

Accepted Manuscript

Optimisation of XAD extraction methodology for the assessment of biodegradation potential of ^{14}C -phenanthrene in soil

Bilqees M. Adedigba, Uchenna O. Ogbonnaya, Gabriela M. Vázquez-Cuevas, Kirk T. Semple



PII: S2352-1864(17)30245-6
DOI: <https://doi.org/10.1016/j.eti.2017.11.011>
Reference: ETI 177

To appear in: *Environmental Technology & Innovation*

Received date: 26 July 2017
Revised date: 26 October 2017
Accepted date: 19 November 2017

Please cite this article as: Adedigba B.M., Ogbonnaya U.O., Vázquez-Cuevas G.M., Semple K.T., Optimisation of XAD extraction methodology for the assessment of biodegradation potential of ^{14}C -phenanthrene in soil. *Environmental Technology & Innovation* (2017), <https://doi.org/10.1016/j.eti.2017.11.011>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 Optimisation of XAD extraction methodology for the assessment of biodegradation potential
2 of ^{14}C -phenanthrene in soil

3

4 Bilqees M. Adedigba¹, Uchenna O. Ogbonnaya^{1,2}, Gabriela M. Vázquez-Cuevas¹ and Kirk T.
5 Semple^{1*}

6

7

8 ¹Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom, LA1
9 4YQ

10

11 ²Institute of Bioresource and Agriculture, Hong Kong Baptist University, Hong Kong.

12

13

14

15

16

17 *Corresponding author: k.semple@lancaster.ac.uk

18

19 **Abstract**

20 This study investigated the use of a hydrophobic resin, amberlite XAD, as a tool for assessing
21 the biodegradation potential of ^{14}C -phenanthrene in soil. The method was optimised in terms
22 of soil/XAD ratio, shaking, extraction time and eluting solvent. The most effective method
23 was then tested on selected XADs, and the performance compared with cyclodextrin (HP- β -
24 CD) and dichloromethane (DCM) extractions suitability to predict phenanthrene
25 biodegradation in soil over 100 d. Results showed that the optimum conditions for the XAD
26 extraction technique are a 2:1 soil/XAD ratio, 100 rpm mixing for 22 h and elution using a
27 DCM:methanol solution (1:1). Mineralisation of ^{14}C -phenanthrene was accurately predicted
28 by HP- β -CD ($r^2 = 0.990$, slope = 0.953, intercept = 1.374) and XAD-4 extractions ($r^2 =$
29 0.989, slope = 0.820, intercept = 6.567), while DCM overestimated the bioaccessibility of
30 ^{14}C -phenanthrene ($r^2 = 0.999$, slope = 1.328, intercept = -49.507). This investigation showed
31 that XAD extraction can be considered a suitable non-exhaustive technique for estimating
32 biodegradability of phenanthrene in soil.

33

34 Keywords: Hydrophobic resins, XAD, HP- β -CD, bioaccessibility

35

36

37 1. Introduction

38 The need to measure the bioaccessibility of anthropogenic chemicals is fast becoming an
39 important step in the risk assessment of contaminated soil and sediment. Bioaccessibility
40 refers to the fraction of contaminant “as that which is available to cross an organism’s
41 cellular membrane from the environment, if the organism has access to the chemical.
42 However, the chemical may be either physically removed from the organism or only
43 bioavailable after a period of time” (Semple et al., 2004). This has also been referred to as the
44 labile and rapidly desorbing fractions (Semple et al., 2007; Ridding et al., 2013; Ortega-Calvo
45 et al., 2015) and has also found an application in assessing the biodegradation potential of
46 hydrocarbons in soil (Semple et al., 2006; 2007), where a number of methods have been
47 tested for their ability to predict contaminant accessibility in soil, including Soxhlet
48 extraction using organic solvents (Kelsey and Alexander, 1997), mild solvents extraction
49 (Kelsey et al., 1997; Reid et al., 2000a; Liste and Alexander, 2002), persulfate oxidation
50 (Cuypers et al., 1999; Dandie et al., 2010), surfactant extraction and solid-phase extraction
51 (Cornelissen et al., 1998; van der Wal et al., 2004). Others include aqueous based extraction
52 using hydropropyl- β -cyclodextrin (HP- β -CD) (Reid et al., 2000b; Papadopoulos et al., 2007;
53 Rhodes et al., 2008) and sub-critical water extraction (Hawthorne et al., 2002; Latawiec et al.,
54 2008). Aqueous based HP- β -CD extraction has received significant attention in the literature
55 and has been found to accurately predict the accessibility of polycyclic aromatic
56 hydrocarbons (PAHs) to microorganisms across a wide range of soil types and conditions
57 (Cuypers et al., 2002; Reid et al., 2000b; Doick et al., 2006; Hickman et al., 2008; Rhodes et
58 al., 2008; 2012; Bernhardt et al., 2013; Yu et al., 2016).

59 Amberlite extra adsorbent material (XAD) is a macrospherical styrene-divinyl non-ionic
60 resin with numerous macropores and large surface area, exhibiting a high affinity to sorb
61 organic compounds that are either dissolved in pore-water or readily desorbable from soil

62 particles (Tan, 2014). The adsorptive capacity of this resin has been proposed to be
63 potentially applicable as a non-exhaustive extraction to estimate the bioaccessibility of
64 organic contaminants in soil and sediment (Puglisi et al., 2003; Lei et al., 2004; Vázquez-
65 Cuevas and Semple, 2016). The hydrophobic nature of the sorption sites located throughout
66 XAD macro spherical structure, represent a chemically attractive sink in which organic
67 contaminants can partition (Lamoureux and Brownawell, 2004). This property has been
68 harnessed to facilitate the detection of trace contaminants concentration in different
69 environmental matrices including pore water (Care et al., 1982; Burnham et al., 1972) and air
70 sampling (Chuang et al., 1999; Wania et al., 2003; Araki et al., 2009). In addition, it has been
71 used to study the desorption and partition behaviour of PAHs (Northcott and Jones, 2000)
72 and polychlorinated biphenyls (PCBs) (Carroll et al., 1994) in soil and sediment,
73 respectively. XAD extraction process can best be described as a passive sampler, where the
74 sorbed HOCs can be eluted with an appropriate organic solvent and then measured
75 appropriately (Wania et al., 2003). Previous reports have described bioaccessibility
76 measurements of HOCs in soils and sediments (Lei et al., 2004; Patterson et al., 2004; Doick
77 et al., 2005; Simpson et al., 2006; Rhodes et al., 2008; 2012), albeit with mixed success.

78 The major factors affecting XAD sorption are thought to include physical and chemical
79 properties of the matrix including pH, temperature, amount and type of humic materials, flow
80 rates and resin sorption capacity (Daignault et al., 1988). However, it is not clear how
81 different physical parameters such as surface area and the resin pore size, might affect the
82 extraction of organic contaminants onto XAD from complex matrices such as soil. In this
83 present study, XAD extraction was optimised in terms of the soil/XAD ratio, the degree of
84 mixing, the choice of eluting solvent and the duration of extraction. Therefore, the aim of this
85 study was to develop an optimised methodology for the use of XAD extractions as a
86 predictor of the bioaccessible fraction of ^{14}C -phenanthrene. More specifically, the duration of

87 extraction, which corresponds to the time required for the desorption/adsorption process to
88 achieve equilibrium. Further, the influence of XAD physical properties on the sorption
89 capacity was tested using four polymeric types of the resin. Finally, the optimised method
90 was validated and compared with DCM and HP- β -CD extractions and the ability to predict
91 the mineralisation of ^{14}C -phenanthrene.

92

93 **2. Materials and methods**

94 **2.1 Materials**

95 ^{12}C -Phenanthrene was obtained from Sigma Aldrich Co, Ltd., UK. ^{9-14}C -Phenanthrene was
96 purchased from American Radiochemical Company, (ARC- USA). Acetone, hexane, and
97 methanol were obtained from Fischer Scientific UK. Dichloromethane (DCM) and toluene
98 were obtained from Rathburn Chemicals and Merck UK, respectively. XAD-2, XAD-4,
99 XAD-16 and XAD-1180N were obtained from Sigma Aldrich and they differed in physic-
100 chemical properties (Table 1), UK and HP- β -CD from Acros Organics UK. Sample oxidiser
101 cocktails (Carbon-Trap and Carbon-Count); Combust-aid; and the Ultima Gold scintillation
102 cocktails were obtained from Perkin Elmer, UK.

103

104 **2.2 Soil preparation**

105 Uncontaminated soil (organic matter = 2.1; pH = 6.5, clay =19.5%, silt = 20%, sand = 60%)
106 was collected from A horizon (0 - 15 cm) at Myerscough College, Preston, U.K. Field moist
107 soil was air-dried and passed through a 2.308 mm sieve to remove stones and other plant
108 debris. Standard for soil spiking with $^{12/14}\text{C}$ -phenanthrene was prepared in acetone to deliver
109 a final concentration of 10 mg kg $^{-1}$ and an activity of 83.3 Bq g $^{-1}$. Soil spiking was performed
110 following a single-step spiking/rehydration technique described by Doick et al. (2003). Soil

111 was stored in amber glass jars and incubated at 21 ± 1 °C in a controlled temperature room
112 until needed.

113

114 **2.3 Determination of total ^{14}C -phenanthrene in soil**

115 The total amount of ^{14}C -phenanthrene activity in soil was determined by combustion
116 (Packard 307 Sample oxidiser) at each sampling period (0, 25, 50 100 d). Soil samples (1 g
117 dw soil; $n = 3$) were weighed into cellulose combustion cones and combusted for three
118 minutes and facilitated by the addition of 200 μl of Combust-aid. $^{14}\text{CO}_2$ released from the
119 combustion was trapped by Carbon-Trap (10 mL) and Carbon-Count (10 mL). Sample
120 oxidiser trapping efficiency was determined before combustion and found to be greater than
121 92 % in all cases. The $^{14}\text{CO}_2$ in the scintillant was quantified by liquid scintillation counting
122 (10 min, LSC- Canberra Packard Tri Carb 2300 TR, UK) after 24 h storage in the dark to
123 correct for chemoluminescence.

124

125 **2.4 Optimisation of extraction parameters**

126 **2.4.1 Soil/XAD ratio and degree of mixing**

127 Soils (2 g dw \pm 0.2; $n = 3$) were weighed into 50 mL Teflon centrifuge tubes. Different
128 amounts (0.2, 0.5, 1 and 2 g) of damp XAD-2 were added to the soils and extracted with a
129 solution of 0.01 M CaCl_2 for 24 h onto an orbital shaker (100 rpm). Following 45 minutes
130 centrifugation at 4000 g , XAD was separated from the slurry and quantitatively removed by
131 decantation through a 50 mL glass funnel plucked with glass wool. The resin was allowed to
132 dry in a fume cabinet and transferred to 40 mL Wheaton glass vials and mixed with 15 mL of
133 DCM:methanol (1:1). Following a 5 min sonication, the vials were transferred to an orbital

134 shaker at 100 rpm to elute the adsorbed chemical from XAD. An aliquot of eluate was
135 sampled into 15 mL Ultima Gold liquid scintillation cocktail and analysed by LSC as
136 previously described.

137 Optimisation of the degree of mixing soil extractions (2 ± 0.25 g dw; $n = 3$) using $1 \text{ g} \pm 0.05$
138 XAD-2 were carried out. Sets of triplicates were placed onto an orbital shaker at 20, 40, 60,
139 80, 100 or 120 rpm for 24 h and processed as described above.

140

141 2.4.2 Elution solvent

142 Extraction was performed as described previously (Section 2.4.1). Triplicate samples of the
143 recovered XAD were eluted with 15 mL of either DCM:methanol (1:1), hexane:acetone
144 (1:1), hexane:acetone (3:1), or toluene:methanol (1:1) as recommended by Northcott and
145 Jones (2000).

146

147 2.4.3 Optimisation of extraction time for selected hydrocarbons

148 The optimisation of the extraction time was performed to ensure system equilibration before
149 termination of extraction process. XAD extractions were performed as described earlier
150 (Section 2.4.1), except that triplicate samples were removed for further analysis at 3, 6, 12,
151 24, 36, 48 or 96 h shaking.

152

153 **2.5 Comparing the adsorption capacities of selected XADs**

154 The physical properties of the selected XAD are presented on Table 1. Spiked soils ($n = 3$)
155 were aged in sealed amber glass jars for 100 d. After 0, 25, 50 and 100 d, samples were
156 removed for XAD extraction. The extraction, recovery and elution of the sorbed chemical

157 were performed based on the results from the optimisation procedure (Section 3.1).

158 Quantification of the ^{14}C -phenanthrene was done by LSC as described previously.

159

160 **2.6 Chemical extractability of ^{14}C -activity from soil**

161 2.6.1 DCM-shake extraction

162 ^{14}C -Phenanthrene was exhaustively extracted from the soil using DCM as a benchmark for
163 individual XAD and HP- β -CD extractions. Soils (1.5 ± 0.05 g dw, $n = 3$) were ground to a
164 fine powder with the addition of equal mass of sodium sulphate as described by
165 Papadopoulos et al. (2007). Ground samples were transferred to 50 mL polycarbonate
166 centrifuge tubes and extracted with 20 mL DCM by mixing onto an orbital shaker at 100 rpm
167 in a controlled temperature room (21 ± 1 °C) for 20 h. An aliquot of eluate was sampled into
168 15 mL Ultima Gold liquid scintillation cocktail and analysed by LSC as previously described.

169

170 2.6.2 Hydroxypropyl- β -cyclodextrin (HP- β -CD) extraction

171 HP- β -CD extractability was determined by aqueous based cyclodextrin extraction following
172 the protocol developed by Reid et al. (2000a). In short, 25 mL of a 50 mM HP- β -CD solution
173 was added to a 50 mL Teflon centrifuge tubes ($n = 3$) containing 1.25 ± 0.05 g (dw) soil
174 (20:1 solution to soil ratio). Tubes were then horizontally placed onto an orbital shaker at 100
175 rpm and mixed for 22 h. Phase separation was achieved by a 60 min centrifugation at $4000 \times$
176 g. An aliquot of the supernatant (5 mL) was mixed with liquid scintillation cocktail and
177 quantified by LSC as previously described.

178

179 2.7 Mineralisation assay

180 Mineralisation of ^{14}C -phenanthrene was assessed over time through a respirometry assay
181 using modified 250 mL Schott bottles (Reid et al., 2001; Semple et al., 2006). Respirometry
182 was performed following the addition of a phenanthrene degrading bacterial inoculum
183 (*Pseudomonas* sp.). Soils (10 g dw, $n = 3$) were placed into the respirometers and mixed with
184 inorganic mineral basal salts solution (MBS) to form a soil:liquid ratio of 1:3 (Doick and
185 Semple, 2003). The bacterial inoculum (*Pseudomonas* sp.), previously isolated from a
186 petroleum contaminated soil capable of utilising phenanthrene as the sole carbon source for
187 growth, was cultured at 0.1 g l⁻¹ phenanthrene in 300 ml sterile minimal basal salt (MBS)
188 solution at 20 °C and at 100 rpm on an orbital shaker (Ogbonnaya et al., 2014). The bacterial
189 inoculum (*Pseudomonas* sp. - 5 ml; ca. 10.28×10^7 - 12.36×10^7 cell g⁻¹ dw) was prepared
190 following the procedure detailed by Ogbonnaya et al. (2014) and added to the respirometers.
191 Bottles were placed onto an orbital shaker (100 rpm) and incubated in a controlled
192 environment room at 21 ± 1 °C. The evolution of $^{14}\text{CO}_2$ following mineralisation was
193 assessed by using a suspended scintillation glass vial containing 1 M NaOH (1 ml) solution.
194 Sampling of the trapped $^{14}\text{CO}_2$ was performed by the periodic replacement of the trap over 14
195 days. Incubated traps were mixed with 5 mL liquid scintillation cocktail and analysed by
196 LSC as previously described.

197

198 2.8 Data presentation and statistical analysis

199 Following blank correction, statistical analyses of data were conducted by ANOVA - post
200 hoc Tukey test, and comparison of relationships between groups was performed using the
201 student's t-test or paired t test, where appropriate (SigmaStat 3.5). Statistical significance of
202 data was assumed at 95% confidence interval ($p < 0.05$). Graphical representation of the data
203 and linear regressions were plotted on SigmaPlot (SPSS version 12.0 for Windows).

204

205 3. Results

206 3.1 Optimisation of extraction conditions

207 3.1.1 Loss of ^{14}C -phenanthrene associated activity in soil

208 Following increases in contact time (0, 25, 50 and 100 d), the total amount of ^{14}C -
209 phenanthrene-associated activity decreased from approximately 100% to a mean of 34%. The
210 significant loss ($p < 0.01$) was recorded between 0 and 25 days contact time, after which
211 there was no further loss ($p > 0.05$) of ^{14}C -phenanthrene activity of the soils during
212 incubation.

213

214 3.1.2 Soil/XAD ratio

215 The total amount of soil associated ^{14}C -phenanthrene extracted by XAD-2 negligibly
216 increased with increasing soil/XAD ratio up to 1 g (Table 2). Although maximum ^{14}C -
217 phenanthrene extractability was achieved using soil/XAD ratio 2:1 (20.73 %), there was no
218 statistical significant difference among ratios ($p > 0.05$). There was a small decrease in the
219 total extractability moving from 2:1 to 1:1, which can be explained by the difficulty in
220 handling 2 g of XAD-2 (20-60 mesh). From this observation, soil/XAD (2:1) was selected for
221 optimum performance. Further analysis of the supernatant for residual ^{14}C -phenanthrene
222 showed that the desorbed chemical was effectively trapped by the XAD, with total ^{14}C -
223 phenanthrene in the supernatant been negligible ($< 1\%$) (Table 2). In addition, residual soil
224 associated ^{14}C -phenanthrene determined by sample oxidation following the removal of the
225 labile fraction showed a mass balance of between 97 % - 103 %, indicating that all the
226 contaminant introduced can be accounted for.

227

228 3.1.3 Degree of mixing

229 The mean total ^{14}C -phenanthrene extractability assessed using 0, 20, 40, 80, 100 and 120 rpm
230 was 22.29 %, 50.33 %, 57.47 %, 68.21 %, 75.08 %, 74.87 %, respectively (Figure 1). ^{14}C -
231 phenanthrene extractability significantly ($p < 0.05$) increased with increasing shaking up to
232 100 rpm. However, ^{14}C -phenanthrene extractability amongst 80, 100, and 120 rpm appeared
233 to be statistically similar ($p > 0.05$). Nonetheless, maximum extractability was obtained using
234 100 rpm (75.08 %). The slight decline in total extractability by increasing the shaking from
235 100 to 120 rpm may be due to increased agitation in the system, which might possibly affect
236 the sorption process. From this observation, a standard agitation of 100 rpm was employed
237 for the rest of this study.

238

239 3.1.4 Eluting solvent

240 Total ^{14}C -phenanthrene recovered from XAD using DCM:methanol (1:1), toluene:methanol
241 (1:1), hexane:acetone (1:1), and hexane:acetone (3:1) were 95.61 ± 5.3 %, 88.45 ± 3.2 %,
242 106.02 ± 4.8 % and 85.54 ± 3.7 %, respectively (Figure 2). The lowest recovery was obtained
243 by using hexane:acetone (3:1), while the maximum recovery (106.02%) was observed with
244 hexane:acetone (1:1). These two solutions were observed to promote the formation of phases
245 in combination with the soil and the resins and therefore not considered as suitable eluting
246 solvents. No significant difference was found between the fraction eluted using
247 DCM:methanol and toluene:methanol ($p > 0.05$). DCM:methanol produced a better recovery
248 (95.61 %) and had less than 2 % residual ^{14}C -phenanthrene detected in the aqueous phase,
249 hence the choice of DCM:methanol (1:1) for the remaining part of this study.

250

251 3.1.5 Extraction time profiles for ^{14}C -phenanthrene

252 To determine the optimum duration for extraction, the kinetics of extractions were
253 investigated. Extraction was performed for 3, 6, 12, 24, 48, 72 or 96 h (Figure 3). The ^{14}C -
254 phenanthrene ($K_{oc} = 4.15$) showed 90 % extractability after 24 h shaking showing
255 equilibration of the desorption/adsorption process in the system. There were no significant
256 differences in extractability at successive time points ($p > 0.05$). Based on these results, the
257 optimum extraction time for ^{14}C -phenanthrene was defined as 22 h.

258

259 **3.2 Comparison of adsorption capacities of for different XAD resins with increased soil-** 260 **phenanthrene contact time.**

261

262 Total ^{14}C -phenanthrene extractability was compared amongst four hydrophobic resins (XAD-
263 2, XAD-4, XAD-16 and XAD-1180N, Table 1), after up to 100 d soil-phenanthrene contact
264 time. Prior to experimental setup, elution efficiencies of DCM:methanol (1:1) on various
265 XADs were determined and found to be approximately 85 %, 95 %, 87 % and 92 %,
266 respectively. Thus, these recovery efficiencies were factored into the final calculations. The
267 amounts of ^{14}C -phenanthrene extracted from freshly spiked soil (0 d) and subsequent ageing
268 times are presented on Table 3. Following increasing soil:phenanthrene contact time, the total
269 ^{14}C -phenanthrene extractability declined significantly ($p < 0.05$) across the four investigated
270 XADs (Table 3). While there were significant differences ($p < 0.05$) between extractabilities
271 after 25, 50, and 100 d in comparison to extractability at 0 d, there was however no
272 significant difference ($p > 0.05$) in the total extractability by the four XADs at each time
273 point. This showed that the sorption capacity of the four XADs was not affected by the

274 differences in the resins pore size and surface area under the conditions by which the present
275 study was conducted.

276

277 **3.3 Chemical extractability of ¹⁴C-phenanthrene and its prediction of bioaccessibility** 278 **with increased soil-PAH contact time**

279 The extractability of ¹⁴C-phenanthrene by DCM, HP-β-CD and XAD-4 was tested at each
280 soil-phenanthrene contact time (0, 25, 50, 100 d). In freshly spiked soil, no significant
281 differences were observed between the amount of ¹⁴C-phenanthrene extracted from the soil
282 by HP-β-CD, DCM and the amount quantified through XAD-4 extractability ($p > 0.05$).

283 Increasing soil-phenanthrene contact time (25, 50 and 100 d) led to a consistent decrease in
284 the amount of ¹⁴C-phenanthrene extraction. However, the extracted ¹⁴C-phenanthrene varied
285 with chemical extractant (Table 3). There was insignificant difference between total ¹⁴C-
286 phenanthrene extracted by individual extractants (DCM, HP-β-CD, XAD-4) after 50 and 100
287 d ($p > 0.05$). However, when the amount of ¹⁴C-phenanthrene extracted by each chemical
288 extractant was compared, it was observed that DCM extracted larger amounts of ¹⁴C-
289 phenanthrene when compared against either HP-β-CD or XAD-4 after increasing contact
290 times (25, 50, 100 d).

291

292 **3.4 Correlation between chemical extraction and extent of ¹⁴C-phenanthrene** 293 **mineralisation with increased soil-PAH contact time**

294 The mineralisation of ¹⁴C-phenanthrene was monitored over 14 days at each contact time (0,
295 25, 50 and 100 d). It was observed that the extent of mineralisation significantly decreased
296 with increasing soil:phenanthrene contact time. The total amount of ¹⁴C-phenanthrene
297 mineralised after spiking (0 d contact time) was significantly higher ($p < 0.05$) when

298 compared to other time points (25, 50, 100 d) (Table 3). Similarly, the amount mineralised
299 after 25 d contact time was significantly higher ($p < 0.05$) than subsequent time points.

300 In order to test the ability of chemical extractions to predict the extent of ^{14}C -phenanthrene
301 mineralisation, individual extractions were compared against the extent of ^{14}C -phenanthrene
302 mineralisation through linear regression (Figure 4). Linear correlation between ^{14}C -
303 phenanthrene mineralisation and DCM-shake extraction across the four contact time points
304 produced a good regression ($r^2 = 0.999$). However, the slope (1.328) and intercept (-49.51)
305 showed that DCM-shake extraction as employed in the current study overestimated
306 bioaccessibility and hence biodegradability of phenanthrene in soil. On the contrary, linear
307 correlation between ^{14}C -phenanthrene mineralisation and HP- β -CD extractions across the
308 four time points produced a good regression was observed to present a close 1:1 relationship
309 ($r^2 = 0.990$, slope = 0.953, intercept = 1.374). Similar trends were also observed when
310 comparing the amount of ^{14}C -phenanthrene extracted from XAD-4 ($r^2 = 0.989$, slope = 0.820,
311 intercept = 6.567).

312 4. Discussion

313 4.1 Optimisation of XAD extraction

314 Prior extraction regime at each contact time (0, 25, 50, 100 d), the remaining ^{14}C -
315 phenanthrene associated activity was determined through soil sample oxidation and the
316 results showed that there was initial significant loss of activity. The initial loss of ^{14}C -
317 phenanthrene activity between 0 and 25 days contact time of incubation was apparently
318 mainly due to microbial degradation and to a minimal extent, volatilisation (Allan et al.,
319 2006; Ogbonnaya et al., 2016). After the 25 days contact time, there was apparently no
320 further loss of ^{14}C -phenanthrene activity through microbial degradation due to unavailability
321 of the contaminant to biodegradation.

322 The condition for optimum XAD extractability of phenanthrene established in the present
323 study indicated that soil:XAD (2:1), extracted for >20 h at 100 rpm using DCM:methanol
324 (1:1) as the eluting solvent was adequate for predicting bioaccessibility of phenanthrene to
325 catabolically active microbial inoculum. Previous studies have been conducted using
326 different conditions. For instance, Patterson et al. (2004) employed 20 h extraction with
327 soil:XAD-4 (2.5:1) and sequential elution first with 10 mL DCM, then 4.95 mL of butanol
328 then 4.95 mL of acetonitrile. The authors reported mixed results, with XAD-4 extraction
329 accurately predicting the biodegradation of naphthalene in one out of four different soils
330 (Patterson et al., 2004). One of the reasons for this behaviour was suggested to be the
331 formation of a coating around XAD grains with fine soil particles (Patterson et al., 2004).
332 However, another reason for the inconsistent performance of the XAD as used in that study
333 could be due to the incorporation of a buffering step by Patterson et al. (2004); where K_2CO_3
334 was added to promote flocculation, thus facilitating the removal of XAD from the soil slurry.
335 In a preliminary study carried out in the present investigation, K_2CO_3 was shown to
336 negatively influence the adsorption process as it enhanced desorption of the sorbed chemical
337 on the XAD resin back into the slurry and promoted the extraction of dissolved organic
338 matter (DOM) from soil (Patterson et al., 2004). These, together with the coating of the XAD
339 surface reported by the authors, might account for the results obtained in that study (Patterson
340 et al., 2004).

341 In another study, Simpson et al. (2006) reported that a 6 h extraction with sediment:XAD-2
342 (1:1) was a poor predictor of the bioavailability of a mixture of low and high molecular
343 weight PAHs when compared against the bioaccumulation of these compounds by the
344 benthic bivalve *Tellina deltaidalis*. Although XAD-2 was observed to overestimate
345 bioaccumulation of most low molecular weight (LMW) PAHs to the sediment organism,
346 Simpson et al. (2006) attributed this behaviour to the toxicity of the hydrocarbons which

347 resulted in the reduction of soil ingestion by the organisms, as well as increasing their
348 mortality. In contrast, Lei et al. (2004) reported that among different sediment:XAD-2 ratios
349 and mixing times, a one week extraction procedure with a 1:1 sediment:XAD-2 ratio was
350 sufficient to predict the microbial degradation of LMW PAHs (2-4 aromatic rings). However,
351 XAD-2 extractions overestimated the biodegradability of higher molecular weight PAHs
352 such as benzo[a]anthracene, chrysene, benzo[b,k]fluoranthene and benzo[a]pyrene (Lei et al.,
353 2004). The current study only examined the applicability of XAD-2, which has a larger pore
354 diameter (100 Å) than XAD-4 (100 Å), capable of accommodating significant amounts of
355 PAHs relative to the biodegradable portions. Hence, this current study showed that the
356 effectiveness of applying XAD as a chemical extractant in soil to predict bioaccessibility is
357 inherently governed by the physic-chemical properties of the adsorbent resin (Juan-Peiro et
358 al., 2012). Furthermore, this study brings to light the plausible applicability of utilising XAD-
359 4 as a preferable mild chemical extractant compared to other XAD resins. Considering the
360 extraction parameters reported by previous authors, the methodology in this current study
361 produced valuable results. The soil:XAD (2:1) for 22 h to predict bioaccessibility of
362 phenanthrene to catabolically active microbial inoculum represents an advancement of
363 previous methodologies, based on the soil:XAD ratio and extraction timing perspectives. The
364 22 h time period was enough to measure the rapidly desorbable portion of ¹⁴C-phenanthrene
365 to optimally locate and sorb onto the accessible XAD adsorption sites. Measuring labile and
366 rapidly desorbable fractions of organic contaminants in soil has been well defined as that
367 which is bioavailable and potentially bioavailable for microbial degradation and represents a
368 sensible descriptor for the biodegradation endpoint of the contaminant in soil (Semple et al.,
369 2004; 2007; Ogbonnaya and Semple, 2013; Ortega-Calvo et al., 2015). The 22 h XAD-4
370 extraction correlated strongly with the already established time duration for extracting low
371 molecular weight (LMW) PAHs using HP-β-CD in soil.

372

373 **4.2 Comparative prediction of ¹⁴C-phenanthrene biodegradability in soil**

374 The mineralisation assay is used to describe the amount of a contaminant that can be
375 degraded by microorganisms in soil (Semple et al., 2006). Given the conditions of the assay,
376 it is the bioaccessible fraction of ¹⁴C-phenanthrene within the soil is mineralised to ¹⁴CO₂,
377 hence, the non-mineralised fraction represent the non-bioaccessible fraction of ¹⁴C-
378 phenanthrene to the degrading inoculum (Semple et al., 2006; Ogbonnaya et al., 2014).
379 Semple et al. (2006) showed that after mineralisation had ended in a respirometry assay set-
380 up that the addition of more phenanthrene degraders did not result in significant increase in
381 mineralisation, in that the initial degraders had utilised the bioaccessible fraction.

382

383 Both the HP-β-CD and the XAD-4 extraction accurately represented the mineralisation of
384 ¹⁴C-phenanthrene while the DCM-shake extraction was observed to overestimate it. This
385 result is in line with previous studies on the prediction of microbial biodegradation of
386 phenanthrene using HP-β-CD shake extractions reported by different authors (Reid et al.,
387 2000b; Swindell and Reid, 2006; Rhodes et al., 2008; Hickman et al., 2008; Rhodes et al.,
388 2012; Ogbonnaya et al., 2014), where close 1:1 linear relationships were typically observed.
389 Single HP-β-CD extraction (20-22 h) successfully removes a combination of the rapidly
390 desorbing and aqueously soluble (labile) fractions of LMW PAHs in soils by forming
391 inclusion complexes within the hydrophobic cavity of HP-β-CD. This measurement can be
392 valuable in predicting biodegradation endpoints in the assessment of contaminated land
393 (Rhodes et al., 2012; Sanchez et al., 2013; Ortega-Calvo et al., 2015; Ogbonnaya et al.,
394 2016). However, prediction of bioaccessibility has been less successful in larger soil
395 organisms, such as earthworms, high molecular weight (HMW) PAHs contaminated soils and
396 soils with relatively high amounts of black carbon (Hickman and Reid, 2005; Barther and

397 Pelletier, 2007; Papadopoulos et al., 2007; Rhodes et al., 2012; Cui et al., 2013; Oyelami et
398 al., 2014). Thus, identifying a supporting technique that can overcome these barriers with
399 similar efficiency would be valuable for the assessment of contaminated land.

400 HP- β -CD works as a complexing agent, whilst XAD works as a mild chemical adsorbent
401 (Cui et al., 2013). The application of XAD extraction for predicting phenanthrene
402 bioaccessibility in soil has not been reported before, though other studies have reported
403 similar trends. Lei et al. (2004) observed the capacity of XAD-2 extraction to predict 2-, 3-,
404 and 4-ring hydrocarbons in sediments, thereby supporting the present findings. Similarly,
405 data from an study carried by Xia et al. (2010) allowed the authors to conclude that residual
406 amounts of ^{14}C -phenanthrene in soil after XAD-2 assisted desorption and biodegradation
407 were similar. Although not considered within the objectives of Xia et al. (2010), these results
408 support the observed in the present investigation suggesting that it is possible to quantify
409 similar amounts of ^{14}C -phenanthrene through XAD assisted extractions and microbial
410 biodegradation. Moreover, other XAD has also been reported to accurately represent the
411 fraction of *p,p'*-dichlorodiphenyl dichloroethylene (*p,p'*-DDE), a by-product of the
412 organochlorine pesticide DDT, available to earthworms (Škulcová et al., 2016). Further,
413 Škulcová et al. (2016) also reported that XAD consistently produced a closer relationship
414 with the bioaccessible fraction than predictors such as HP- β -CD.

415 **5. Conclusions**

416 The optimum conditions for the XAD extraction technique are a 2:1 soil/XAD ration, 100
417 rpm mixing for 22 h and elution using a DCM:methanol solution (1:1). Furthermore, this
418 methodology was highly comparable with HP- β -CD extraction technique in terms of its
419 accuracy predicting the microbial degradation of aromatic hydrocarbons in soil. Therefore, it
420 can be concluded that XAD extraction closely mimics the mass transfer mechanisms that

421 govern the bioaccessibility of hydrophobic organic contaminants in soil and sediment.
422 Further testing and validation is however required to establish its place as a reliable tool for
423 assessing biodegradation potential of a wide range of soil and sediment-bound organic
424 contaminants, and hence its application in the assessment of contaminated land
425 bioremediation potential as well as in the risk assessment of non-polar organic chemicals.

426 **Acknowledgment**

427 The authors would like to thanks to the UK Department for International Development
428 (DFID) for funding this project through a doctoral scholarship administered by the
429 Association of Commonwealth Universities (ACU). GVC would like to thank the Mexican
430 National Council for Science and Technology (CONACyT) for their financial support
431 through a scholarship (No. 313807) to pursue postgraduate studies at the Lancaster
432 Environment Centre, Lancaster University.

433

434 **References**

- 435 Allan, I. J., Semple, K. T., Hare, R. & Reid, B. J. 2006. Prediction of mono- and polycyclic
436 aromatic hydrocarbon degradation in spiked soils using cyclodextrin extraction.
437 *Environmental Pollution*, 144, 562–571.
- 438 Araki, Y., Tang, N., Ohno, M., Kameda, T., Toriba, A. & Hayakawa, K. 2009. Analysis of
439 Atmospheric Polycyclic Aromatic Hydrocarbons and Nitropolycyclic Aromatic
440 Hydrocarbons in Gas/Particle Phases Separately Collected by a High-Volume Air
441 Sampler Equipped with a Column Packed with Xad-4 Resin. *Journal of Health
442 Science* 55(1), 77-85.
- 443 Barthe, M. & Pelletier, E. 2007. Comparing bulk extraction methods for chemically available
444 polycyclic aromatic hydrocarbons with bioaccumulation in worms. *Environmental
445 Chemistry*, 4, 271–283.
- 446 Bernhardt, C., Derz, K., Kördel, W. & Terytze, K. 2013. Applicability of Non-Exhaustive
447 Extraction Procedures with Tenax and Hpcd. *Journal of Hazardous Materials*, 261,
448 711-717.
- 449 Burnham, A. K., Calder, G. V., Fritz, J. S., Junk, G. A., Svec, H. J. & Willis, R. 1972.
450 Identification and Estimation of Neutral Organic Contaminants in Potable Water.
451 *Analytical Chemistry*, 44(1), 139-142.
- 452 Ortega-Calvo, J. J., Harmsen, J., Parsons, J. R., Semple, K. T., Aitken, M. D., Ajao, C.,
453 Eadsforth, C., Galay-Burgos, M., Naidu, R., Oliver, R. & Peijnenburg, W. J. 2015.
454 From bioavailability science to regulation of organic chemicals. *Environmental
455 Science and Technology*, 49, 10255–10264.
- 456 Care, R., Morrison, D. & Smith, J. F. 1982. On the Limit of Detection of Traces of Volatile
457 Organics in Water Using Amberlite Xad-2 Resin. *Water Research*, 16(5), 663-665.
- 458 Carroll, K., Harkness, M., Bracco, A. & Balcarcell, R. 1994. Application of Permanent
459 Polymer Diffusional Model to the Desorption of Polychlorinated-Biphenyls from
460 Hudson River Sediments. *Environmental Science & Technology*, 28(2), 253-258.
- 461 Chuang, J., Wilson, N. & Lewis, R. 1999. Methodology of Ambient Air Monitoring for
462 Polycyclic Aromatic Hydrocarbons. *Fresenius Environmental Bulletin*, 8(9-10), 547-
463 556.
- 464 Cornelissen, G., Van Noort, P. C. M. & Govers, H. A. J. 1998. Mechanism of Slow
465 Desorption of Organic Compounds from Sediments: A Study Using Model Sorbents
466 *Environmental Science and Technology*, 32(20), 3124-3131.
- 467 Cui, X., Mayer, P. & Gan, J. 2013. Methods to assess bioavailability of hydrophobic organic
468 contaminants: Principles, operations, and limitations. *Environmental Pollution*, 172,
469 223-234.

- 470 Cuypers, C., Pancras, T., Grotenhuis, T. & Rulkens, W. 2002. The Estimation of Pah
471 Bioavailability in Contaminated Sediments Using Hydroxypropyl-B-Cyclodextrin and
472 Triton X-100 Extraction Techniques. *Chemosphere*, 46(8), 1235-1245.
- 473 Cuypers, M. P., Grotenhuis, J. T. C. & Rulkens, W. H. 1999. Prediction of Pah
474 Bioavailability in Soils and Sediments by Persulfate Oxidation. In: LEESON, A. &
475 ALLEMAN, B. C. (eds.) *5th International In Situ and On-Site Bioremediation*
476 *Symposium*. San Diego, California, U.S.A.
- 477 Daignault, S. A., Noot, D. K., Williams, D. T. & Huck, P. M. 1988. A Review of the Use of
478 Xad Resins to Concentrate Organic Compounds in Water. *Water Research*, 22(7),
479 803-813.
- 480 Dandie, C. E., Weber, J., Aler, S., Adetutu, E. M., Ball, A. S. & Juhasz, A. L. 2010.
481 Assessment of Five Bioaccessibility Assays for Predicting the Efficacy of Petroleum
482 Hydrocarbon Biodegradation in Aged Contaminated Soils. *Chemosphere*, 81(9),
483 1061-1068.
- 484 Doick, K. & Semple, K. 2003. The Effect of Soil: Water Ratios on the Mineralisation of
485 Phenanthrene: Lnpl Mixtures in Soil. *Fems Microbiology Letters*, 220(1), 29-33.
- 486 Doick, K. J., Clasper, P. J., Urmann, K. & Semple, K. T. 2006. Further Validation of the
487 Hpcd-Technique for the Evaluation of Pah Microbial Availability in Soil.
488 *Environmental Pollution*, 144(1), 345-354.
- 489 Doick, K. J., Lee, P. H. & Semple, K. T. 2003. Assessment of Spiking Procedures for the
490 Introduction of a Phenanthrene- Lnpl Mixture into Field- Wet Soil. *Environmental*
491 *Pollution*, 126(3), 399-406.
- 492 Hawthorne, S. B., Poppendieck, D. G., Grabanski, C. B. & Loehr, R. C. 2002. Comparing
493 Pah Availability from Manufactured Gas Plant Soils and Sediments with Chemical
494 and Biological Tests. 1. Pah Release During Water Desorption and Supercritical
495 Carbon Dioxide Extraction. *Environmental Science and Technology*, 36(22), 4795-
496 4803.
- 497 Hickman, Z. A. & Reid, B. J. 2005. Towards a more appropriate water based extraction for
498 the assessment of organic contaminant availability. *Environmental Pollution*, 138(2),
499 299-306.
- 500 Hickman, Z. A., Swindell, A. L., Allan, I. J., Rhodes, A. H., Hare, R., Semple, K. T. & Reid,
501 B. J. 2008. Assessing Biodegradation Potential of Pahs in Complex Multi-
502 Contaminant Matrices. *Environmental Pollution*, 156(3), 1041-1045.
- 503 Juan-Peiro, L., Bernhammer, A., Pastor, A. & de la Guardia, M. 2013. The use of amberlite
504 adsorbents for green chromatography determination of volatile organic compounds in
505 air. *Journal of Analytical Methods in Chemistry*, (2012) 728143.

- 506 Kelsey, J. W. & Alexander, M. 1997. Declining Bioavailability and Inappropriate Estimation
507 of Risk of Persistent Compounds. *Environmental Toxicology and Chemistry*, 16(3),
508 582-585.
- 509 Kelsey, J. W., Kottler, B. D. & Alexander, M. 1997. Selective Chemical Extractants to
510 Predict Bioavailability of Soil-Aged Organic Chemicals. *Environmental Science &*
511 *Technology*, 31(1), 214-217.
- 512 Lamoureux, E. M. & Brownawell, B. J. 2004. Influence of Soot on Hydrophobic Organic
513 Contaminant Desorption and Assimilation Efficiency. *Environmental Toxicology and*
514 *Chemistry*, 23(11), 2571-2577.
- 515 Latawiec, A. E., Swindell, A. L. & Reid, B. J. 2008. Environmentally Friendly Assessment of
516 Organic Compound Bioaccessibility Using Sub-Critical Water. *Environmental*
517 *Pollution*, 156(2), 467-473.
- 518 Lei, L., Suidan, M. T., Khodadoust, A. P. & Tabak, H. H. 2004. Assessing the Bioavailability
519 of Pahs in Field-Contaminated Sediment Using Xad-2 Assisted Desorption.
520 *Environmental Science and Technology*, 38(6), 1786-1793.
- 521 Liste, H. H. & Alexander, M. 2002. Butanol Extraction to Predict Bioavailability of Pahs in
522 Soil. *Chemosphere*, 46(7), 1011-1017.
- 523 Northcott, G. L. & Jones, K. C. 2000. In Situ Xad Resin Adsorption Method for Determining
524 Partition Coefficients and Desorption Kinetics of Soil Aged Pahs. *Abstracts of Papers*
525 *American Chemical Society*, 220(Part 1), ENVR 113.
- 526 Ogbonnaya, U. & Semple, K. T. (2013). Impact of biochar on organic contaminants in soil: a
527 tool for mitigating risk? *Agronomy*, 3(2), 349-375.
- 528 Ogbonnaya, O. U., Adebisi, O. O. & Semple, K. T. 2014. The Impact of Biochar on the
529 Bioaccessibility of ¹⁴C-Phenanthrene in Aged Soil. *Environmental Science: Processes*
530 *& Impacts*, 16(11), 2635-2643.
- 531 Ogbonnaya, U. O., Thomas, J., Fasina, S. A. & Semple, K. T. 2016. Impact of two
532 contrasting biochars on the bioaccessibility of 14 C-naphthalene in soil.
533 *Environmental Technology and Innovation*, 6, 80-93.
- 534 Oyelami, A., Elegbede, B. & Semple, K. 2014. Impact of different types of activated carbon
535 on the bioaccessibility of ¹⁴C-phenanthrene in sterile and non-Sterile Soils.
536 *Environments* 1, 137-156.
- 537 Papadopoulos, A., Paton, G. I., Reid, B. J. & Semple, K. T. 2007. Prediction of Pah
538 Biodegradation in Field Contaminated Soils Using a Cyclodextrin Extraction
539 Technique. *Journal of Environmental Monitoring*, 9(6), 516-522.

- 540 Patterson, C. J., Semple, K. T. & Paton, G. I. 2004. Non-Exhaustive Extraction Techniques
541 (Neets) for the Prediction of Naphthalene Mineralisation in Soil. *FEMS Microbiology*
542 *Letters*, 241(2), 215-220.
- 543 Puglisi, E., Patterson, C. J. & Paton, G. I. 2003. Non-Exhaustive Extraction Techniques
544 (Neets) for Bioavailability Assessment of Organic Hydrophobic Compounds in Soils.
545 *Agronomie*, 23(8), 755-756.
- 546 Reid, B. J., Jones, K. C. & Semple, K. T. 2000a. Bioavailability of Persistent Organic
547 Pollutants in Soils and Sediments—a Perspective on Mechanisms, Consequences and
548 Assessment. *Environmental Pollution*, 108(1), 103-112.
- 549 Reid, B. J., Macleod, C. J. A., Lee, P. H., Morriss, A. W. J., Stokes, J. D. & Semple, K. T.
550 2001. A Simple ¹⁴C-Respirometric Method for Assessing Microbial Catabolic
551 Potential and Contaminant Bioavailability. *FEMS Microbiology Letters*, 196(2), 141-
552 146.
- 553 Reid, B. J., Semple, K. T. & Jones, K. C. 1998. Prediction of Bioavailability of Persistent
554 Organic Pollutants by a Novel Extraction Technique. *Contaminated Soil '98 - 6th*
555 *International FZK/TNO Conference on Contaminated soil (ConSoil 98)*. Edinburgh,
556 Scotland.
- 557 Reid, B. J., Stokes, J. D., Jones, K. C. & Semple, K. T. 2000. Nonexhaustive Cyclodextrin-
558 Based Extraction Technique for the Evaluation of Pah Bioavailability. *Environmental*
559 *Science & Technology*, 34(15), 3174-3179.
- 560 Reid, B. J., Stokes, J. D., Jones, K. C. & Semple, K. T. 2000b. Nonexhaustive Cyclodextrin-
561 Based Extraction Technique for the Evaluation of Pah Bioavailability. *Environmental*
562 *Science and Technology*, 34(15), 3174-3179.
- 563 Rhodes, A. H., Dew, N. M. & Semple, K. T. 2008. Relationship between Cyclodextrin
564 Extraction and Biodegradation of Phenanthrene in Soil. *Environmental Toxicology*
565 *and Chemistry*, 27(7), 1488-1495.
- 566 Rhodes, A. H., Riding, M. J., McAllister, L. E., Lee, K. & Semple, K. T. (2012). Influence of
567 activated charcoal on desorption kinetics and biodegradation of phenanthrene in soil.
568 *Environmental Science and Technology*, 46(22), 12445-12451.
- 569 Sánchez-Trujillo, M. A., Morillo, E., Villaverde, J. & Lacorte, S. 2013. Comparative effects
570 of several cyclodextrins on the extraction of PAHs from an aged contaminated soil.
571 *Environmental Pollution*, 178, 52-58.
572
- 573 Semple, K. T., Doick, K. J., Jones, K. C., Burauel, P., Craven, A. & Harms, H. 2004.
574 Defining Bioavailability and Bioaccessibility of Contaminated Soil and Sediment Is
575 Complicated. *Environmental Science and Technology*, 38(12), 228A-231A.

- 576 Semple, K. T., Dew, N. M., Doick, K. J. & Rhodes, A. H. 2006. Can microbial
577 mineralization be used to estimate microbial availability of organic contaminants in
578 soil? *Environmental Pollution*, 140, 164-172.
- 579 Semple, K. T. Doick, K. J. Wick, L. Y. & Harms, H. 2007. Microbial interactions with
580 organic contaminants in soil: Definitions, processes and measurement. *Environmental*
581 *Pollution*, 150, 166–176.
- 582 Škulcová, L., Neuwirthová, J., Hofman, L. & Bielská. 2016. Assessment of the biological
583 and chemical bioavailability of the freshly spiked and aged DDE in soil.
584 *Environmental Pollution*, 212,105-112
- 585 Simpson, S. L., Burston, V. L., Jolley, D. F. & Chau, K. 2006. Application of Surrogate
586 Methods for Assessing the Bioavailability of Paks in Sediments to a Sediment
587 Ingesting Bivalve. *Chemosphere*, 65(11), 2401-2410.
- 588 Swindell, A. L. & Reid, B. J. 2006. Comparison of Selected Non-Exhaustive Extraction
589 Techniques to Assess Pah Availability in Dissimilar Soils. *Chemosphere*, 62(7),
590 1126-1134.
- 591 Tan, K. H. 2014. *Humic Matter in Soil and the Environment: Principles and Controversies*,
592 Florida, U.S.A.: CRC Press.
- 593 Van Der Wal, L., Jager, T., Fleuren, R. H. L. J., Barendregt, A., Sinnige, T. L., Van Gestel,
594 C. A. M. & Hermens, J. L. M. 2004. Solid-Phase Microextraction to Predict
595 Bioavailability and Accumulation of Organic Micropollutants in Terrestrial
596 Organisms after Exposure to a Field-Contaminated Soil. *Environmental Science and*
597 *Technology*, 38(18), 4842–4848.
- 598 Vázquez-Cuevas, G. M. & Semple, K. T. 2016. Measurement of Hydrocarbon Bioavailability
599 in Soil. In: MCGENITY, T. J., TIMMIS, K. N. & NOGALES FERNÁNDEZ, B.
600 (eds.) *Hydrocarbon and Lipid Microbiology Protocols*. Totowa, NJ: Humana Press.
- 601 Wania, F., Shen, L., Lei, Y. D., Teixeira, C. & Muir, D. C. G. 2003. Development and
602 Calibration of a Resin-Based Passive Sampling System for Monitoring Persistent
603 Organic Pollutants in the Atmosphere. *Environmental Science & Technology*, 37(7),
604 1352-1359.
- 605 Xia, X., Li, Y., Zhou, Z. & Feng, C. 2010. Bioavailability of Adsorbed Phenanthrene by
606 Black Carbon and Multi-Walled Carbon Nanotubes to *Agrobacterium*. *Chemosphere*,
607 78(11), 1329-1336.
- 608 Yu, L., Vázquez-Cuevas, G., Duan, L. & Semple, K. T. 2016. Buffered Cyclodextrin
609 Extraction of [Formula Omitted]-Phenanthrene from Black Carbon Amended Soil.
610 *Environmental Technology & Innovation*, 6, 177-184.

612 **List of tables**

613 Table 1: Important physic-chemical properties of selected XADs

614 Table 2: Total ^{14}C -phenanthrene extracted by different soil/XAD-2 ratios. The residual ^{14}C -
615 activity in the supernatant and soil pellets is also presented. Values represent the mean \pm
616 standard error of the mean ($n = 3$). Data with the same lower case letter down the column
617 indicates no statistical significant difference ($p > 0.05$)

618 Table 3. ^{14}C -phenanthrene extracted by four XADs: XAD-2, XAD-4, XAD-16, and XAD-
619 1180N; in comparison with DCM, HP- β -CD and total estent of mineralisation at 0, 25, 50
620 and 100 d soil-PAH contact time. Values represent the mean \pm standard error of the mean (n
621 = 3). Data with the same lower case letter down the column indicates no statistical significant
622 difference ($p > 0.05$)

623 **List of figures**

624 Figure 1. Effect of shaking at 0 (A), 20 (B), 40 (C), 80 (D), 100 (E), 120 (F) rpm on the
625 desorption and XAD-2 extractability of ^{14}C -phenanthrene from freshly spiked soil. Error bars
626 represent the standard error of the mean ($n = 3$).

627 Figure 2. Comparison of elution efficiencies of DCM:methanol (1:1, A); toluene:methanol
628 (1:1, B); hexane:acetone (1:1, C); hexane:acetone (3:1, D) for the removal of XAD sorbed
629 ^{14}C -phenanthrene. Errors bars represent the standard error of the mean ($n = 3$).

630 Figure 3. Summarised XAD-2 extraction-time profiles for ^{14}C -phenanthrene. Error bars
631 represent the standard error of the mean ($n = 3$). Data with the same lower case letter down
632 the column indicates no statistical significant difference ($p > 0.05$).

633 Figure 4. Relationship between ^{14}C -phenanthrene mineralisation and DCM (●), HP- β -CD
634 (○) and XAD-4 (▼) extractions in soil over 100 d soil-phenanthrene contact time. Error bars
635 represent the standard error of the mean ($n = 3$).

636 Tables

637 **Table 1.** General physical and chemical properties of selected Amberlite XADs according to
638 the manufacturer

Properties	XAD-2	XAD-4	XAD-16	XAD-1180N
Appearance	White solid	Opaque solid	Translucent solid	White solid
Type	Polymeric	Polymeric	Polymeric	Polymeric
Mesh size (mm)	20 - 60	20 - 60	20 - 60	20 - 50
Density (g mL ⁻¹)	1.02	1.02	1.03	1.03
Surface area (mm ²)	300	600	750	900
Pore diameter (Å)	100	50	300	600

639

640 **Table 2.** Total ^{14}C -phenanthrene extracted by different soil/XAD-2 ratios. The residual ^{14}C -
 641 activity in the supernatant and soil pellets is also presented. Values represent the mean \pm
 642 standard error of the mean ($n = 3$). Data with the same lower case letter down the column
 643 indicates no statistical significant difference ($p > 0.05$).

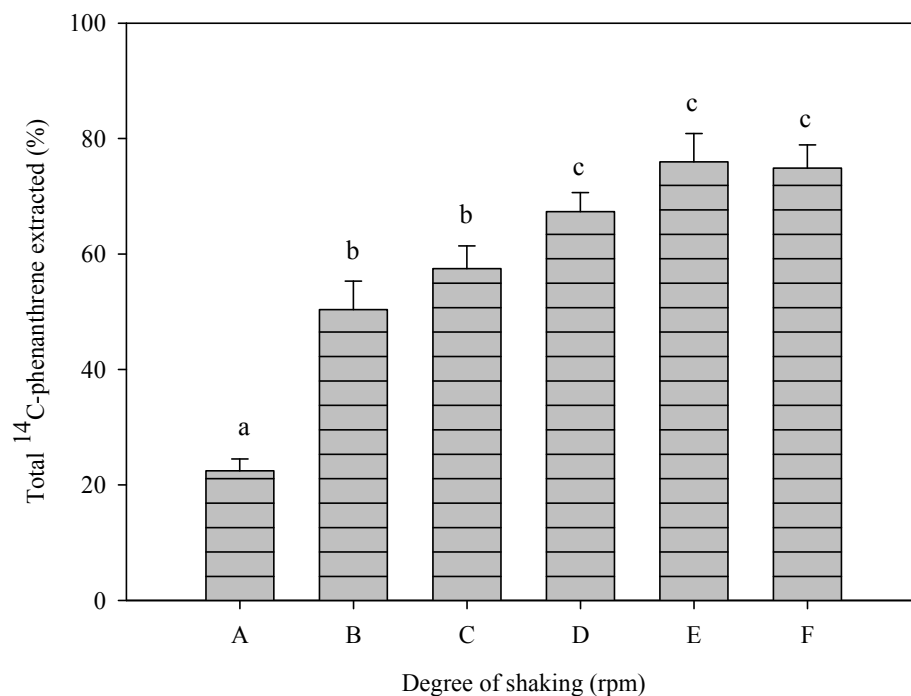
Soil/XAD ratio	Resin added (g)	Amount of ^{14}C -phenanthrene (%)			
		XAD-2	Supernatant	Soil residue	Mass balance*
10/1	0.2	16.63 \pm 0.4 ^a	0.57 \pm 0.1 ^a	84.11 \pm 2.8 ^a	101.31 \pm 3.2
4/1	0.5	18.68 \pm 2.3 ^a	0.51 \pm 0.0 ^a	79.58 \pm 3.5 ^a	98.77 \pm 4.3
2/1	1.0	20.73 \pm 1.2 ^a	0.66 \pm 0.1 ^a	80.64 \pm 3.5 ^a	102.03 \pm 2.6
1/1	2.0	18.85 \pm 1.5 ^a	0.41 \pm 0.1 ^a	82.17 \pm 4.0 ^a	101.43 \pm 2.8

644 *Equals total XAD-2 extracted + residual ^{14}C -in the supernatant + residual ^{14}C in soil.

645 **Table 3.** ^{14}C -phenanthrene extracted by four XADs: XAD-2, XAD-4, XAD-16, and XAD-1180N; in compar
 646 extent of mineralisation at 0, 25, 50 and 100 d soil-PAH contact time. Values represent the mean \pm standard e
 647 the same lower case letter down the column indicates no statistical significant difference ($p > 0.05$).

Soil-PAH contact time (d)	XAD-extractable (%)				HP- β -CD (%)	DCM (%)
	XAD-2	XAD-4	XAD-16	XAD-1180N		
0	85.82 \pm 3.5 ^{bA}	85.72 \pm 5.0 ^{bA}	83.76 \pm 3.2 ^{bA}	89.48 \pm 4.5 ^{bA}	78.98 \pm 4.5 ^{aA}	94.91 \pm 5.92 ^{cA}
25	27.74 \pm 1.2 ^{aB}	30.22 \pm 2.1 ^{bB}	25.83 \pm 1.9 ^{aB}	26.06 \pm 1.8 ^{aB}	32.25 \pm 3.9 ^{bB}	63.99 \pm 3.21 ^{cB}
50	16.99 \pm 1.0 ^{aC}	18.05 \pm 1.8 ^{aC}	15.78 \pm 1.1 ^{aC}	15.19 \pm 1.0 ^{aC}	23.35 \pm 1.7 ^{bC}	53.08 \pm 3.48 ^{cC}
100	16.82 \pm 2.4 ^{aC}	19.15 \pm 2.3 ^{aC}	17.53 \pm 2.1 ^{aC}	16.11 \pm 1.1 ^{aC}	18.97 \pm 2.9 ^{aD}	51.82 \pm 3.80 ^{bC}

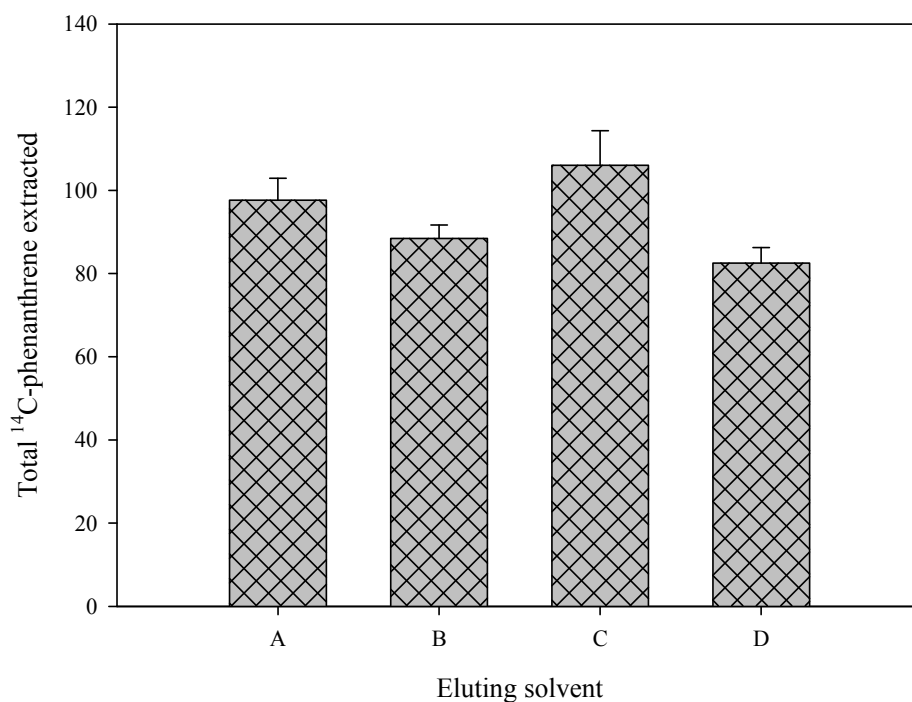
648 a: No statistical significant difference ($p > 0.05$) amongst extractions and mineralisation in soil within each contact time; A: No
 649 within each extraction or during mineralisation across different contact times; b or c: Statistically higher ($p < 0.05$) extents of extra
 650 contact time; B or C: Statistically higher ($p < 0.05$) extents of extraction or mineralisation in soil across different contact times.

651 **Figures**

652

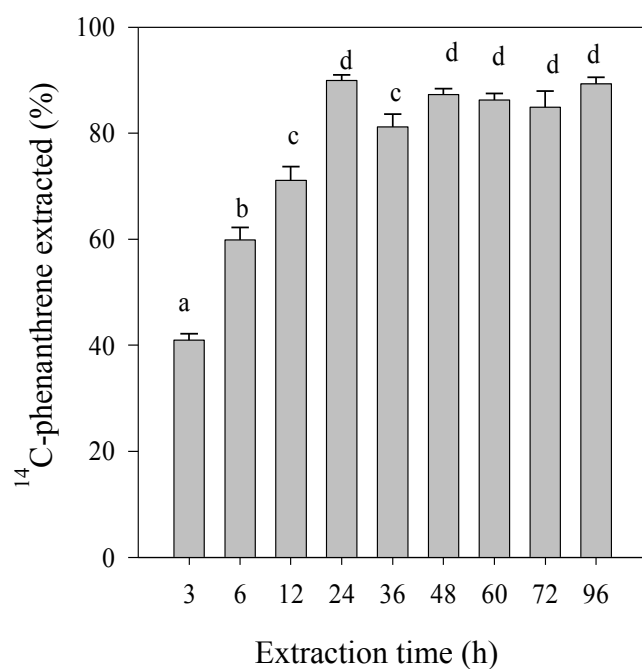
653 **Figure 1.** Effect of shaking at 0 (A), 20 (B), 40 (C), 80 (D), 100 (E), 120 (F) rpm on the
654 desorption and XAD-2 extractability of ¹⁴C-phenanthrene from freshly spiked soil. Error bars
655 represent the standard error of the mean ($n = 3$).

656



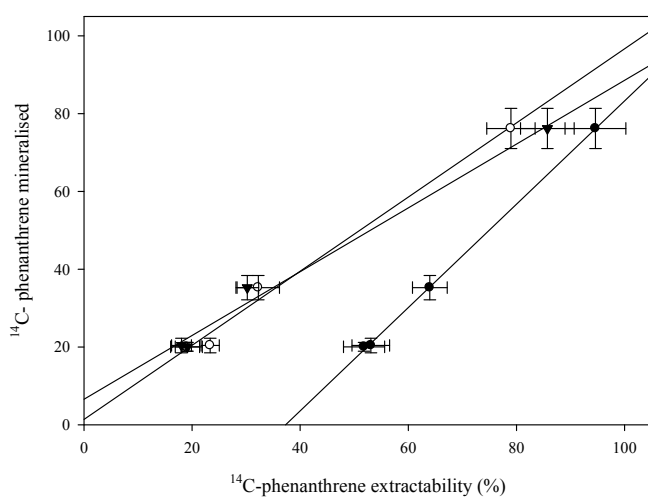
657

658 **Figure 2.** Comparison of elution efficiencies of DCM:methanol (1:1, A); toluene:methanol
659 (1:1, B); hexane:acetone (1:1, C); hexane:acetone (3:1, D) for the removal of XAD sorbed
660 ¹⁴C-phenanthrene. Errors bars represent the standard error of the mean ($n = 3$).



661

662 **Figure 3.** Summarised XAD-2 extraction-time profiles for ¹⁴C-phenanthrene. Error bars
663 represent the standard error of the mean ($n = 3$). Data with the same lower case letter down
664 the column indicates no statistical significant difference ($p > 0.05$).



665

666 **Figure 4.** Relationship between ^{14}C -phenanthrene mineralisation and DCM (\bullet), HP- β -CD
667 (\circ) and XAD-4 (\blacktriangledown) extractions in soil over 100 d soil-phenanthrene contact time. Error bars
668 represent the standard error of the mean ($n = 3$).

Highlights

- Organic solvent extraction was more effective than XAD at removing phenanthrene from soil
- The extraction of phenanthrene using XAD was comparable to the of HPCD from soil
- Mineralisation of ^{14}C -phenanthrene was predicted by HPCD and XAD-4 extractions