mineralisation with increase in soil-PAH
411 contact time is in agreement with other related studies [2,22,25,37,55].

412

413 Semple et al. [26] clearly described bioavailability as a good descriptor of the rate of
414 biodegradation of an organic contaminant; whilst bioaccessibility described the
415 biodegradation end-point. Based on these definitions, the rates and extents of ^{14}C -
416 naphthalene mineralisation were individually compared to its HPCD, CaCl_2 and
417 methanol extractability to utilise a suitable chemical extraction technique to determine
418 the bioavailability and bioaccessibility of ^{14}C -naphthalene in biochar-amended soils.
419 Linear regression was used to statistically test correlation between CaCl_2 , HPCD and
420 methanol extracts to rates and extents of ^{14}C -naphthalene mineralisation by indigenous

421 soil microflora. CaCl₂ extraction estimated the maximum rates of ¹⁴C-naphthalene
422 mineralisation (bioavailable) in all soils irrespective of biochar concentrations.
423 Previous studies demonstrated that HPCD extractability of PAHs represents its
424 bioavailable fraction [22,57,58]. However, HPCD and methanol extractions
425 overestimated bioavailability, thus they don't illustrate the chemically active fraction
426 but HPCD extraction illustrated the bioaccessible fractions irrespective of the
427 concentration and type of biochar. The interior cavity of HPCD is hydrophobic in
428 nature and capable of forming complexes with HOCs, whilst its exterior is hydrophilic
429 in nature [59,60]. A HPCD initiated 'host-guest' complex [61] means that HPCD can
430 readily form inclusion complex with naphthalene [62], enabling the extraction of the
431 bioaccessible fraction of the contaminant in soil [21,22,25,29,37] irrespective of
432 biochar concentration. This is because, HPCD can access the macroporous exterior
433 cavity of biochar where majority of PAHs are often entrapped [46] and form
434 complexes with the compounds of question for extraction. Additionally, the
435 macroporous cavity is also accessible to microorganisms for biodegradation of
436 naphthalene. In contrast, Rhodes et al. [2] showed that hydrophobicity and
437 microporosity of activated charcoal extensively reduces the extractability of HPCD
438 from hydrophobic matrices. The other chemical extraction techniques (CaCl₂ and
439 methanol) underestimated and overestimated the extents of ¹⁴C-naphthalene
440 mineralisation, respectively. This study validates the applicability of HPCD extraction
441 to predict extents of PAH biodegradation soils where biochar has been incorporated to
442 reduce bioaccessibility and the corresponding risk of exposure.

443

444 **Conclusions**

445 This current study tested extractability of ¹⁴C-naphthalene spiked soils containing 2
446 different biochar particles (BioC1 and BioC2). Despite the influence of individual
447 biochar on biodegradation of naphthalene, HPCD extraction was capable of predicting
448 the extents of mineralisation and influence of biochar on biodegradation, whilst CaCl₂
449 extraction predicted the maximum rate of mineralisation. Thus extending the use of
450 HPCD extraction to biochar amended soils. Additionally, this study has demonstrated
451 that biochar reduces the bioaccessibility of naphthalene in soil and this depends on its
452 production process and feedstock which affects physical properties. Thus, with
453 different biochar concentrations and porous nature, the risk of contaminants in soil can
454 be reduced and yet HPCD can predict the extent of biodegradation of the
455 contaminants. Biochar being cheaper than AC can be used in PAH contaminated land
456 sites to immobilise contaminants. However, this study is based on single spiked soil,
457 field contaminated soils can contain mixtures of contaminants and are exposed to
458 more hostile conditions. Further research should focus on the applicability of biochar
459 in field contaminated soils.

460

461 **Acknowledgements**

462 The authors are thankful to Petroleum Development and Technology Fund for
463 funding. The biochar analysis (pore size distribution) was done by Dr. Beau Webber
464 of Lab-Tools Ltd, Kent, UK. Additionally, we thank Dr. Aller Fernandez for her
465 comments and advice.

466

467

468

469

470

471 **References**

- 472 1. A.A. Koelmans, M.T.O. Jonker, G. Cornelissen, T.D. Bucheli, P.C.M. Van
473 Noort, O. Gustafsson, Review. Black carbon: The reverse of its dark side.
474 *Chemosphere* 63 (2006) 365-377.
475
- 476 2. A.H. Rhodes, A. Carlin, K.T. Semple, Impact of black carbon in the extraction
477 and mineralisation of phenanthrene in soil. *Environ. Sci. Technol.* 42 (2008a)
478 740-745.
479
- 480 3. M.W.I. Schmidt, N.G. Noack, Black carbon in soils and sediments: Analysis,
481 distribution, implications and current challenges. *Global Biogeochem. Cycles*
482 14 (2000) 777-793.
483
- 484 4. J. Lehmann, M. Rondon, Bio-char soil management on highly weathered soils
485 in the humid tropics, in N. Uphoff, A.S. Ball, E. Fernandes, H. Harren, O.
486 Husson, M. Liang, C. Palm, J. Pretty, P. Sanchez, N. Sanginga, J. Thies,
487 (Eds.), *Biological Approaches to Sustainable Soil Systems*. CRC Press Boca
488 Raton, FL, 2006 pp. 517-530.
489
- 490 5. B. Glaser, J. Lehmann, W. Zech, Ameliorating physical and chemical
491 properties of highly weathered soils in the tropics with charcoal – A review.
492 *Biol. Fert. Soils* 35 (2002) 219-230.
493
- 494 6. H. Asai, B.K. Samson, H.M. Stephan, K. Songyikhangsuthor, K. Homma, Y.
495 Kiyono, Y. Inoue, T. Shiraiwa, T. Horie, Biochar amendment techniques for
496 upland rice production in northern Laos. *Field Crops Res.* 111 (2009) 81-84.
497
- 498 7. J. Lehmann, J. Gaunt, M. Rondon, Bio-char sequestration in terrestrial
499 ecosystems – A review. *Mitig. Adapt. Strat. Global Change* 11 (2006) 403-
500 427.
501
- 502 8. B. Chen, M. Yuan, Enhanced sorption of polycyclic aromatic hydrocarbons by
503 soil amended with biochar. *J. Soils & Sediments* 11 (2011) 62-71.
504
- 505 9. S.E. Hale, K. Hanley, J. Lehman, A.R. Zimmerman, G. Cornelissen, Effects of
506 chemical, biological, and physical aging as well as soil addition on the sorption
507 of pyrene to activated carbon and biochar. *Environ. Sci. Technol.* 45 (2011)
508 10445-10453.
509
- 510 10. K. Sun, K. Ro, M. Guo, J. Novak, H. Mashayekhi, B. Xing, Sorption of
511 bisphenol A, 17 α -ethinyl estradiol and phenanthrene on thermally and
512 hydrothermally produced biochars. *Bioresource Technol.* 102 (2011) 5757-
513 5763.

514

515 11. Y. Chai, R.J. Curriet, J.W. Davist, M. Wilken, G.D. Martin, V.N. Fishman, U.
516 Ghosh, Effectiveness of activated carbon and biochar in reducing the
517 availability of polychlorinated Dibenzo-*p*-dioxins/Dibenzofurans in soil.
518 Environ. Sci. Technol. 46 (2012) 1035-1043.

519

520 12. U. Ogbonnaya, K.T. Semple, Impact of biochar on organic contaminants in
521 soil. A tool for mitigating risk? Agronomy 3 (2013) 349-375.

522

523 13. J.E. Thies, M.C. Rillig, 2009. Characteristics of biochar: biological properties,
524 in, J. Lehmann, S. Joseph, (Eds.), Biochar for environmental management.
525 Earth scan, USA, (2009) pp. 85-105.

526

527 14. D. Özçimen, A. Ersoy-Meriçboyu, Characterisation of biochar and bio-oil
528 samples obtained from carbonization of various biomass materials. Renew.
529 Energ. 35 (2010) 1319-1324.

530

531 15. L. Beesley, E. Moreno-Jiménez, J.L. Gomez-Eyles, Effects of biochar and
532 greenwaste compost amendments on mobility, bioavailability and toxicity of
533 inorganic and organic contaminants in a multi-element polluted soil. Environ.
534 Pollut. 158 (2010) 2282-2287.

535

536 16. G. Marchal, K.E.C. Smith, A. Rein, A. Winding, S. Trapp, U.G. Karlson,
537 Comparing the desorption and biodegradation of low concentrations of
538 phenanthrene sorbed to activated carbon, biochar and compost. Chemosphere
539 90 (2013) 1767-1778.

540

541 17. F. Verheijen, S. Jeffrey, A.C. Bastos, M. van der Velde, I. Diafas, Biochar
542 application to soil. A critical scientific review of effects on soil properties,
543 processes and functions. JRC Scientific and Technical Reports 2010 pp. 1-166.

544

545 18. K.C. Das, M. Garcia-Perez, B. Bibens, N. Melear, Slow pyrolysis of poultry
546 litter and pine woody biomass: Impact of chars and bio-oils on microbial
547 growth. J. Environ. Sci. Health: Part A 43 (2008) 714-724.

548

549 19. S. Sohi, E. Lopez-Capel, E. Krull, R. Bol, Biochar's roles in soil and climate
550 change: A review of research needs. CSIRO Land and Water Science Report
551 05/09, (2009) 64 pp.

552

553 20. B.J. Reid, J.D. Stokes, K.C. Jones, K.T. Semple, Nonexhaustive cyclodextrin-
554 based extraction technique for the evaluation of PAH bioavailability. Environ.
555 Sci. Technol. 34 (2000) 3174-3179.

556

- 557 21. C.J. Patterson, K.T. Semple, G.I. Paton, Non-exhaustive extraction techniques
558 (NEET) for the prediction of naphthalene mineralisation in soil. *FEMS*
559 *Microbiol. Lett.* 241 (2004) 215-220.
560
- 561 22. I.J. Allan, K.T. Semple, R. Hare, B.J. Reid, Prediction of mono- and
562 polycyclic aromatic hydrocarbon degradation in spiked soils using
563 cyclodextrin extraction. *Environ. Pollut.* 144 (2006) 562-571.
564
- 565 23. K.J. Doick, P.J. Clasper, K. Urmann, K.T. Semple, Further validation of the
566 HPCD-technique for the evaluation of PAH microbial availability in soil.
567 *Environ. Pollut.* 144 (2006) 345-354.
568
- 569 24. A.H. Rhodes, N.M. Dew, K.T. Semple, Relationship between cyclodextrin
570 extraction and biodegradation of phenanthrene in soil. *Environ. Toxicol.*
571 *Chem.* 27 (2008b) 1488-1495.
572
- 573 25. U.O. Ogbonnaya, O.O. Adebisi, K.T. Semple, Impact of biochar on the
574 bioaccessibility of ¹⁴Cphenanthrene in soil. *Environ. Sci.: Processes Impacts*
575 16 (2014) 2635 – 2643.
576
- 577 26. K.T. Semple, K.J. Doick, L.Y. Wick, H. Harms, Review: Microbial
578 interactions with organic contaminants in soil: Definitions, processes and
579 measurements. *Environ. Pollut.* 150 (2007) 166-176.
580
- 581 27. K.J. Doick, N.M. Dew, K.T. Semple, Linking catabolism to cyclodextrin
582 extractability: Determination of the microbial availabilities of PAHs in soil.
583 *Environ. Sci. Technol.* 39 (2005) 8858-8864.
584
- 585 28. J.L. Stroud, G.I. Paton, K.T. Semple, Predicting the biodegradation of target
586 hydrocarbons in the presence of mixed contaminants in soil. *Chemosphere* 74
587 (2009) 563-567.
588
- 589 29. J.D. Stokes, A. Wilkinson, B.J. Reid, K.C. Jones, K.T. Semple, 2005a.
590 Prediction of polycyclic aromatic hydrocarbon biodegradation in contaminated
591 soils using an aqueous hydroxypropyl- β -cyclodextrin extraction technique.
592 *Environ. Toxicol. Chem.* 24 (2005a) 1325-1330.
593
- 594 30. A. Papadopoulos, G.I. Paton, B.J. Reid, K.T. Semple, Prediction of PAH
595 biodegradation in field contaminated soils using a cyclodextrin extraction
596 technique. *J. Environ. Monitor.* 9 (2007) 516-522.
597
- 598 31. C. Cuypers, T. Pancras, T. Grotenhuis, W. Rulkens, The estimation of PAH
599 bioavailability in contaminated sediments using hydroxypropyl- β -cyclodextrin
600 and triton X-100 extraction techniques. *Chemosphere* 46 (2002) 1235-1245.

- 601
602 32. G.I. Paton, B.J. Reid, K.T. Semple, Application of a luminescence-based
603 biosensor for assessing naphthalene biodegradation in soils from a
604 manufactured gas plant. *Environ. Pollut.* 157 (2009) 1643 – 1648.
605
- 606 33. J.M. Novak, I. Lima, B. Xing, J.W. Gaskin, C. Steiner, K.C. Das, M.
607 Ahmedna, D. Rehrah, D.W. Watts, W.J. Busscher, H. Schomberg, H., 2009.
608 Characterization of designer biochar produced at different temperatures and
609 their effects on loamy sand. *Ann. Environ. Sci.* 3 (2009) 195-206.
610
- 611 34. X. Cao, W. Harris, Properties of dairy-manure-derived biochar pertinent to its
612 potential use in remediation. *Bioresource Technol.* 101 (2010) 5222-5228.
613
- 614 35. J.B.W. Webber, P. Corbett, K.T. Semple, U. Ogbonnaya, S.W. Teel, C.A.
615 Masiello, Q.J. Fisher, J.J. Valenza II, Y.Q. Song, Q. Hu, An NMR study of
616 porous rock and biochar containing organic material. *Micropor. Mesopor. Mat.*
617 178 (2013) 94-98.
618
- 619 36. K.J. Doick, P.H. Lee, K.T. Semple, Assessment of spiking procedures for the
620 introduction of a phenanthrene-LNAPL mixture into field-wet soil. *Environ.*
621 *Pollut.* 126 (2003) 399-406.
622
- 623 37. A.H. Rhodes, L.E. McAllister, K.T. Semple, 2010. Linking desorption kinetics
624 to phenanthrene biodegradation in soil. *Environ. Pollut.* 158 (2010) 1348-
625 1353.
626
- 627 38. B.J. Reid, C.J.A. MacLeod, P.H. Lee, A.W.H. Morriss, J.D. Stokes, K.T.
628 Semple, A simple ¹⁴C-respirometric method for assessing microbial catabolic
629 potential and contaminant bioavailability. *FEMS Microbiol. Lett.* 196 (2001)
630 141-146.
631
- 632 39. J.D. Stokes, G.I. Paton, K.T. Semple, Behaviour and assessment of
633 bioavailability of organic contaminants in soil: relevance for risk assessment
634 and remediation. *Soil Use Manage.* 21 (2005b) 475-486.
635
- 636 40. W.C. Ghiorse, J.B. Herrick, R.L. Sandoli, E.L. Madsen, Natural selection of
637 PAH-degrading bacterial guilds at coal-tar disposal sites. *Environ. Health*
638 *Persp.* 103 (1995) 107-111.
639
- 640 41. N.C.M. Gomes, L.K. Borges, R. Paranhos, F.N. Pinto, E. Krögerrecklenfort,
641 L.C.S. Mendonça-Hagles, K. Smalla, 2007. Diversity of *ndo* genes in
642 mangrove sediments exposed to different sources of polycyclic aromatic
643 hydrocarbon pollution. *Appl. Environ. Microbiol.* 73 (2007) 7392-7399.
644

- 645 42. C. Steiner, W.G. Teixeira, J. Lehmann, T. Nehls, J.L.V. de Macedo, W.E.H.
646 Blum, W. Zech, Long term effects of manure, charcoal and mineral
647 fertilization on crop production and fertility on highly weathered Central
648 Amazonian upland soil. *Plant Soil* 291 (2007) 275-290.
649
- 650 43. S. Sohi, E. Lopez-Capel, E. Krull, R. Bol, A review of biochar and its use and
651 function in soil. *Adv. Agron.* 105 (2010) 47-82.
652
- 653 44. G. Cornelissen, O. Gustafsson, T.D. Bucheli, M.T.O. Jonker, A.A. Koelmans,
654 P.C.M. Van Noort, Extensive sorption of organic compounds to black carbon,
655 coal, and kerogen in sediments and soils: Mechanisms and consequences for
656 distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* 39
657 (2005) 6881-6895.
658
- 659 45. B. Chen, Z. Chen, Sorption of naphthalene and 1-naphthol by biochars of
660 orange peels with different pyrolytic temperatures. *Chemosphere* 76, (2009)
661 127-133.
662
- 663 46. M. Obst, P. Grathwohl, A. Kappler, O. Eibl, N. Peranio, T. Gocht,
664 Quantitative high-resolution mapping of phenanthrene sorption to black
665 carbon particles. *Environ. Sci. Technol.* 45 (2011) 7314-7322.
666
- 667 47. Z. Zhou, H. Sun, W. Zhang, Desorption of polycyclic aromatic hydrocarbons
668 from aged and unaged charcoals with and without modification of humic acids.
669 *Environ. Pollut.* 158 (2010) 1916-1921.
670
- 671 48. K.A. Spokas, J.M. Novak, C.E. Stewart, K.B. Cantrell, M. Uchimiya, M.G.
672 DuSaire, K.S. Ro, Qualitative analysis of volatile organic compounds on
673 biochar. *Chemosphere* 85 (2011) 869-882.
674
- 675 49. L.C. Bornemann, R.S. Kookana, G. Welp, Differential sorption behaviour of
676 aromatic hydrocarbons on charcoal prepared at different temperatures from
677 grass and wood. *Chemosphere* 67 (2007) 1033-1042.
678
- 679 50. H. Zhang, K. Lin, H. Wang, J. Gan, Effect of *Pinus radiata* derived biochars on
680 soil sorption and desorption of phenanthrene. *Environ. Pollut.* 158 (2010)
681 2821-2825.
682
- 683 51. J.J. Pignatello, B. Xing, Critical review: Mechanisms of slow sorption of
684 organic chemicals to natural particles. *Environ. Sci. Technol.* 30 (1996) 1-11.
685
- 686 52. H. Sun, Z. Zhou, Impacts of charcoal characteristics on sorption of polycyclic
687 aromatic hydrocarbons. *Chemosphere* 71 (2008) 2113-2120.
688

- 689 53. J. Dec, K. Haider, A. Benesi, V. Rangaswamy, A. Schaeffer, U. Pluecken, J.M.
690 Bollag, Analysis of soil-bound residues of ¹³C-labeled fungicide cyprodinil by
691 NMR spectroscopy. *Environ. Sci. Technol.* 31 (1997) 1128-1135.
692
- 693 54. F. Sopeña, K.T. Semple, S. Sohi, G. Bending, Assessing the chemical and
694 biological accessibility of the herbicide isoproturon in soil amended with
695 biochar. *Chemosphere* 88 (2012) 77-83.
696
- 697 55. A.H. Rhodes, M.J. Riding, L.E. McAllister, K. Lee, K.T. Semple, Influence of
698 activated charcoal on desorption kinetics and biodegradation of phenanthrene
699 in soil. *Environ. Sci. Technol.* 46 (2012) 12,445-12,451.
700
- 701 56. G. James, D.A. Sabatini, C.T. Chiou, D. Rutherford, A.C. Scott, K.
702 Karapanagioti, Evaluating phenanthrene sorption on various wood chars.
703 *Water Res.* 39 (2005) 549–558.
704
- 705 57. A.L. Swindell, B.J. Reid, Influence of a NAPL on the loss and biodegradation
706 of ¹⁴C-phenanthrene residues in two dissimilar soils. *Chemosphere* 66 (2007)
707 332-339.
708
- 709 58. P. Oleszczuk, Application of hydroxypropyl (β) cyclodextrin to evaluation of
710 polycyclic aromatic hydrocarbon losses during sewage sludge composting. *J.*
711 *Environ. Sci. Health Part A* 43 (2008) 10-17.
712
- 713 59. K. Uekama, F. Hirayama, T. Irie, Cyclodextrin drug carrier systems. *Chem.*
714 *Rev.* 98 (1998) 2045-2076.
715
- 716 60. A. Vyas, S. Saraf, S. Saraf, Cyclodextrin based novel drug delivery systems. *J.*
717 *Incl. Phenom. Macro. Chem.* 62 (2008) 23-42.
718
- 719 61. R. Auzely-Velty, Self-assembling polysaccharide systems based on
720 cyclodextrin complexation: Synthesis, properties and potential applications in
721 the biomaterials field. *Comptes Rendus Chimie* 14 (2011) 167-177.
722
- 723 62. W. Sliwa, T. Girek, Noncovalently-bound cyclodextrin dimers and related
724 compounds. *Review. Chem. Heterocycl. Comp.* 41 (2005) 1343-1361.
725
726
727
728
729
730
731
732
733

734 Table 1 Physiochemical properties of uncontaminated Myerscough (sandy loam) soil

| Soil particle analysis | | | | | | pH | | Elemental analysis |
|------------------------|------|------|-------------------|-----|------|-------------------|-------------------|--------------------|
| Texture | Clay | Silt | Sand ^a | | | dH ₂ O | CaCl ₂ | OM ^b |
| | | | C | M | F | | | |
| Sandy loam | 19.5 | 20.0 | 0.12 | 6.9 | 53.3 | 6.53 | 5.18 | 2.7 |

735

736 ^a coarse, medium, and fine sand, ^b organic matter content (%)

737

738

739

740

741

742

743

744

745

746

747

748

749

750

751

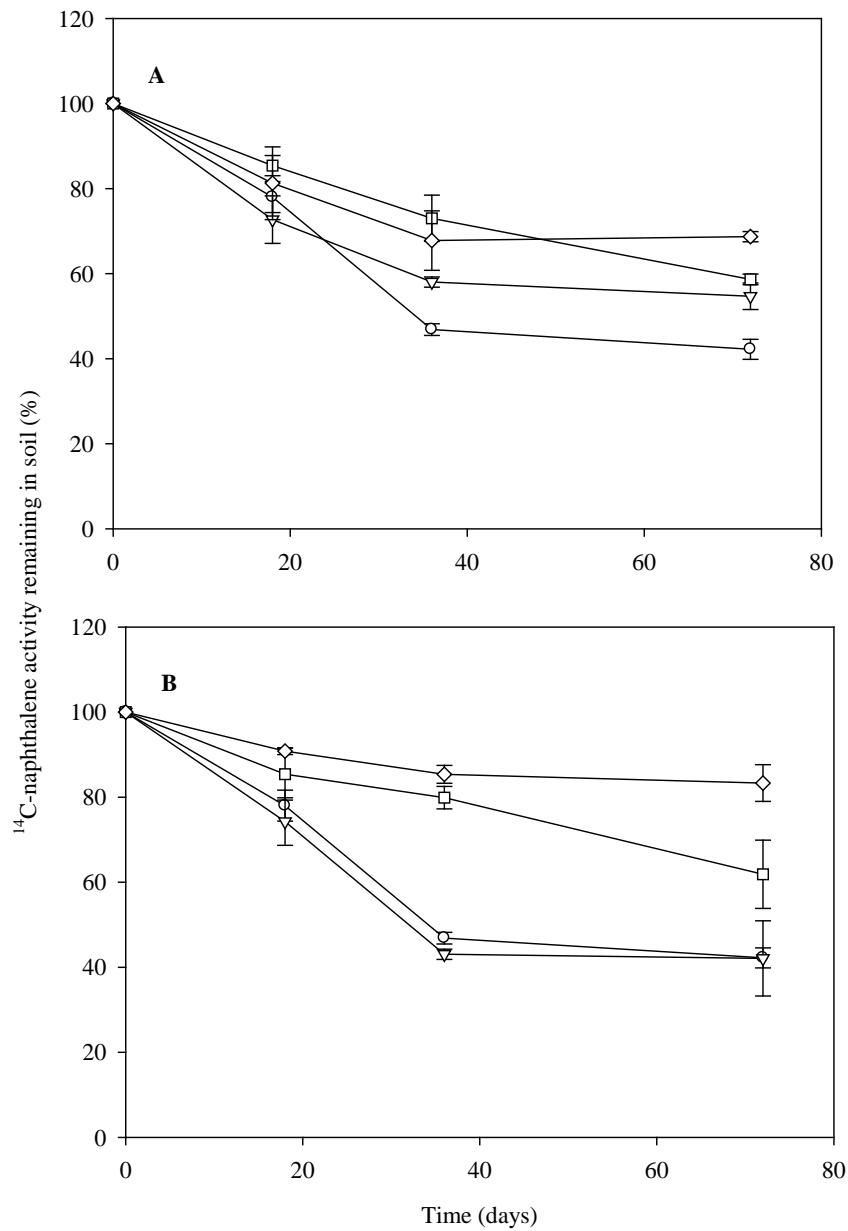
752

753

754

755

756



757

758 Figure 1 Total ¹⁴C-naphthalene-associated activity remaining in soil amended with 0%
 759 (○), 0.1% (∇), 0.5% (□) and 1% (◇) BioC1 (A) and BioC2 (B) over 72 days
 760 incubation period. Error bars represent standard deviation (*n* = 3)

761

762

763

764

765

766

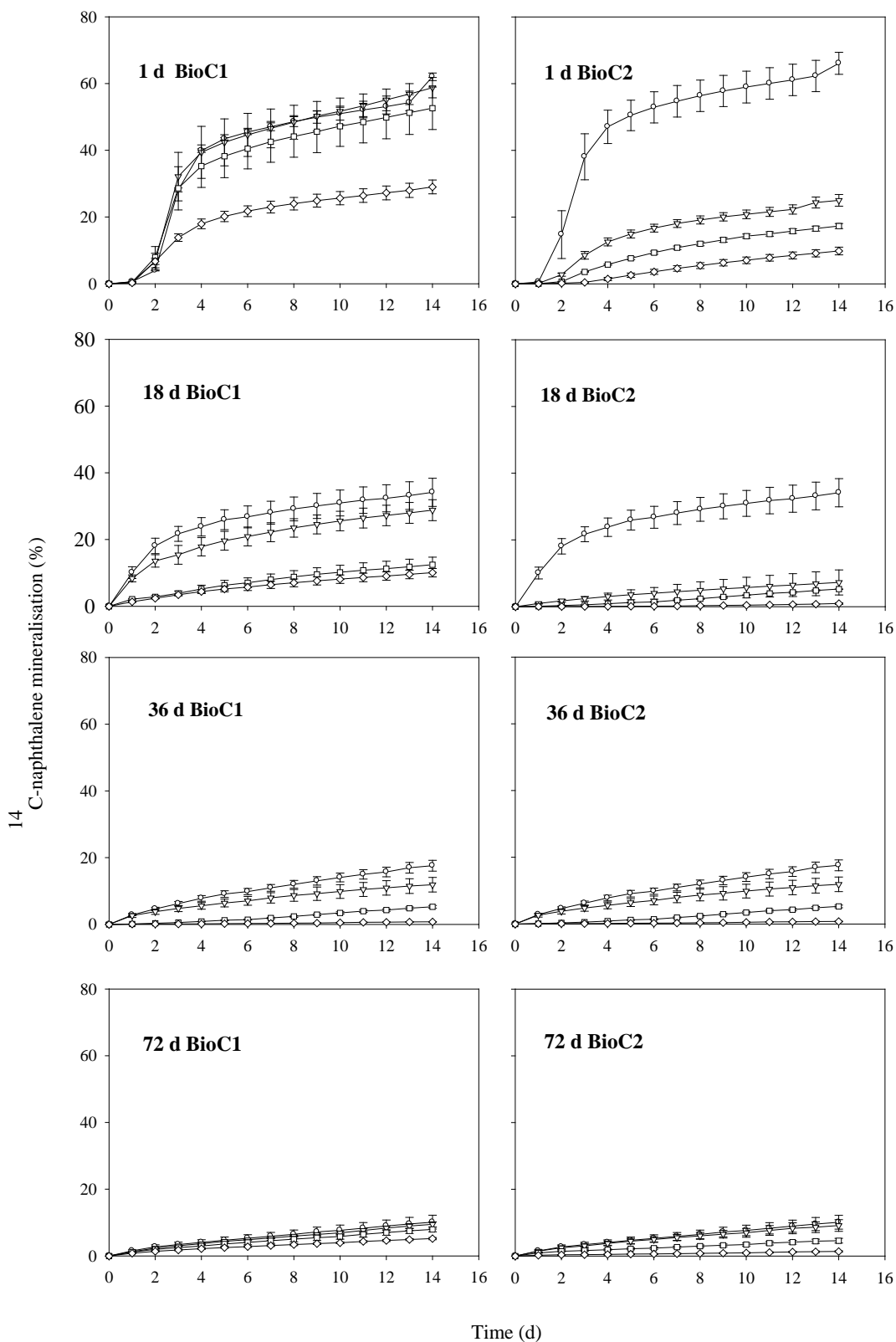
767

768

769 Table 2 ¹⁴C-naphthalene extracted (%) by CaCl₂, HPCD and methanol ± standard
 770 deviation of triplicate samples (*n* = 3)

| Soil-PAH contact (d) | Biochar type | Amendment (%) | CaCl ₂ | HPCD | Methanol |
|----------------------|--------------|---------------|-------------------|--------------|--------------|
| 1 | BioC1 | 0 | 40.42 ± 0.62 | 72.92 ± 0.34 | 86.55 ± 4.98 |
| | | 0.1 | 34.25 ± 0.12 | 67.53 ± 0.39 | 72.05 ± 2.37 |
| | | 0.5 | 26.13 ± 2.28 | 50.28 ± 1.01 | 74.88 ± 3.17 |
| | BioC2 | 1 | 10.10 ± 3.45 | 46.63 ± 2.36 | 61.23 ± 1.81 |
| | | 0.1 | 10.18 ± 0.94 | 39.89 ± 0.14 | 74.00 ± 0.74 |
| | | 0.5 | 1.56 ± 0.11 | 22.18 ± 0.24 | 67.84 ± 2.99 |
| | | 1 | 1.54 ± 0.64 | 7.90 ± 0.36 | 58.52 ± 4.18 |
| 18 | BioC1 | 0 | 14.21 ± 0.42 | 34.57 ± 3.15 | 62.80 ± 1.55 |
| | | 0.1 | 8.59 ± 0.10 | 21.85 ± 1.01 | 55.51 ± 5.91 |
| | | 0.5 | 7.02 ± 1.77 | 11.53 ± 1.97 | 55.60 ± 2.45 |
| | BioC2 | 1 | 4.16 ± 0.59 | 11.29 ± 2.19 | 54.00 ± 5.53 |
| | | 0.1 | 3.14 ± 0.55 | 11.74 ± 3.28 | 61.80 ± 2.32 |
| | | 0.5 | 2.85 ± 0.50 | 10.21 ± 1.21 | 65.21 ± 2.40 |
| | | 1 | 1.41 ± 0.10 | 4.07 ± 0.59 | 56.49 ± 5.34 |
| 36 | BioC1 | 0 | 6.49 ± 0.83 | 17.41 ± 1.68 | 32.30 ± 1.48 |
| | | 0.1 | 6.33 ± 1.48 | 12.90 ± 2.87 | 33.30 ± 6.23 |
| | | 0.5 | 3.72 ± 1.03 | 12.10 ± 2.82 | 53.40 ± 5.71 |
| | BioC2 | 1 | 2.35 ± 0.05 | 7.66 ± 1.15 | 49.26 ± 2.76 |
| | | 0.1 | 3.40 ± 0.80 | 12.59 ± 2.71 | 57.10 ± 2.59 |
| | | 0.5 | 1.93 ± 0.21 | 6.62 ± 0.71 | 73.70 ± 3.14 |
| | | 1 | 1.66 ± 0.09 | 4.55 ± 0.21 | 56.90 ± 4.62 |
| 72 | BioC1 | 0 | 5.99 ± 1.07 | 17.34 ± 1.34 | 26.30 ± 0.72 |
| | | 0.1 | 4.66 ± 0.28 | 12.96 ± 1.03 | 24.45 ± 1.65 |
| | | 0.5 | 4.44 ± 0.83 | 14.93 ± 2.33 | 43.65 ± 4.73 |
| | BioC2 | 1 | 3.33 ± 0.76 | 8.27 ± 1.28 | 69.33 ± 7.09 |
| | | 0.1 | 2.54 ± 0.28 | 10.89 ± 1.06 | 34.45 ± 3.03 |
| | | 0.5 | 2.58 ± 0.57 | 6.49 ± 2.11 | 59.28 ± 0.34 |
| | | 1 | 1.29 ± 0.08 | 5.04 ± 0.12 | 50.40 ± 3.58 |

771
 772
 773
 774
 775
 776
 777



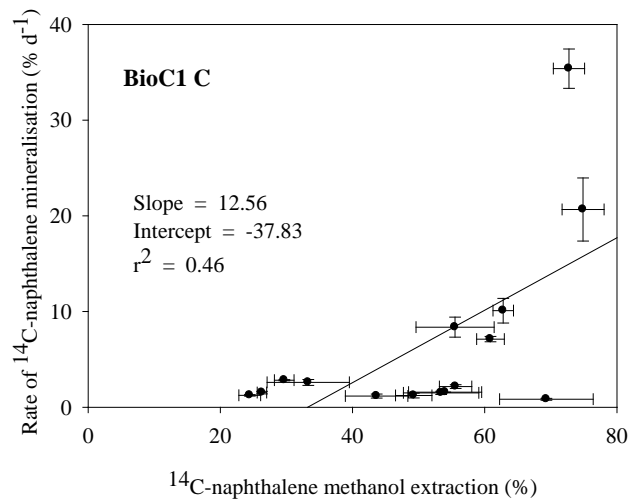
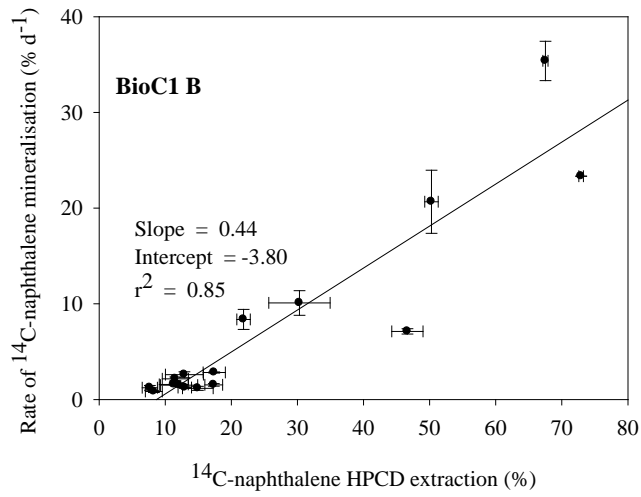
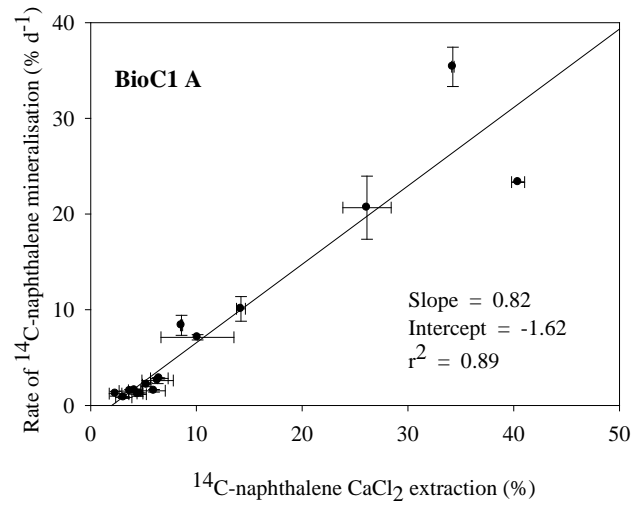
778

Time (d)

779 Figure 2 Mineralisation of ^{14}C -naphthalene in Myerscough soil amended with 0% (\circ),
 780 0.1% (∇), 0.5% (\square) and 1% (\diamond) of BioC1 and BioC2. Error bars represent standard
 781 error of mineralisation (SEM) of triplicate samples ($n = 3$).

782 Table 3 Mineralisation of ¹⁴C-naphthalene in Myerscough soil amended with 0%,
 783 0.1%, 0.5% and 1 % of biochar 1 and 2 ± standard error of mineralisation (SEM) of
 784 triplicate samples (*n* = 3)

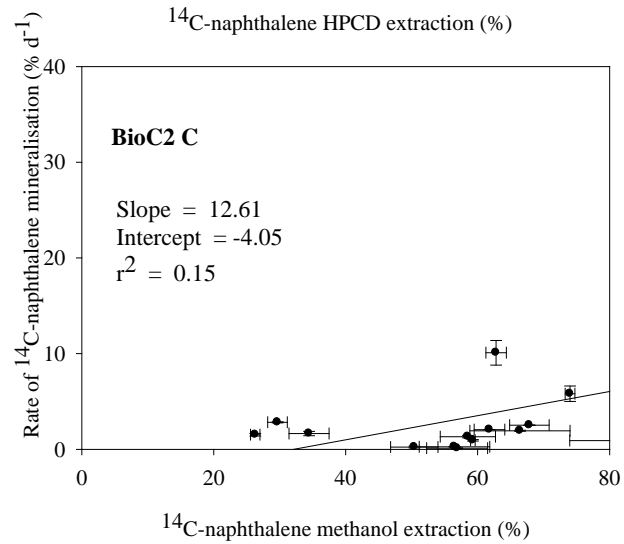
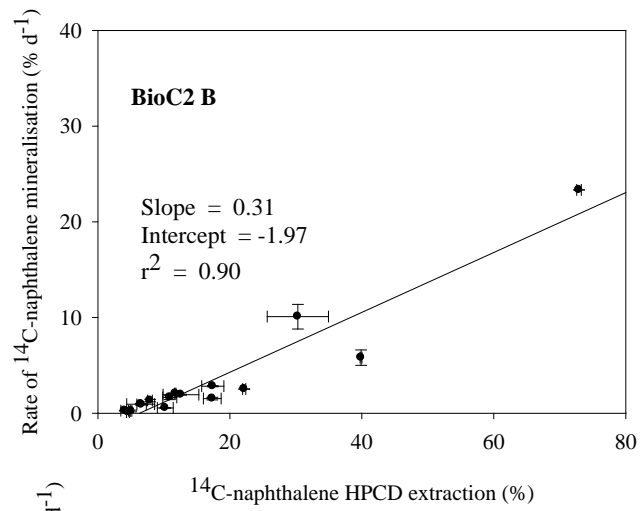
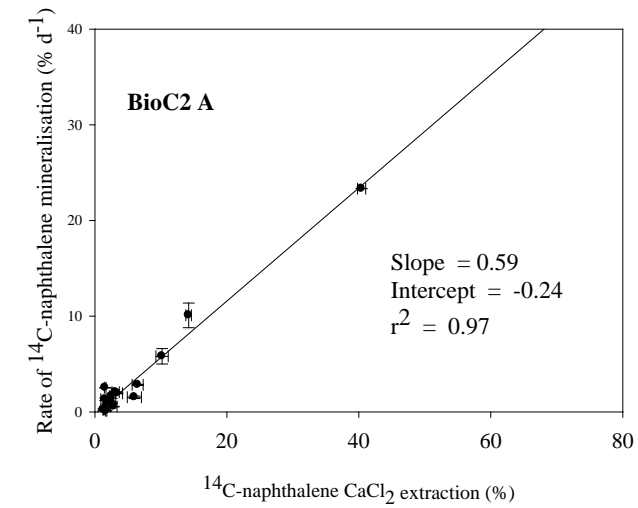
| Soil-PAH contact (d) | Biochar type | Amendment (%) | Lag phase (d) | Maximum rates (d ⁻¹) | Total extent | |
|----------------------|--------------|---------------|---------------|----------------------------------|--------------|--------------|
| 1 | BioC1 | 0 | 3.04 ± 0.01 | 23.33 ± 0.01 | 62.03 ± 1.15 | |
| | | 0.1 | 2.87 ± 0.19 | 35.38 ± 2.06 | 58.78 ± 3.06 | |
| | | 0.5 | 2.72 ± 0.28 | 20.66 ± 3.30 | 52.62 ± 6.38 | |
| | BioC2 | 1 | 2.77 ± 0.13 | 7.12 ± 0.28 | 29.04 ± 2.08 | |
| | | 0.1 | 3.51 ± 0.11 | 5.81 ± 0.81 | 25.04 ± 1.74 | |
| | | 0.5 | 4.65 ± 0.05 | 2.52 ± 0.03 | 17.34 ± 0.75 | |
| | | | 1 | 8.70 ± 1.04 | 1.32 ± 0.13 | 9.87 ± 1.16 |
| | 18 | BioC1 | 0 | 1.51 ± 0.08 | 10.09 ± 1.29 | 34.14 ± 2.00 |
| | | | 0.1 | 1.62 ± 0.08 | 8.37 ± 1.04 | 28.80 ± 3.14 |
| 0.5 | | | 5.25 ± 0.83 | 2.16 ± 0.20 | 12.45 ± 2.30 | |
| BioC2 | | 1 | 6.50 ± 0.91 | 1.57 ± 0.01 | 10.09 ± 1.27 | |
| | | 0.1 | 9.11 ± 0.58 | 2.06 ± 0.01 | 7.28 ± 0.12 | |
| | | 0.5 | 14.21 ± 0.33 | 0.55 ± 0.03 | 5.28 ± 0.03 | |
| | | | 1 | N/A | 0.27 ± 0.03 | 0.95 ± 0.23 |
| 36 | | BioC1 | 0 | 3.23 ± 0.01 | 2.82 ± 0.06 | 17.58 ± 1.62 |
| | | | 0.1 | 3.68 ± 0.68 | 2.59 ± 0.31 | 15.88 ± 2.10 |
| | 0.5 | | 5.82 ± 0.02 | 1.50 ± 0.17 | 10.84 ± 0.20 | |
| | BioC2 | 1 | N/A | 1.22 ± 0.25 | 5.81 ± 0.63 | |
| | | 0.1 | 4.63 ± 1.41 | 1.94 ± 0.05 | 11.89 ± 2.19 | |
| | | 0.5 | N/A | 0.91 ± 0.01 | 5.36 ± 0.39 | |
| | | | 1 | N/A | 0.12 ± 0.04 | 0.78 ± 0.11 |
| | 72 | BioC1 | 0 | 10.21 ± 2.85 | 1.53 ± 0.14 | 10.13 ± 2.08 |
| | | | 0.1 | 7.31 ± 0.30 | 1.24 ± 0.08 | 9.47 ± 0.78 |
| 0.5 | | | 9.71 ± 1.22 | 1.17 ± 0.21 | 7.94 ± 0.72 | |
| BioC2 | | 1 | N/A | 0.83 ± 0.10 | 5.20 ± 0.33 | |
| | | 0.1 | 8.97 ± 0.66 | 1.65 ± 0.23 | 7.32 ± 0.25 | |
| | | 0.5 | N/A | 0.94 ± 0.08 | 4.62 ± 0.70 | |
| | | | 1 | N/A | 0.23 ± 0.09 | 1.40 ± 0.22 |



785

786 Figure 3 Correlation between maximum rate ^{14}C -naphthalene mineralised and ^{14}C -
 787 naphthalene extracted with (A) CaCl_2 (B) HPCD (C) methanol after 24 h with BioC1
 788 amendment.

789

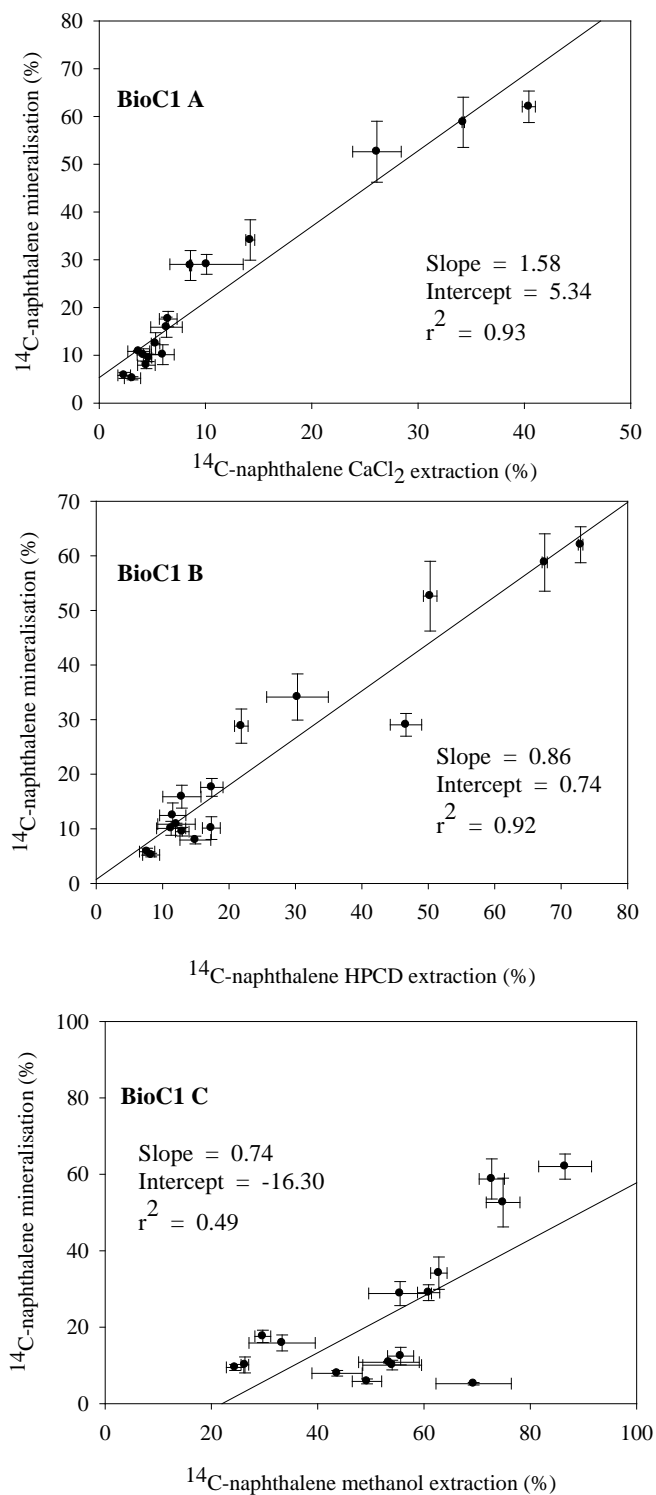


790

791 Figure 4 Correlation between maximum rate ^{14}C -naphthalene mineralised and ^{14}C -
 792 naphthalene extracted with (A) CaCl_2 (B) HPCD (C) methanol after 24 h with BioC2
 793 amendment.

794

795



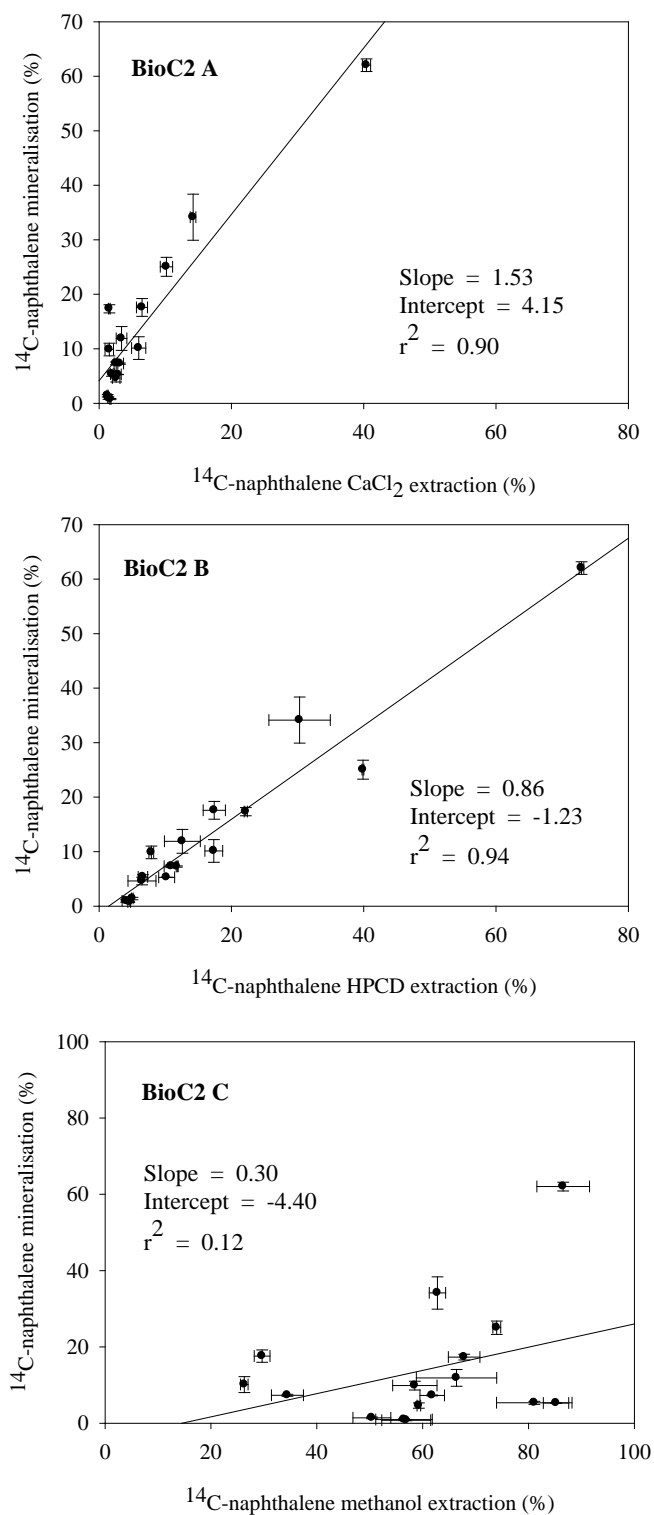
797

798 Figure 5 Correlation between extent of ^{14}C -naphthalene mineralised and ^{14}C -
 799 naphthalene extracted with (A) CaCl_2 (B) HPCD (C) methanol after 24 h with BioC1
 800 amendment.

801

802

803



804

805 Figure 6 Correlation between extent of ¹⁴C-naphthalene mineralised and ¹⁴C-
806 naphthalene extracted with (A) CaCl₂ (B) HPCD (C) methanol after 24 h with BioC2
807 amendment.