

1 Time-Dependent Remobilisation of Non-  
2 Extractable Benzo[a]pyrene Residues in  
3 Contrasting Soils: Effects of Aging, Spiked  
4 Concentration, and Soil Properties

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## 12 **ABSTRACT**

13 The environmental and health risks associated with ‘non-extractable’ residues (NERs) of  
14 polycyclic aromatic hydrocarbons in soils and their potential for remobilisation remain largely  
15 unexplored. In this novel study, sequential solvent extractions were employed to interrogate  
16 time-dependent remobilisation of benzo[a]pyrene (B[a]P) NERs and associated kinetics after  
17 re-equilibration (REQ) periods lasting 30 d in four artificially-spiked soils aged for up to 200  
18 days. Following sequential extractions of the re-equilibrated soils, remobilisation of B[a]P  
19 NERs was observed and further confirmed by decreases in the absolute amounts of B[a]P  
20 recovered following methanolic saponification after REQ. Remobilisation may occur through  
21 slow intercompartmental partitioning of more sequestered into less sequestered B[a]P fractions  
22 in soils. The amounts of B[a]P remobilised in soils decreased throughout aging following first-  
23 order kinetics and the rates of decrease were slow but 2 to 4 times faster than those of  
24 extractable B[a]P before re-equilibration. Sandy-clay-loam soils with large amounts of hard  
25 organic carbon exhibited less NER remobilisation compared to sandy soils. The amounts of  
26 remobilised B[a]P decreased significantly ( $p < 0.05$ ) with aging. Specifically, butanol-  
27 remobilised B[a]P in soils spiked at 10 mg/kg and 50 mg/kg B[a]P ranged from 0.15 to 0.39  
28 mg/kg and 0.67 to 2.30 mg/kg, respectively, after 200 d of aging.

## 29 INTRODUCTION

30 The putative remobilisation, impact, and significance of ‘non-extractable’ residues (NERs)  
31 of polycyclic aromatic hydrocarbons (PAHs) in soils remain largely unexplored. Polycyclic  
32 aromatic hydrocarbons are ubiquitous hydrophobic organic contaminants (HOCs) in the  
33 environment and are of global concern. Of the 16 PAHs listed by the United States  
34 Environmental Protection Agency (USEPA) as priority pollutants, Benzo[a]pyrene is often  
35 the risk-driver at most PAH-contaminated sites due to its persistence, toxicity,  
36 carcinogenicity, mutagenicity and potential for bioaccumulation <sup>1,2</sup>. Common sources of  
37 PAHs, with soil serving as a sink, include coking and coal firing plants, vehicular emissions,  
38 bush fires, fossil fuel burning, and crude oil spillage <sup>3</sup>. The amounts of PAHs in soils can be  
39 wide-ranging depending on land use and proximity to industrial activities <sup>4,5</sup>. Average total  
40 concentration of the 16 USEPA PAHs in soils collected near gasworks sites can range from  
41 300 to over 8000 mg/kg <sup>6</sup>, and many people living close to these sites may be exposed.

42 In soil, PAHs may be lost through volatilisation, photolysis, leaching, microbial  
43 degradation, and biological uptake <sup>7</sup>. Importantly, PAHs are readily sequestered in soil as  
44 they are highly hydrophobic. Sequestration of PAHs in soils occurs through partitioning into  
45 soft and hard organic carbon, clay, organo-mineral complexes, as well as diffusion into  
46 micropores, or strong sequestration to carbonaceous geosorbents such as black carbon <sup>8,9</sup>.  
47 Detailed mechanisms of HOC sequestration have been described elsewhere <sup>8,10</sup>. With aging,  
48 the amounts of readily available PAH fractions decrease while those of sequestered fractions  
49 increase <sup>11</sup>. Even with successive exhaustive solvent extractions, the sequestered fractions in  
50 soils are not extracted substantially and referred to as ‘NERs’ <sup>12</sup>.

51 The NERs of HOCs in soils are sometimes regarded as unimportant in risk assessment <sup>13</sup>.  
52 The focus of traditional risk assessments has been on total-extractable concentrations which

53 will be generally greater than bioavailable concentrations. It is assumed that NERs will  
54 always be strongly and irreversibly sequestered to soil particles <sup>13</sup>. However, recent  
55 knowledge reveals that total NERs in soil could be of different types depending on binding  
56 mechanisms <sup>12, 14</sup>. Type I NERs are entrapped and adsorbed residues which are non-  
57 covalently bound in soil, with low to high stability but also with low to high potential for  
58 release as parent compounds or metabolites. Type II NERs are covalently bound, highly  
59 stable and with low potential for release. Type III or biogenic NERs are residues formed from  
60 microbial metabolic activities in soils and are ultimately incorporated into SOM; their release  
61 bears no risks to human and ecological health. Differentiation of NERs in soils ensures that  
62 potential risks associated with exposure are not overestimated. Methods such as silylation,  
63 methanolic saponification, and microbial turnover to biomass modelling have been proposed  
64 for the differentiation of the NER types; however, these methods are complex, laborious and  
65 still need to be widely validated <sup>12</sup>. Investigating potential remobilisation of undifferentiated  
66 NERs can still provide relevant information on NER fate in soils, which is useful for the risk  
67 assessment and management of contaminated lands <sup>13</sup>. Further, the concentrations of  
68 potentially remobilised NERs in soils must be considered for risk assessment purposes.

69 A number of earlier studies focused on the potential release of soil-bound pesticide residues  
70 to sentinel organisms, such as earthworms and plants <sup>15</sup>. For PAHs, Eschenbach et al. <sup>16</sup>  
71 reported that non-extractable <sup>14</sup>C-PAH residues in long-term contaminated soils were not  
72 remobilised by biological (fungal and microbial) and physical treatments. Enhanced release  
73 of non-extractable PAH residues in soil by the influence of low molecular weight organic  
74 acids in plant root exudates has been reported <sup>17, 18</sup>. Low molecular weight organic acids such  
75 as citric acid chelates metal cations within mineral-SOM complexes in soil, thereby  
76 facilitating release of sequestered PAHs within the SOM <sup>17</sup>. Following removal of labile PAH  
77 fractions from sediments, non-labile PAHs repartitioned from solid into liquid phases during

78 an incubation period of 30 d as facilitated by solid-phase (Amberlite XAD-2 resin) extraction  
79 <sup>19</sup>. Contaminant repartitioning may have implications for the long-term fate of PAH NERs in  
80 soils since associated risks may be underestimated. Still, the nature and acceptability of  
81 potential risks will depend on the concentrations of the repartitioned contaminants in soil.

82 The cited studies have either only used a few soils or did not report concentrations of NER  
83 remobilised and its significance, especially regarding its potential to cause significant harm  
84 from a risk assessment perspective. Importantly, the potential remobilisation of NERs in  
85 soils, after a re-equilibration period, which would likely be time-dependent and may vary  
86 with soil properties and PAH concentrations has not been investigated. This is the first study  
87 to examine the time-dependent remobilisation of B[a]P NERs following sequential solvent  
88 extractions of four artificially-spiked soils.

## 89 **EXPERIMENTAL METHODS**

90 **Chemicals.** Analytical grade B[a]P (> 96% purity) was obtained as crystalline solid from  
91 Sigma-Aldrich Pty Ltd., Sydney, Australia. Also sourced from the same supplier were p-  
92 Terphenyl-d14, analytical grade acetone (Ace), acetonitrile (ACN), 1-BuOH (BuOH, density  
93 = 0.81 g/ml,  $\geq$  99.4%), dichloromethane (DCM), methanol (MeOH, HPLC Grade), toluene  
94 (Tol, 99.8%), potassium hydroxide (KOH), sodium persulphate, hydrochloric acid, and silica  
95 sand. Hexane (Hex, HPLC grade) was purchased from Fisher Scientific, Loughborough, UK.

96 **Design.** Twenty-five surface and 7 sub-surface soils previously collected from 25 sites  
97 located in Australia and characterised <sup>20</sup> were used in this study (Table S1, Supplementary  
98 Information (SI)). The data for the physico-chemical properties (pH, EC, TOC, DOC, CEC,  
99 sand, silt, and clay contents) of the 32 soils were subjected to a principal component (PC)  
100 analysis to select soils with differing properties. Four soils (I, M, B, N) were selected based  
101 on average PC scores, relating to varying soil properties, and calculated following the  
102 Anderson-Rubin Method <sup>21</sup>. Soils I (Kurosol), M (Ferrosol), B (Black Vertisol), and N  
103 (Tenosol) were classified according to the USDA textural classification as sandy-loam,  
104 sandy-clay-loam, sandy-clay-loam, and loamy-sand, respectively. Soil organic matter  
105 contents of soils I (13.3), M (21.4%), B (11.0%), and N (4.8%) were estimated by loss on  
106 ignition <sup>22</sup>. The contents of hard organic carbon (Hard OC) in acid-hydrolysed soils were  
107 quantified (by combustion at 1350 °C using LECO CNS analyser) after removing soft carbon  
108 by wet oxidation with persulphate according to a previously described method <sup>23</sup>. The  
109 difference between TOC and hard OC contents estimated Soft OC contents in soils <sup>23</sup>.  
110 Thermogravimetric analysis (TGA) was also used to determine the contents of nonpyrogenic  
111 OC (weight losses at 200–470 °C) and black carbon (BC)-rich fractions (weight losses at  
112 470–600 °C) in soils based on thermal stability of these fractions <sup>24</sup>.

113 For the B[a]P studies, each air-dried soil was spiked at 10 mg/kg and 50 mg/kg B[a]P  
114 following methods described previously<sup>22</sup>. The soils were rehydrated with deionised water to  
115 their field moisture contents (25% to 40% on dry weight basis) and incubated in sealed amber  
116 glass in the dark at  $22 \pm 3$  °C for 14, 33, 66, 120, and 200 d. Spiked silica sand was treated in  
117 a similar manner to test spike recovery. All treatments are presented in Figure S1. It was  
118 expected that substantial NER would be formed after 33 d of aging<sup>22, 23</sup>. After aging,  
119 approximately 1 g soil subsamples ( $n = 12$ ) were dried at 37.5 °C and subjected to a  
120 sequential extraction to completely remove readily available B[a]P fractions first using  
121 BuOH and then DCM/Ace extraction as described previously<sup>22</sup>. Since pre-extracted soils  
122 were to be further re-equilibrated, anhydrous Na<sub>2</sub>SO<sub>4</sub> was not used (which would also include  
123 grinding) for drying soil prior to DCM/Ace extraction. This was to avoid any artefact, such as  
124 disintegration of micropores and drying-out of soils, which could impact on re-equilibration  
125<sup>25</sup>. All percentage extractability and sequestration determinations were calculated based on  
126 initial amounts of B[a]P spiked into soil (10 mg/kg or 50 mg/kg), except where stated  
127 otherwise stated.

$$128 \text{ Extractability (\%)} = \left( \frac{\text{Amount of B[a]P Extracted by Solvent (mg)}}{\text{Amount of B[a]P Spiked into Soil (mg)}} \right) \times 100\% \quad (1)$$

129 After exhaustive extraction, soils were randomised into three batches of 4 replicate samples  
130 per soil (Figure S1).

131 For each soil, no significant difference ( $p > 0.05$ ) was found between mean total  
132 extractability (percent BuOH + DCM/Ace extractability) among the three batches using one-  
133 way ANOVA (Figure S2), as well as variances between batches using Levene's Test<sup>21</sup>.  
134 Standard error of the mean for each batch ( $n = 4$ ) was generally 5% or less, and the mean  
135 concentration of each batch was not statistically different ( $p < 0.05$ ) from the mean of original  
136 samples ( $n = 12$ ). Therefore, each batch ( $n = 4$ ) was statistically indistinguishable from the

137 original, and independent of the other batches within the same soil group. The first batch of  
138 pre-extracted soils (Batch A) containing NERs was hydrolysed using methanol/potassium  
139 hydroxide (MeKOH) to extract the highly sequestered B[a]P residues<sup>26, 27</sup>. The second (B)  
140 and third (C) batches ( $n = 4$  each) were re-wetted to field moisture contents in the same  
141 centrifuge bottle used for sequential extraction. The sample bottles were then capped and  
142 allowed to re-equilibrate in the dark at  $25.5 \pm 1.0$  °C for 30 d and 60 d, respectively. After re-  
143 equilibration (REQ), soils were dried at 37.5 °C and the sequential extraction was repeated.  
144 The additional fractions of B[a]P that were extracted after REQ constituted the remobilised  
145 fractions.

146 For each of BuOH and DCM/Ace extractions, the amount of B[a]P (mg/kg) extracted after  
147 REQ was then added to that prior to REQ to obtain cumulative extractability (mg/kg or %).  
148 Benzo[a]pyrene extractability prior to and after REQ in the soils were then compared. If  
149 cumulative B[a]P extractability (%) after REQ was significantly greater ( $p < 0.05$ ) than that  
150 prior to REQ, this suggested that previous NER had become remobilised after the REQ  
151 period.

152 **HPLC Analysis of B[a]P.** The concentrations of B[a]P in extracts filtered with 0.45 µm  
153 polytetrafluoroethylene (PTFE) syringe filters were determined with an Agilent 1100 Series  
154 HPLC equipped with a fluorescence detector (excitation wavelength = 230 nm and emission  
155 wavelength = 460 nm) as in our previous study<sup>22</sup>.

156 **Modelling Extractability Kinetics of Aging and Estimation of Half-Life of B[a]P Loss**  
157 **in Soil.** Benzo[a]pyrene Extractability versus aging data in this study generally showed first-  
158 order exponential decreases<sup>28</sup>. A 2 parameter first-order kinetics equation served to model  
159 decrease in B[a]P with aging in BuOH, total and MeKOH extractability of B[a]P before and  
160 after REQ as well as the decrease in the remobilised amounts<sup>23, 29</sup>.



161 
$$B[a]Pt = B[a]Po * e^{-kt} \text{ (2)}$$

162 Where B[a]Po and B[a]Pt are the percentage of B[a]P extracted at time 0 d and *t* d of aging,  
163 respectively, and *k* is the rate constant of the decrease in the extractable fraction (*k*, d<sup>-1</sup>). The  
164 non-linear curve fitting package of Origin software (Microcal Software Inc., Northampton,  
165 MA, USA, version 6) was employed for modelling and obtaining B[a]Po and *k* values  
166 through 100 iterations. The half-lives of B[a]P loss in the soils were also estimated.

167 **Quality Assurance and Quality Control.** A standard calibration curve using 10 calibration  
168 points (0.1 ng/ml to 1 µg/ml) was used to determine extracted B[a]P concentrations and  
169 consistently gave R<sup>2</sup> greater than 0.999. Detection limit (LOD) using the linear regression  
170 method was calculated from the lowest detectable concentrations (0.1, 0.5, 1, 5, and 10  
171 ng/ml) using 8 replicates for each concentration. The LOD and limit of quantitation were 0.07  
172 ng/ml and 0.21 ng/ml, respectively. Background concentrations of B[a]P in the four soils and  
173 silica sand were below LOD. There were at least 4 replicate analyses per treatment and not  
174 more than 12 per soil sample. Solvent-rinsed clean glass bottles with PTFE-lined caps were  
175 utilised, and they were tightly-capped throughout the experiment. In a preliminary test, p-  
176 Terphenyl-d14 was used as a surrogate to monitor B[a]P losses in freshly spiked soils  
177 throughout experimental steps, including spiking, drying, extraction, vacuum concentration,  
178 and analysis. Recoveries ranging from 80.9 ± 9.3% to 101.9 ± 17.6% were obtained. Potential  
179 B[a]P losses through sample pre-treatment were not corrected for in this study.

180 **Operational Definitions and Data Evaluation.** All operational definitions are presented in  
181 Table S2. The statistical software used for data analysis and graphing were SPSS Statistics  
182 (IBM Corp, version 24) and Origin (Microcal Software Inc., Northampton, MA, USA,  
183 version 6). No data transformations were applied. A one-way ANOVA was used to test the  
184 effects of aging or soil properties on B[a]P extractability before and after REQ at *p* < 0.05.  
185 Tukey HSD and Games Howell post hoc tests were used to identify within group variances

186 when the assumption of homogeneity of variance (Levene's Test) was met or not <sup>21</sup>. Where  
187 data were not normally distributed (as tested by the Shapiro-Wilk Normality Test), Kruskal  
188 Wallis test was applied. A 2-way ANOVA was used to assess the effects of REQ and soil  
189 properties on B[a]P extractability in the 4 soils when Levene's Test of Equality of Error  
190 Variances was not significant ( $p > 0.05$ ). A simple linear regression was used to evaluate the  
191 relationships between soil properties and potentially remobilised B[a]P, and the rate constants  
192 obtained from kinetics modelling.

## 193 RESULTS AND DISCUSSION

194 **Interpretation of Solvent-Extractable B[a]P Fractions.** A detailed interpretation of B[a]P  
195 fractions extracted by the solvents utilised is presented in Table S3. Briefly, BuOH-  
196 extractable B[a]P refers to fractions in soils that may be readily available to microbes,  
197 earthworms, and the human gut <sup>11, 30-33</sup>. Subsequent extraction with DCM/Ace relates to  
198 B[a]P fractions that are weakly and strongly adsorbed but not physically entrapped in soils <sup>11,</sup>  
199 <sup>14, 34</sup>. Total-extractable B[a]P is the sum of fractions extracted by BuOH and DCM/Ace.  
200 Methanolic saponification of pre-extracted soils (after DCM/Ace extraction) results in partial  
201 cleavage of ester and ether bonds of soil humic matter <sup>12, 14, 27</sup>. After cleavage, sequestered  
202 B[a]P molecules in soils may be partially released <sup>25, 26</sup>, resulting in greater recovery.  
203 Potential mechanisms for increases in the extractability of NERs after methanolic  
204 saponification are detailed elsewhere <sup>12, 25, 27</sup>.

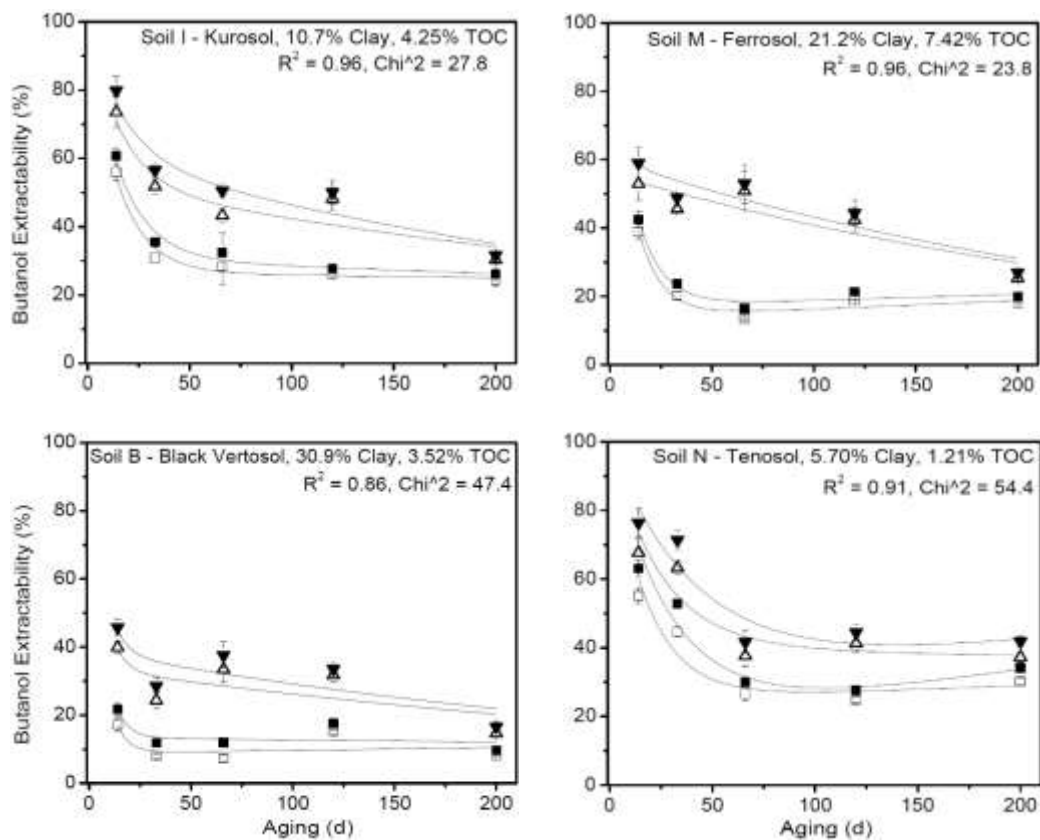
205 **Spike Recovery.** Spike recovery of B[a]P in silica sand was consistently above 95%. In the  
206 4 soils, recovery ranged from  $93.3 \pm 1.5$  to  $118.8 \pm 0.0\%$  ( $n = 2$ ) immediately after spiking  
207 with 1 mg/kg B[a]P; and from  $60.6 \pm 5.6$  to  $87.9 \pm 5.1\%$  ( $n = 2$ ) and  $84.2 \pm 3.4$  to  $97.3 \pm$   
208  $8.0\%$  ( $n = 2$ ) at 10 and 50 mg/kg B[a]P, respectively, 2 d after spiking. The average standard  
209 deviation in recovery for each soil at both spiked concentrations was less than 10%  
210 throughout the experiment and comparable to other studies <sup>11, 25</sup>. Data variability was  
211 attributed to the widely different soil properties <sup>9</sup>. Mass balances for all of the soils, before  
212 and after REQ, are presented in Figure S3. The incomplete mass balances of B[a]P in soils  
213 after 200 d of aging may be attributed to very strongly sequestered B[a]P residues in soils that  
214 were not extractable by MeKOH. The different soil properties in addition to the difficulty of  
215 artificially spiking HOCs, may result in spike heterogeneity in soils. Except for soil B (sandy-  
216 clay-loam) which showed a relatively low recovery, percentage recovery as well as the

217 observed data variability were similar to those documented in other studies<sup>25, 29</sup>, and thus  
218 validated the spiking and extraction procedures. Benzo[a]pyrene is intrinsically hydrophobic  
219 (Log octanol-water partition coefficient > 6.0) and has a very low aqueous solubility<sup>35</sup>,  
220 which may limit its transfer to the soil surface or aqueous phase, resulting in low  
221 bioavailability. Further, because of its strong partitioning to soil, low volatility, low water  
222 solubility and its molecular structure, B[a]P will be resistant to biodegradation<sup>8, 36, 37</sup>, and  
223 therefore persist in soil<sup>10</sup>. Low PAH extractabilities have also been reported in organic  
224 matter rich-clayey soils which were freshly spiked<sup>30</sup>, and incomplete mass balances of B[a]P  
225 in soils after 160 d and 200 d of aging<sup>25, 29</sup>. Overall, decrease in B[a]P extractability after  
226 aging was attributed to sequestration processes<sup>29</sup>, although losses from biodegradation may  
227 not be completely disregarded.

#### 228 **Differences in B[a]P Extractability in Soils with Aging before re-equilibration.**

229 Generally, B[a]P extractability in soils spiked at 50 mg/kg was consistently greater than in  
230 soils spiked at 10 mg/kg (Figure 1 and Table S4), which agrees with previous studies<sup>38</sup>.  
231 Total-extractable B[a]P was also greater than BuOH-extractable B[a]P. For BuOH, the  
232 amounts of B[a]P extracted from each soil significantly decreased ( $p < 0.001$ ) with aging, as  
233 shown by the first-order kinetic model ( $R^2 = 0.80 - 0.94$ ) (Figure 1 and Table S5). The  
234 modelled  $k$  values (Table S5) agreed with those ( $10^{-3}$ ) reported for slowly-desorbing PAH  
235 fractions<sup>23, 39</sup>, and did not differ ( $p > 0.05$ ) between the spiked B[a]P concentrations. In  
236 contrast, subsequent DCM/Ace-extractable B[a]P significantly increased ( $p < 0.05$ ) with  
237 aging, especially in the sandy-clay-loam organic matter-rich soil B and organic matter-rich  
238 soil M, suggesting that B[a]P became quickly and progressively sequestered in the soils. The  
239 amounts of total-extractable B[a]P (mg/kg) generally decreased rapidly ( $p < 0.05$ ), whereas  
240 NERs increased (Table S4). Although total B[a]P extractability tended to decrease

241 continuously or plateau beyond 33 and 66 d of aging, subsequent changes were not generally  
242 significant ( $p > 0.05$ ).

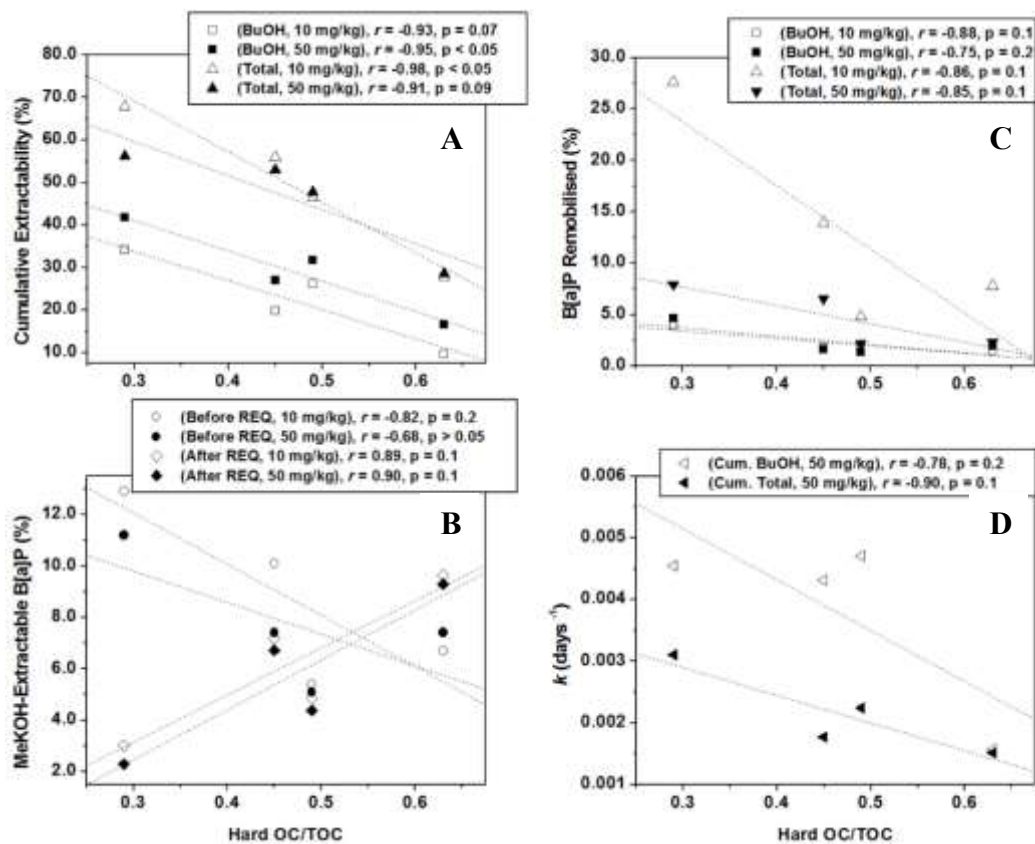


243

244 **Figure 1.** Exponential curve-fitting of observed B[a]P BuOH extractability in soil before and  
245 after re-equilibration. Open and shaded symbols represent B[a]P concentrations (%) before  
246 and after re-equilibration, respectively. Spiked B[a]P concentrations: 10 mg/kg ( $\square$ ) and 50  
247 mg/kg ( $\Delta$ ). TOC is total organic carbon. Values are means of 4 replicates  $\pm$  standard  
248 deviations.

249 Readily available PAHs in soils often tend to decrease or stabilise after approximately 30 d of  
250 aging<sup>23</sup>. Soils with various sites for PAH sequestration, such as black carbon, organic  
251 materials in soils, and clay, are likely to show greater PAH sequestration than soils without or  
252 with less of these sequestration sites<sup>38</sup>. The fractions of hard OC as a percentage of TOC, and  
253 the contents of clay in soils showed negative correlations ( $r > -0.90$ ) with B[a]P extractability

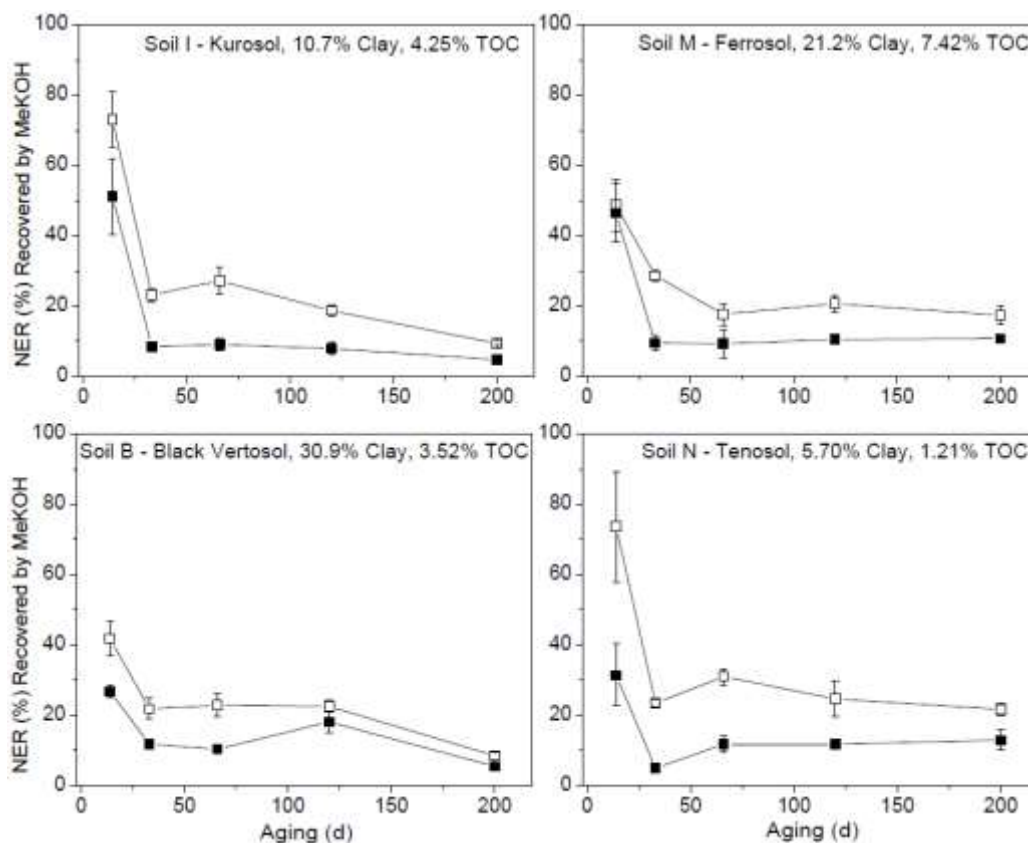
254 (Figure 2A and Figure S4), suggesting decreased B[a]P extractability in soils with large  
 255 amounts of hard OC.



256  
 257 **Figure 2.** Correlations between hard OC fractions in soils and B[a]P extractability and  
 258 sequestration. Cum. is cumulative extractability after re-equilibration (REQ).

259 Detectable amounts of highly sequestered B[a]P were found in all soils from 14 d of aging  
 260 (Table S4) indicating rapid B[a]P sequestration. Benzo[a]pyrene NERs ranged from 5.8 – 8.0  
 261 mg/kg (10 mg/kg B[a]P) and from 25.8 – 36.9 mg/kg (50 mg/kg B[a]P) after 200 d of aging.  
 262 At 200 d of aging, B[a]P NERs recovered by MeKOH ranged from 8 – 22% (Figure 3), with  
 263 the more sandy soils N and I showing larger recoveries than the sandy-clay-loam soil B or  
 264 organic matter-rich soil M (Figure 3). This indicates that B[a]P residues in more sandy soils  
 265 with less amounts of hard OC were less sequestered (Figure 2B), and may have greater  
 266 potential to be remobilised than in the sandy-clay-loam organic matter-rich soils (Figure 2C).  
 267 The remaining B[a]P residues (78 to 92%) not extractable by MeKOH were regarded as very

268 strongly sequestered with very low potential for remobilisation.



269

270 **Figure 3.** Amount (%) of B[a]P NER recovered by MeKOH before and after re-equilibration.

271 Only soils spiked at 10 mg/kg B[a]P. Open and shaded symbols represent B[a]P

272 concentrations (%) before and after re-equilibration, respectively. TOC is total organic

273 carbon. Values are means of 4 replicates  $\pm$  standard deviations.

274 No obvious correlations were observed between soft OC fractions and B[a]P extractability

275 and sequestration. Also, the strong positive relationships between hard OC and BC-rich

276 fractions (Figure S5), as well as between soft OC and nonpyrogenic OC (Figure S5) indicate

277 that increasing amounts of recalcitrant OC fractions in soils influenced B[a]P extractability

278 and sequestration in soils, as with clay contents (Figure S4). In addition, a strong positive

279 relationship ( $r > 0.8$ ) was observed between the recalcitrant fractions and clay contents which

280 may indicate that B[a]P sequestration sites in the soils may be a continuum comprising

281 mostly hard OC fractions, clay, and clay-associated OC fractions (Figure S6). Therefore, the

282 extents of B[a]P extractability and sequestration in the soils may differ depending on its  
283 interactions with these sequestration sites. A wider range of soils may be needed to validate  
284 the observed relationships, hence interpretation of soil properties and B[a]P extractability and  
285 sequestration in this study should be treated with caution.

286 Over time, the decreasing trend in total B[a]P extractability or increasing trend in the NERs  
287 further indicates progressive B[a]P sequestration, particularly in soils with larger amounts of  
288 hard OC fractions, clay or clay-associated OC fractions. Aging effects on B[a]P extractability  
289 in soils before REQ have been well reported<sup>11, 22, 25</sup>. The interested reader is directed to the  
290 SI for further discussions.

#### 291 **Differences in B[a]P Remobilisation in Soils with Aging and Associated Rates of Loss.**

292 *Effects of Re-equilibration Time.* The re-equilibration time of 30 or 60 d significantly  
293 influenced ( $p < 0.05$ ) the amounts of B[a]P extracted by each of the solvents, as cumulative  
294 extractability after REQ was consistently greater than prior to REQ in all 4 soils (Table S4).  
295 However, the effects of either a 30 d REQ or a 60 d REQ on B[a]P extractability at 14 and 33  
296 d of aging were statistically similar ( $p > 0.05$ ) (Figures S7 and S8). Hence, pre-extracted soils  
297 after 33 d of aging were re-equilibrated for 30 d only. Another study also reported that the  
298 amounts of anthracene, benzo[k]fluoranthene and B[a]P repartitioned in an XAD-treated  
299 sediment did not differ after 30 or 90 d of incubation<sup>19</sup>.

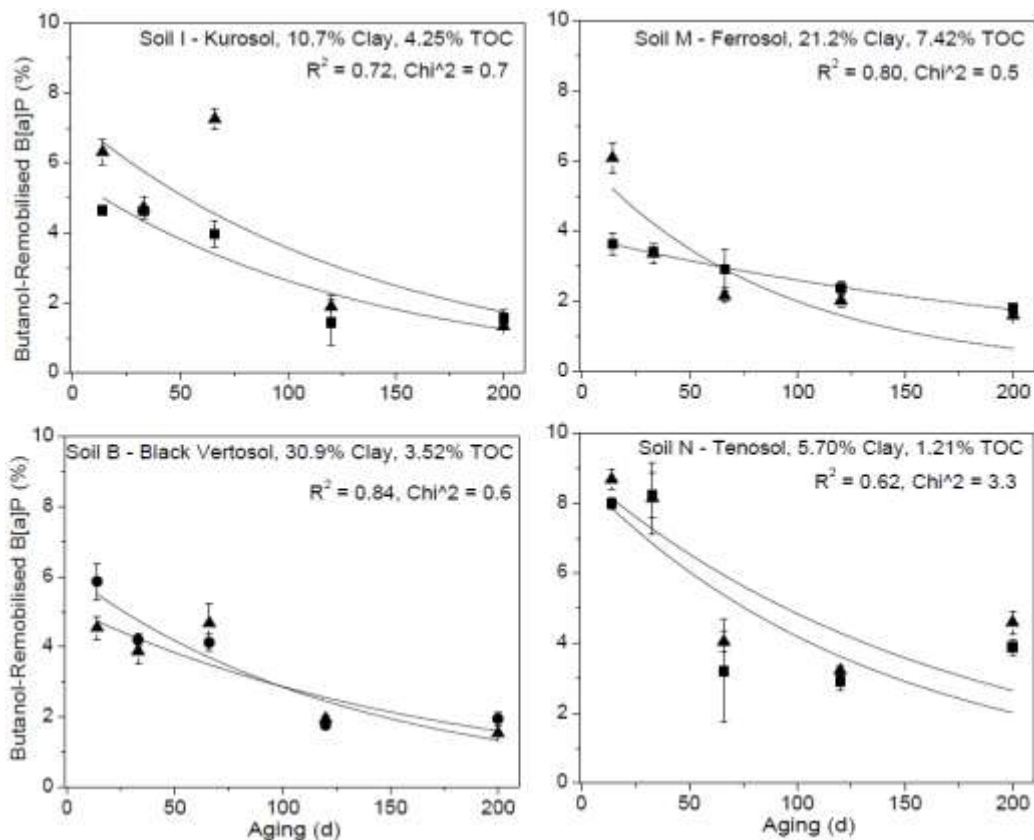
300 The increase in cumulative total-extractable B[a]P after REQ at each aging period in this  
301 current study also implied that B[a]P NERs remaining in soils after REQ were significantly  
302 more diminished ( $p < 0.001$ ) when compared to the NERs before REQ. This is a further  
303 indication that B[a]P NERs became re-extractable after REQ. The amounts of total-  
304 remobilised B[a]P in soils were greater than BuOH-remobilised B[a]P (Figures 2C and 3),  
305 however, aging significantly decreased the amounts of B[a]P remobilised (Table S6).



306 The rates ( $k$ ,  $d^{-1}$ ) at which the remobilised B[a]P fractions decreased over time range from  
307  $0.0040 \pm 0.0001 - 0.007 \pm 0.002$ , and  $0.006 \pm 0.003 - 0.011 \pm 0.005$  in soils spiked with 10  
308 mg/kg and 50 mg/kg B[a]P, respectively (Table S5). Although the  $k$  values of the remobilised  
309 B[a]P fractions aligned with  $k$  values before REQ ( $10^{-3} d^{-1}$ ), the former were 2 to 4 times  
310 faster than the latter. This suggests that, over time, potentially remobilised B[a]P in soils may  
311 decrease very rapidly, particularly in long-term field-contaminated soils. Correlational  
312 analyses also suggest that the rates at which remobilisable B[a]P fractions decrease over time  
313 would be slower in soils with larger hard OC and clay contents (Figure 2D and Figure S9),  
314 further indicating that potentially remobilisable amounts are not changing substantially over  
315 time since B[a]P NERs are more strongly sequestered in these soils.

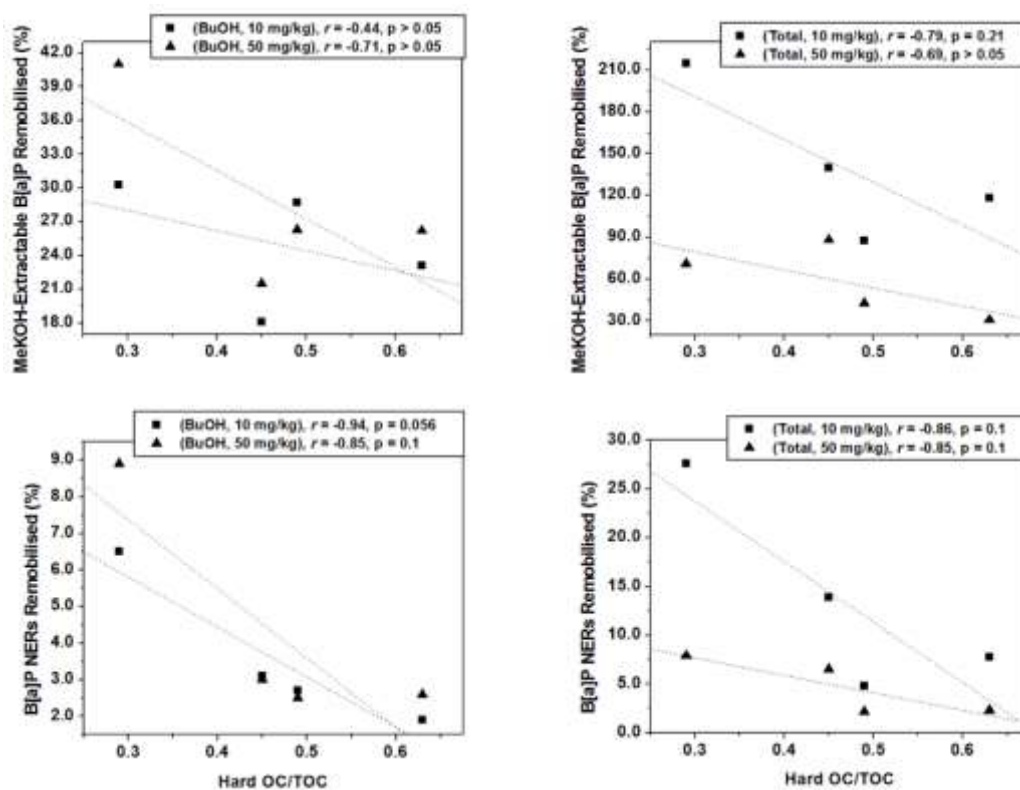
316 *Effects of Spiked Concentrations and Soil Properties.* Student's t-test showed that the  
317 amounts (%) of B[a]P remobilised after REQ by BuOH from each soil spiked at 10 and 50  
318 mg/kg differed particularly during earlier aging times (Figure 4). The amounts of B[a]P  
319 NERs remobilised by BuOH from 14 d to 200 d of aging ranged from 2 – 7% (10 mg/kg  
320 B[a]P) and 2 – 9% (50 mg/kg B[a]P) (Figure 4), and were largest in the more sandy soils.  
321 After 200 d of aging, the amounts of B[a]P remobilised by BuOH in the 4 soils were reduced  
322 and generally similar (Figure 4). For the subsequent DCM/Ace extraction, the amounts  
323 (mg/kg) of B[a]P remobilised increased significantly ( $p < 0.05$ ) especially until 66 d in soils  
324 I, M, and B after which remobilised B[a]P (mg/kg) decreased significantly ( $p < 0.05$ ) or  
325 remained unchanged. Amounts of B[a]P remobilised in soil N increased significantly ( $p <$   
326  $0.05$ ) from 14 d (0.26 mg/kg) to 200 d (2.37 mg/kg), especially at 10 mg/kg B[a]P. Increases  
327 in the DCM/Ace-remobilisable B[a]P with aging further indicate the loss BuOH-  
328 remobilisable B[a]P in soils. The amounts (mg/kg) of total-remobilised (BuOH + DCM/Ace)  
329 generally decreased ( $p < 0.05$ ) from 14 d to 200 d of aging as with BuOH extractability  
330 (Table S6). The amounts of B[a]P totally remobilised differed significantly ( $p < 0.05$ )

331 between all 4 soils at each aging time; these amounts ranged from 8% (0.5 mg/kg) to 46%  
 332 (2.8 mg/kg) of B[a]P NERs (10 mg/kg B[a]P) and from 4% (1.1 mg/kg) to 15% (4.0 mg/kg)  
 333 (50 mg/kg B[a]P) throughout the aging period. The amounts of B[a]P NERs totally  
 334 remobilised were again generally greater in soils I and N compared to soils M and B (Figure  
 335 5), further indicating the influence of soil properties.



336  
 337 **Figure 4.** Exponential curve-fitting of B[a]P remobilised (%) after re-equilibration through  
 338 aging in soils. Spiked B[a]P concentrations: 10 mg/kg (■) and 50 mg/kg (▲). TOC is total  
 339 organic carbon. Values are means of 4 replicates  $\pm$  standard deviations.  
 340 Only soils M and N spiked at 10 mg/kg had greater amounts of remobilised B[a]P (mg/kg)  
 341 after 200 d of aging ( $1.39 \pm 0.05$  and  $2.76 \pm 0.03$ , respectively) compared to that remobilised  
 342 at 14 d ( $1.08 \pm 0.06$  and  $1.06 \pm 0.03$ ), although remobilised amounts remained low. Overall,  
 343 BuOH- and total-remobilisable B[a]P (mg/kg) were small in the four soils, especially after  
 344 200 d of aging.

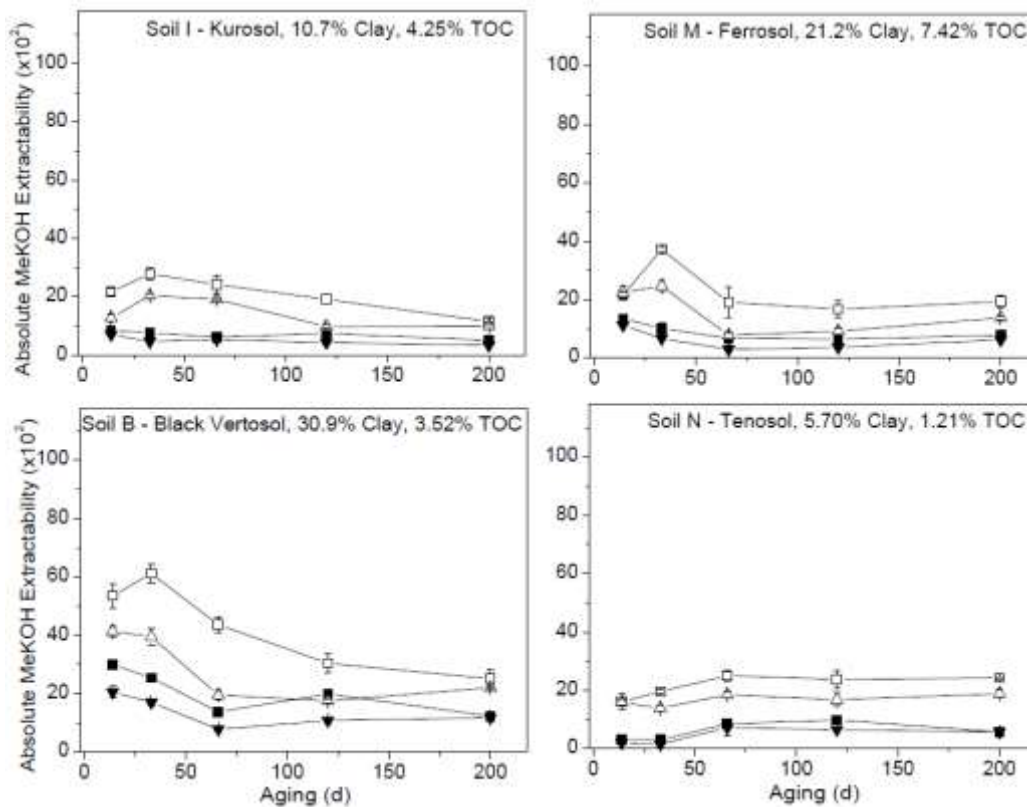
345 Further, there were negative relationships between the amounts of B[a]P remobilised from  
 346 B[a]P NERs or from highly sequestered residues recovered by MeKOH and the fractions of  
 347 hard OC in soils (Figure 5), and clay (Figure S9). Intercompartmental repartitioning of B[a]P  
 348 NERs from strongly sequestered sites (BC-rich or hard OC-rich) into weakly sequestered  
 349 sites where they are readily available may occur at very slow rates<sup>19</sup>. Hence, the amounts of  
 350 repartitioned and potentially remobilisable B[a]P were small, particularly in aged sandy-clay  
 351 loam soils with BC-rich fractions.



352  
 353 **Figure 5.** Remobilised B[a]P fractions relative to the amounts of highly sequestered B[a]P  
 354 NERs recovered by MeKOH and the amounts of NERs in soils.

355 After remobilisation in soils which had been re-equilibrated, the highly sequestered B[a]P  
 356 residues (MeKOH-extractable) decreased significantly ( $p < 0.05$ ) compared to those before  
 357 REQ (Tables S4 and S6), as well as the percentage of the NERs recovered (Figure 3). This  
 358 was an interesting finding because it indicated remobilisation of NERs. The decreasing trend

359 of the NERs after REQ further supports B[a]P remobilisation from NERs in pre-extracted  
 360 soils. Also, increasing absolute amounts of the sequestered fractions (Table S2) indicate  
 361 progressive transfer of B[a]P into more strongly sequestered fractions over time<sup>26</sup>. In this  
 362 study, absolute amounts of sequestered fractions were smaller after REQ than before REQ  
 363 (Figure 6), indicating remobilisation of B[a]P NERs.



364

365 **Figure 6.** Absolute amounts of the highly sequestered B[a]P recovered before and after re-  
 366 equilibration. Open and shaded symbols represent absolute amounts before and after re-  
 367 equilibration, respectively. Spiked B[a]P concentrations: 10 mg/kg (□) and 50 mg/kg (Δ).

368 TOC is total organic carbon. Values are means of 4 replicates  $\pm$  standard deviations and were  
 369 normalised ( $\times 10^2$ ).

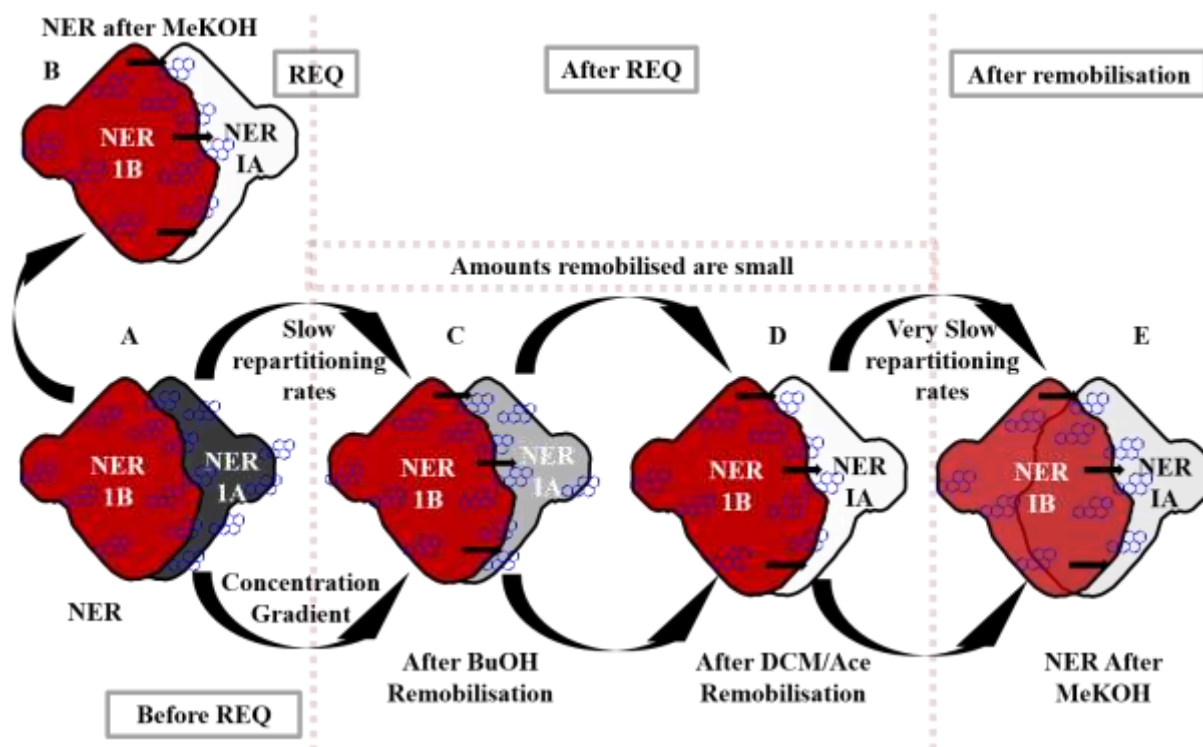
370 **Estimated Half-Life of B[a]P Loss in Soil.** The estimated half-lives of B[a]P loss in soils  
 371 based on BuOH extractability, before and after REQ, ranged from 93 – 442 and 91– 221 d at  
 372 10 and 50 mg/kg spiked concentrations, respectively (Table S5). For total extractability

373 (Table S5), the half-lives of B[a]P loss in soils ranged from 125 – 866 d (10 mg/kg B[a]P)  
374 and from 92 – 459 d (50 mg/kg). The half-lives of B[a]P loss were generally shorter for the  
375 more sandy soils I and N compared to the sandy-clay-loam organic matter-rich soil B and  
376 organic matter-rich soil M (Table S5) especially at 50 mg/kg, further confirming the  
377 influence of soil properties and B[a]P concentration on B[a]P loss in soils. The half-life of  
378 B[a]P loss in soils estimated in this study agrees with other studies' findings: for example,  
379 208 – 254 d in sterile sewage-sludge amended soil with radiolabelled B[a]P aged for 525 d <sup>25</sup>;  
380 45 – 181 d in soils aged for 160 d <sup>29</sup>; and 1155 d in more than a decade old aged  
381 contaminated agricultural soil <sup>28</sup>.

382 **Towards a Potential Mechanism of Remobilisation of B[a]P NERs in Soils.** This study  
383 did not focus on differentiating NERs into their different types. The MeKOH-extractable B[a]P  
384 fractions may be associated with Type I NERs in soils. The NERs described here may be linked  
385 with hard OC fractions, clay, or clay-associated OC in soils. The influence of hard OC and  
386 clay-associated OC fractions on PAH sequestration has been previously documented <sup>8, 23, 40, 41</sup>.  
387 Hard OC fractions contribute substantially to PAH desorption hysteresis <sup>8, 9</sup>. Clay surfaces with  
388 OC patches may also have better sequestration capacities for PAHs in soils than bare SOM or  
389 clay <sup>42</sup>. Due to the heterogeneous nature of sequestration sites in soils <sup>43</sup>, non-covalently  
390 sequestered PAHs may be potentially released at different rates and extents. This may depend  
391 on the strength of associated interactions (Van der Waals, hydrophobic, hydrogen bonding, and  
392  $\pi - \pi$  interactions) <sup>12</sup>. These interactions are influenced by the amounts and quality of OC (hard  
393 or soft OC) and clay type (e.g. montmorillonite and kaolin) <sup>44-46</sup>. Hence, Type I NERs (parent  
394 compounds and metabolites) in this study may not be discreet fractions. Instead they may be a  
395 continuum of NERs subjected to different degrees of sequestration in soils where Type 1A is  
396 less sequestered than Type 1B (Figure 7).

397 Relative to the amounts of highly sequestered fractions (MeKOH-extractable) before REQ,  
398 the amounts of B[a]P remobilised ranged from 18% to 30% (10 mg/kg B[a]P) and from 21%  
399 to 41% (50 mg/kg B[a]P) (Figure 5). However, amounts of B[a]P totally-remobilised from the  
400 MeKOH-extractable fractions ranged from 86% to 214% (10 mg/kg) and 31% to 71% (50  
401 mg/kg) (Figure 5). These observations suggest that small to large percentages of the highly  
402 sequestered fractions recovered before REQ by MeKOH were remobilised. The very high  
403 B[a]P remobilisation (up to 214%) in some soils indicated that the sequestered fractions in soils  
404 before REQ (Type 1A) may have been replenished by repartitioning of very strongly  
405 sequestered fractions (Type 1B) during the REQ period. This is explained in more detail below.

406 Following total remobilisation (BuOH + DCM/Ace) of B[a]P after REQ (Figure 7), the  
407 amounts of B[a]P NERs remaining in soils ranged from 4.4 mg/kg to 7.2 mg/kg (10 mg/kg  
408 B[a]P), and from 23.5 mg/kg to 35.8 mg/kg (50 mg/kg B[a]P). However, the amounts of B[a]P  
409 NERs in soils recovered by MeKOH after total remobilisation ranged from 5% (0.25 mg/kg)  
410 to 13% (0.5 mg/kg) at 10 mg/kg B[a]P, and from 3% (0.9 mg/kg) to 8% (1.9 mg/kg) at 50  
411 mg/kg B[a]P (Figure 4E). These small amounts (0.25 to 0.5 mg/kg) of highly sequestered B[a]P  
412 fractions (MeKOH-extractable) that were still recoverable after total-remobilisation (10  
413 mg/kg) (Figure 7), even though these fractions were completely depleted previously (i.e.  
414 214%), may indicate that very small amounts of more strongly sequestered B[a]P NER  
415 fractions (Type 1B) repartitioned slowly into the less sequestered forms (Type 1A) with very  
416 low potential to be remobilised (Figure 4E). Slow repartitioning rates ( $3.40 \times 10^{-3}$ ) of strongly  
417 sequestered PAHs into less sequestered PAH fractions in field-contaminated sediments have  
418 been reported<sup>19</sup>. Overall, the amounts remobilised in soils after 200 d of aging were very small,  
419 and may be much smaller in long-term PAH-contaminated soils.



420

421 **Figure 7.** Proposed mechanism of remobilisation of B[a]P non-extractable residues (NERs) in  
 422 soils after re-equilibration (REQ) through slow intercompartmental partitioning from strongly  
 423 sequestered to less sequestered fractions. BuOH = butanol, DCM/Ace =  
 424 dichloromethane/acetone, MeKOH = methanolic saponification. A = non-covalently  
 425 sequestered B[a]P NER (parent compound or metabolites) comprising Types IA (less strongly  
 426 sequestered) or Type IB (more strongly sequestered) before REQ; B = Soil humic matter  
 427 disaggregated by MeKOH partially releasing B[a]P NERs (Likely Type IA); MeKOH-  
 428 extractable B[a]P before REQ is an indication of the amounts of highly sequestered B[a]P that  
 429 are potentially remobilisable following a REQ period. As a consequence of removing total-  
 430 extractable fractions in soils, a concentration gradient results between readily available and  
 431 sequestered compartments. During REQ of B[a]P NERs in soils, sequestered B[a]P (Type 1A)  
 432 may repartition into readily available fractions at slow rates and low extents. C. Small amounts  
 433 of BuOH-extractable B[a]P after the repartitioning process; D. Small amounts of total-  
 434 extractable B[a]P after the repartitioning process. The remaining soil residue at this point is

435 similar to that after MeKOH extraction in B; E. MeKOH-extractable B[a]P relating to the  
436 previously repartitioned fractions that have not been completely depleted after DCM/Ace  
437 extraction (Type 1A) and/or newly repartitioned fractions (very low amounts and very slow  
438 rates) from strongly sequestered fractions (Type 1B) after complete depletion of previously  
439 repartitioned fractions. Note that the amounts of B[a]P NERs in this scheme may be  
440 overestimated, and that amounts repartitioned and potentially remobilised are likely to be very  
441 small and associated rates very slow, particularly in long-term contaminated soils. Also note  
442 that Type IA and Type 1B were depicted as discreet fractions for simplicity; in reality, they are  
443 a continuum.

444 **Does the very limited Remobilisation of B[a]P NERs in Soils have Implications for**  
445 **Contaminated Land Decision-Making?** Understanding the potential for remobilisation of  
446 NERs in soils may be important in risk assessment <sup>47</sup>, particularly where NER concentrations  
447 exceed regulatory guideline values (5.8 to 36.9 mg/kg in this study); however, only a very  
448 small portion of the NERs may be remobilisable (Type I NERs). Therefore, it is important to  
449 consider the concentrations of NERs released or remobilised in soils for effective risk  
450 assessment and contaminated land decision-making, rather than consider the potential for  
451 release only. In Australia, for example, site-specific risk assessment and characterisation of  
452 contaminated soils considers bioaccessibility <sup>48</sup>, particularly for metals and metalloids <sup>49</sup>. The  
453 recently amended health investigation levels (HILs) for B[a]P in contaminated soils range  
454 from 3 to 40 mg/kg, based on B[a]P toxic equivalence factors, and are dependent on land use  
455 <sup>50</sup>. Clearly, the concentrations of B[a]P NERs remobilised in soils by BuOH and exhaustive  
456 extractions were generally below 3 mg/kg in this study. From a potential remobilisation  
457 perspective, if only BuOH-extractable concentrations were considered, all soils would pass  
458 the HIL criteria and risks to human health may be acceptable. However, not all soils would  
459 pass the criteria if total-extractable concentrations were used. Based on this study, B[a]P



460 NERs in soils have the potential to be released at low concentrations over a long period of  
461 time further reducing associated risks to human and environmental health.

#### 462 ASSOCIATED CONTENT

463 **Supporting Information.** The supporting information is available free of charge. Additions  
464 to Results and Discussion including: differences in B[a]P extractability in soils with aging;  
465 preliminary trends between soil properties and B[a]P extractability and sequestration; and  
466 supporting figures and tables; and references.

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479 Notes

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488 REFERENCES

- 489 1. *Agents Classified by the IARC Monographs*; In Working Group on the Evaluation of  
490 Carcinogenic Risks to Humans: International Agency for Research on Cancer: Lyon, France,  
491 2012; <https://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-14.pdf>.
- 492 2. Lemieux, C. L.; Long, A. S.; Lambert, I. B.; Lundstedt, S.; Tysklind, M.; White, P. A.  
493 Cancer risk assessment of polycyclic aromatic hydrocarbon contaminated soils determined  
494 using bioassay-derived levels of Benzo[a]pyrene equivalents. *Environ. Sci. Technol.* **2015**, *49*,  
495 (3), 1797-1805; DOI 10.1021/es504466b.
- 496 3. Nguyen, T. C.; Loganathan, P.; Nguyen, T. V.; Vigneswaran, S.; Kandasamy, J.; Slee,  
497 D.; Stevenson, G.; Naidu, R. Polycyclic aromatic hydrocarbons in road-deposited sediments,  
498 water sediments, and soils in Sydney, Australia: Comparisons of concentration distribution,  
499 sources and potential toxicity. *Ecotox. Environ. Safe.* **2014**, *104*, 339-348; DOI  
500 10.1016/j.ecoenv.2014.03.010.
- 501 4. Cachada, A.; Ferreira da Silva, E.; Duarte, A. C.; Pereira, R. Risk assessment of urban  
502 soils contamination: The particular case of polycyclic aromatic hydrocarbons. *Sci. Total*  
503 *Environ.* **2016**, *551–552*, 271-284; DOI <http://dx.doi.org/10.1016/j.scitotenv.2016.02.012>.
- 504 5. Krauss, M.; Wilcke, W.; Zech, W. Availability of polycyclic aromatic hydrocarbons  
505 (PAHs) and polychlorinated biphenyls (PCBs) to earthworms in urban soils. *Environ. Sci.*  
506 *Technol.* **2000**, *34*, (20), 4335-4340; DOI 10.1021/es001137s.
- 507 6. Thavamani, P.; Megharaj, M.; Krishnamurti, G. S. R.; McFarland, R.; Naidu, R. Finger  
508 printing of mixed contaminants from former manufactured gas plant (MGP) site soils:  
509 Implications to bioremediation. *Environ. Int.* **2011**, *37*, (1), 184-189; DOI  
510 10.1016/j.envint.2010.08.017.

- 511 7. Semple, K.; Morriss, A.; Paton, G. Bioavailability of hydrophobic organic  
512 contaminants in soils: Fundamental concepts and techniques for analysis. *Eur. J. Soil Sci.* **2003**,  
513 *54*, (4), 809-818; DOI <https://doi.org/10.1046/j.1351-0754.2003.0564.x>.
- 514 8. Cornelissen, G.; Gustafsson, O.; Bucheli, T.; Jonker, M.; Koelmans, A.; Van Noort, P.  
515 Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and  
516 soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation.  
517 *Environ. Sci. Technol.* **2005**, *39*, (18), 6881-6895; DOI 10.1021/es050191b.
- 518 9. Luthy, R.; Aiken, G.; Brusseau, M.; Cunningham, S.; Gschwend, P.; Pignatello, J.;  
519 Reinhard, M.; Traina, S.; Weber, W.; Westall, J. Sequestration of hydrophobic organic  
520 contaminants by geosorbents. *Environ. Sci. Technol.* **1997**, *31*, (12), 3341-3347; DOI  
521 10.1021/es970512m.
- 522 10. Pignatello, J. J.; Xing, B. S. Mechanisms of slow sorption of organic chemicals to  
523 natural particles. *Environ. Sci. Technol.* **1996**, *30*, (1), 1-11; DOI 10.1021/es940683g.
- 524 11. Duan, L. C.; Palanisami, T.; Liu, Y. J.; Dong, Z. M.; Mallavarapu, M.; Kuchel, T.;  
525 Semple, K. T.; Naidu, R. Effects of ageing and soil properties on the oral bioavailability of  
526 benzo[a]pyrene using a swine model. *Environ. Int.* **2014**, *70*, 192-202; DOI  
527 10.1016/j.envint.2014.05.017.
- 528 12. Kastner, M.; Nowak, K. M.; Miltner, A.; Trapp, S.; Schaffer, A. Classification and  
529 modelling of nonextractable residue (NER) formation of xenobiotics in soil - A synthesis. *Crit.*  
530 *Rev. Env. Sci. Tec.* **2014**, *44*, (19), 2107-2171; DOI 10.1080/10643389.2013.828270.
- 531 13. Umeh, A. C.; Duan, L.; Naidu, R.; Semple, K. T. Residual hydrophobic organic  
532 contaminants in soil: Are they a barrier to risk-based approaches for managing contaminated  
533 land? *Environ. Int.* **2017**, *98*, 18-34; DOI <https://doi.org/10.1016/j.envint.2016.09.025>.
- 534 14. Eschenbach, A. Characterization of non-extractable residues for their risk assessment  
535 in soil with special regard to pharmaceuticals. In *International Workshop - Pharmaceuticals in*

- 536 *Soil, Sludge and Slurry*, Dessau, 2013;  
537 [https://www.umweltbundesamt.de/sites/default/files/medien/376/dokumente/eschenbach\\_presentation.pdf](https://www.umweltbundesamt.de/sites/default/files/medien/376/dokumente/eschenbach_presentation.pdf).  
538
- 539 15. Gevao, B.; Mordaunt, C.; Semple, K. T.; Pearce, T. G.; Jones, K. C. Bioavailability of  
540 nonextractable (bound) pesticide residues to earthworms. *Environ. Sci. Technol.* **2001**, *35*, (3),  
541 501-507; DOI 10.1021/es000144d.
- 542 16. Eschenbach, A.; Wienberg, R.; Mahro, B. Fate and stability of nonextractable residues  
543 of [(14)C]PAH in contaminated soils under environmental stress conditions. *Environ. Sci.*  
544 *Technol.* **1998**, *32*, (17), 2585-2590; DOI 10.1021/es9708272.
- 545 17. Gao, Y.; Yuan, X.; Lin, X.; Sun, B.; Zhao, Z. Low-molecular-weight organic acids  
546 enhance the release of bound PAH residues in soils. *Soil Tillage Res.* **2015**, *145*, 103-110; DOI  
547 <https://doi.org/10.1016/j.still.2014.09.008>.
- 548 18. Gao, Y.; Hu, X.; Zhou, Z.; Zhang, W.; Wang, Y.; Sun, B. Phytoavailability and  
549 mechanism of bound PAH residues in field contaminated soils. *Environ. Pollut.* **2017**, *222*,  
550 465-476; DOI <https://doi.org/10.1016/j.envpol.2016.11.076>.
- 551 19. Birdwell, J. E.; Thibodeaux, L. J. PAH repartitioning in field-contaminated sediment  
552 following removal of the labile chemical fraction. *Environ. Sci. Technol.* **2009**, *43*, (21), 8092-  
553 8097; DOI 10.1021/es9016798.
- 554 20. Duan, L. C.; Naidu, R. Effect of ionic strength and index cation on the sorption of  
555 phenanthrene. *Water Air Soil Poll.* **2013**, *224*, (12), 1700; DOI [https://doi.org/10.1007/s11270-](https://doi.org/10.1007/s11270-013-1700-4)  
556 [013-1700-4](https://doi.org/10.1007/s11270-013-1700-4).
- 557 21. Field, A. P. *Discovering Statistics Using IBM SPSS Statistics: And Sex And Drugs And*  
558 *Rock 'N' Roll*. Sage: Los Angeles, U.S.A., 2013.

- 559 22. Umeh, A. C.; Duan, L.; Naidu, R.; Semple, K. T. Comparison of single- and sequential-  
560 solvent extractions of total extractable benzo[a]pyrene fractions in contrasting soils. *Anal.*  
561 *Chem.* **2018**; DOI 10.1021/acs.analchem.8b03387.
- 562 23. Luo, L.; Lin, S.; Huang, H. L.; Zhang, S. Z. Relationships between aging of PAHs and  
563 soil properties. *Environ. Pollut.* **2012**, *170*, 177-182; DOI 10.1016/j.envpol.2012.07.003.
- 564 24. Edmondson, J. L.; Stott, I.; Potter, J.; Lopez-Capel, E.; Manning, D. A. C.; Gaston, K.  
565 J.; Leake, J. R. Black carbon contribution to organic carbon stocks in urban soil. *Environ. Sci.*  
566 *Technol.* **2015**, *49*, (14), 8339-8346; DOI 10.1021/acs.est.5b00313.
- 567 25. Northcott, G. L.; Jones, K. C. Partitioning, extractability, and formation of  
568 nonextractable PAH residues in soil. 1. Compound differences in aging and sequestration.  
569 *Environ. Sci. Technol.* **2001**, *35*, (6), 1103-1110; DOI 10.1021/es000071y.
- 570 26. Northcott, G. L.; Jones, K. C. Validation of procedures to quantify nonextractable  
571 polycyclic aromatic hydrocarbon residues in soil. *J. Environ. Qual.* **2003**, *32*, (2), 571-582;  
572 DOI doi:10.2134/jeq2003.5710.
- 573 27. Eschenbach, A.; Kastner, M.; Bierl, R.; Schaefer, G.; Mahro, B. Evaluation of a new,  
574 effective method to extract polycyclic aromatic-hydrocarbons from soil samples. *Chemosphere*  
575 **1994**, *28*, (4), 683-692; DOI 10.1016/0045-6535(94)90219-4.
- 576 28. Doick, K. J.; Klingelmann, E.; Burauel, P.; Jones, K. C.; Semple, K. T. Long-term fate  
577 of polychlorinated biphenyls and polycyclic aromatic hydrocarbons in an agricultural soil.  
578 *Environ. Sci. Technol.* **2005**, *39*, (10), 3663-3670; DOI 10.1021/es048181i.
- 579 29. Duan, L. C.; Naidu, R.; Liu, Y. J.; Palanisami, T.; Dong, Z. M.; Mallavarapu, M.;  
580 Semple, K. T. Effect of ageing on benzo[a]pyrene extractability in contrasting soils. *J. Hazard.*  
581 *Mater.* **2015**, *296*, 175-184; DOI 10.1016/j.jhazmat.2015.04.050.

- 582 30. Swindell, A. L.; Reid, B. J. Comparison of selected non-exhaustive extraction  
583 techniques to assess PAH availability in dissimilar soils. *Chemosphere* **2006**, *62*, (7), 1126-  
584 1134; DOI 10.1016/j.chemosphere.2005.05.047.
- 585 31. Gomez-Eyles, J. L.; Collins, C. D.; Hodson, M. E. Relative proportions of polycyclic  
586 aromatic hydrocarbons differ between accumulation bioassays and chemical methods to predict  
587 bioavailability. *Environ. Pollut.* **2010**, *158*, (1), 278-284; DOI 10.1016/j.envpol.2009.07.012.
- 588 32. *PAH Interactions with Soil and Effects on Bioaccessibility and Bioavailability to*  
589 *Humans*; Final report Department of Defense Strategic Environmental Research and  
590 Development Program (SERDP) project ER-1743: Exponent, Inc., Boulder, U.S.A., 2017;  
591 <https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Risk>  
592 [Assessment/ER-1743](https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Risk).
- 593 33. Liste, H.; Alexander, M. Butanol extraction to predict bioavailability of PAHs in soil.  
594 *Chemosphere* **2002**, *46*, (7), 1011-1017; DOI 10.1016/S0045-6535(99)00216-7.
- 595 34. Macleod, C. J. A.; Semple, K. T. Sequential extraction of low concentrations of pyrene  
596 and formation of non-extractable residues in sterile and non-sterile soils. *Soil Biol. Biochem.*  
597 **2003**, *35*, (11), 1443-1450; 10.1016/S0038-0717(03)00238-4.
- 598 35. Wild, S. R.; Jones, K. C. Polynuclear aromatic hydrocarbons in the United Kingdom  
599 environment: A preliminary source inventory and budget. *Environ Pollut.* **1995**, *88*, (1), 91-  
600 108; DOI 10.1016/0269-7491(95)91052-M.
- 601 36. Juhasz, A.; Naidu, R. Bioremediation of high molecular weight polycyclic aromatic  
602 hydrocarbons: A review of the microbial degradation of benzo[a]pyrene.  
603 *Int. Biodeterior. Biodegradation* **2000**, *45*, (1-2), 57-88; DOI 10.1016/S0964-8305(00)00052-  
604 4.

- 605 37. Mahmoudi, N.; Slater, G.F.; Juhasz, A.L. Assessing limitations for PAH biodegradation  
606 in long-term contaminated soils using bioaccessibility assays. *Water Air Soil Poll.* **2013**, *224*,  
607 (2), 1411; DOI <https://doi.org/10.1007/s11270-012-1411-2>.
- 608 38. Macleod, C.; Semple, K. Influence of contact time on extractability and degradation of  
609 pyrene in soils. *Environ. Sci. Technol.* **2000**, *34*, (23), 4952-4957; DOI 10.1021/es000061x.
- 610 39. Rhodes, A. H.; McAllister, L. E.; Semple, K. T. Linking desorption kinetics to  
611 phenanthrene biodegradation in soil. *Environ. Pollut.* **2010**, *158*, (5), 1348-1353; DOI  
612 10.1016/j.envpol.2010.01.008.
- 613 40. Yang, Y.; Shu, L.; Wang, X. L.; Xing, B. S.; Tao, S. Impact of de-ashing humic acid  
614 and humin on organic matter structural properties and sorption mechanisms of phenanthrene.  
615 *Environ. Sci. Technol.* **2011**, *45*, (9), 3996-4002; 10.1021/es2003149.
- 616 41. Rhodes, A.; Carlin, A.; Semple, K. Impact of black carbon in the extraction and  
617 mineralization of phenanthrene in soil. *Environ. Sci. Technol.* **2008**, *42*, (3), 740-745; DOI  
618 10.1021/es071451n.
- 619 42. Vogel, C.; Mueller, C. W.; Hoschen, C.; Buegger, F.; Heister, K.; Schulz, S.; Schloter,  
620 M.; Kogel-Knabner, I. Submicron structures provide preferential spots for carbon and nitrogen  
621 sequestration in soils. *Nat Commun.* **2014**, *5*, 2947; DOI 10.1038/ncomms3947.
- 622 43. Murphy, E. M.; Zachara, J. M. The role of sorbed humic substances on the distribution  
623 of organic and inorganic contaminants in groundwater. *Geoderma* **1995**, *67*, (1-2), 103-124;  
624 DOI 10.1016/0016-7061(94)00055-F.
- 625 44. Feng, X. J.; Simpson, A. J.; Simpson, M. J. Investigating the role of mineral-bound  
626 humic acid in phenanthrene sorption. *Environ. Sci. Technol.* **2006**, *40*, (10), 3260-3266; DOI  
627 10.1021/es0521472.



- 628 45. Hur, J.; Schlautman, M. A. Influence of humic substance adsorptive fractionation on  
629 pyrene partitioning to dissolved and mineral-associated humic substances. *Environ. Sci.*  
630 *Technol.* **2004**, *38*, (22), 5871-5877; DOI 10.1021/es049790t.
- 631 46. Hwang, S.; Cutright, T. Effect of expandable clays and cometabolism on PAH  
632 biodegradability. *Environ. Sci. Pollut. Res.* **2003**, *10*, (5), 277-280; DOI  
633 10.1065/espr2003.08.167.
- 634 47. Calderbank, A. The occurrence and significance of bound pesticide-residues in soil.  
635 *Rev. Environ. Contam. T.* **1989**, *108*, 71-103; DOI [https://doi.org/10.1007/978-1-4613-8850-](https://doi.org/10.1007/978-1-4613-8850-0_2)  
636 [0\\_2](https://doi.org/10.1007/978-1-4613-8850-0_2).
- 637 48. Semple, K. T.; Doick, K. J.; Jones, K. C.; Burauel, P.; Craven, A.; Harms, H. Peer  
638 reviewed: Defining bioavailability and bioaccessibility of contaminated soil and sediment is  
639 complicated. *Environ. Sci. Technol.* **2004**, *38*, (12), 228A-231A; DOI 10.1021/es040548w.
- 640 49. Ortega-Calvo, J.-J.; Harmsen, J.; Parsons, J. R.; Semple, K. T.; Aitken, M. D.; Ajao,  
641 C.; Eadsforth, C.; Galay-Burgos, M.; Naidu, R.; Oliver, R.; Peijnenburg, W. J. G. M.; Römbke,  
642 J.; Streck, G.; Versonnen, B. From bioavailability science to regulation of organic chemicals.  
643 *Environ. Sci. Technol.* **2015**, *49*, (17), 10255–10264; DOI 10.1021/acs.est.5b02412.
- 644 50. *The derivation of HILs for PAHs and total phenols*: National environment protection  
645 (assessment of site contamination) amendment measure; National Environment Protection  
646 Council (NEPC): Canberra, Australia, 2013;  
647 [https://www.legislation.gov.au/Details/F2013C00288/Html/Volume\\_11](https://www.legislation.gov.au/Details/F2013C00288/Html/Volume_11).