

1

2 **Influence of pH, electrical conductivity and ageing on the**
3 **extractability of benzo[a]pyrene in two contrasting soils**

4

5 Fanbo Meng^{a,b}, Xiaodong Yang^{b,c}, Luchun Duan^{b,d*}, Ravi Naidu^{b,d}, Md Nuruzzaman^b, Kirk T.

6 Semple^e

7 ^a Institute of Soil, Jinan Environmental Research Academy, Jinan 250102, China

8 ^b Global Centre for Environmental Remediation (GCER), ATC Building, the University of
9 Newcastle, Callaghan Campus, NSW 2308, Australia

10 ^c Department of Geography & Spatial Information Technology, Ningbo University, Ningbo
11 315211, China

12 ^d Cooperative Research Centre for Contamination Assessment and Remediation of the
13 Environment (CRC CARE), the University of Newcastle, Callaghan Campus, NSW 2308,
14 Australia

15 ^e Lancaster Environmental Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom

16

17 * **Corresponding author:** Luchun Duan, Global Centre for Environmental Remediation
18 (GCER), ATC Building, University of Newcastle, Callaghan Campus, NSW 2308, Australia;

19 e-mail: luchun.duan@newcastle.edu.au

20 **Abstract**

21 Higher soil pH and electrical conductivity (EC) were suspected to result in higher extractability
22 and bioavailability of benzo[a]pyrene (B[a]P) in soils. In this study, we investigated the
23 influence of pH, EC and ageing on the extractability of B[a]P in two contrasting soils (varied
24 largely in soil texture, clay mineralogy and organic carbon content) over 4 months. Dilute
25 sodium hydroxide (0.2 mol L⁻¹) and sodium chloride (0.1 mol L⁻¹) solutions were used to adjust
26 soil pH and EC either separately or simultaneously. Extractability of B[a]P in these soils was
27 monitored using a mild solvent extraction using butanol (BuOH, end-over-end shake over 24
28 hours), and an exhaustive mix-solvent extraction using dichloromethane/acetone (DCM/Ace,
29 v:v = 1:1) facilitated by sonication and a subsequent NaOH saponification method following
30 the DCM/Ace extraction. Results showed that increased pH and/or EC significantly increased
31 the B[a]P extractability in the sandy soil (GIA). Variance analysis of contribution of pH and/or
32 EC modification and ageing time on changes in B[a]P extractability indicated that in GIA more
33 than 55% and over 25% of the changes in B[a]P extractability was attributed to increased
34 pH&EC and pH only respectively. While ageing resulted in more than 85% of the change in
35 B[a]P extractability in the clayey soil (BDA), following by increased pH&EC (contribution less
36 than 15%). Large amount of non-extractable residue (NER) were formed over the ageing period,
37 up to 94.6% and 78.8% in GIA/BDA and its modified soils, respectively. Significant
38 correlations were observed between B[a]P BuOH extractability and the exhaustive sequential
39 extraction using DCM/Ace followed by NaOH saponification for all soils ($p < 0.001$). With
40 slopes of the correlations close to 1, our results indicated that the simple mild solvent BuOH

- 41 extraction was equivalent to the complex sequential DCM/Ace and NaOH saponification
- 42 extraction in these soils.
- 43 *Keywords:* B[a]P, extractability, soil, ageing, pH, EC

44 **1. Introduction**

45 Polycyclic aromatic hydrocarbons (PAHs), is a group of organic compounds that consists
46 of two or more fused benzene rings. They arise mainly as combustion by-products of organic
47 materials, and are prevalent in both industrial and agricultural soils (Ortega-Calvo et al., 2013).
48 PAHs are well known for their teratogenic, carcinogenic and mutagenic properties as well as
49 their toxicity to living organisms (Ma et al., 2012). Benzo[a]pyrene (B[a]P), a five-ring PAH,
50 which has been well-characterised for its carcinogenic potency compared to other PAHs has
51 been frequently used as an indicator of potential risk of PAHs to the environment and human
52 health (Pufulete et al., 2004; Pardo et al., 2016). B[a]P is highly hydrophobic and very resistant
53 to biodegradation and is therefore very persistent in soils. Once incorporated into soil, B[a]P
54 tends to sorb to the surfaces of the solid surface, especially the organic components, and then
55 undergoes sorptive diffusion into minute pores of soil particles over time (ageing process),
56 exhibiting reduced bioavailability (Duan et al., 2015; Cipullo et al., 2018).

57 The contaminant bioavailability is defined as the fraction of the total amount that is 'freely
58 available' in a medium for uptake i.e., able to cross the cellular membrane of an organism at a
59 given point of time (Semple et al., 2004). Therefore, it is the bioavailable fraction, rather than
60 the total contaminant in soils, that is critical for defining exposure, uptake and the consequent
61 risk to the environmental receptors, and could be used to assess the effectiveness or feasibility
62 of bioremediation technologies (Li et al., 2013). Many researchers have observed an 'ageing
63 effect' of hydrophobic organic contaminants (HOCs), such as PAHs in soil (Duan et al., 2015;
64 Meng and Chi, 2017; Ye et al., 2019). Such process are often determined by increased sorption
65 or decreased desorption which are controlled by several factors including soil properties (e.g.

66 organic matter quality and quantity, cation-exchange capacity, pH, electrical conductivity (EC),
67 nanoporosity and soil disaggregation), physio-chemical characteristics of the organic
68 contaminants, (e.g. their hydrophobicity, stability and co-existing compounds or source
69 material), as well as the environmental factors, (e.g. ageing time, temperature, precipitation,
70 wetting and drying circles, freezing-thawing and sterilised or not) (Maliszewska-Kordybach,
71 2005; Riding et al., 2013; Yu et al., 2018).

72 From a human health risk assessment perspective, bioavailability of HOCs is the amount of
73 compound that is desorbed from soil through desorption processes under physiological
74 conditions, which is available for uptake into the circulatory system (Ruby et al., 1996; Kramer
75 and Ryan, 2000), which needs to be estimated using in vivo animal studies, such as rat or swine
76 models. However, animal studies are expensive and time-consuming, sometimes are not even
77 possible due to ethics issues, hence these type of data are scarce. Among the limited studies,
78 our previous research investigated the influence of soil properties on the oral bioavailability
79 (BA) of B[a]P in soils, in which eight soils with significantly varied soil properties were
80 investigated using a swine model (Duan et al., 2014). Despite being spiked at the same
81 concentration (at 50 mg kg⁻¹), BA of B[a]P in most soils estimated after 90 days of ageing
82 ranged from 20% to 60%, which was significantly lower than that estimated in freshly spiked
83 silica sand. Significant negative correlations were identified between relative bioavailability of
84 B[a]P in soil (RB, compared to that in freshly spiked silica sand used as reference material that
85 assists comparison between different soils) and two specific soil properties, namely FPAC (fine
86 particle associated carbon) and PF < 6 nm (meso-pore size less than 6 nm fraction) in most soils.
87 In addition, there were two obvious outlier soils, both with elevated pH and EC. These soils

88 showed much higher oral bioavailability compared to the general correlation(s) (Duan et al.,
89 2014). One of the outlier soils was a subsurface soil (GIB, sampled from 20 cm to 40 cm depth
90 from the surface). Its surface soil (GIA), which had lower pH and EC values, however, was part
91 of the set of soils that showed strong significant correlation with FPAC and meso pores < 6 nm.
92 These results strongly indicated that higher soil pH and EC may result in the higher B[a]P
93 bioavailability in soil GIB compared to GIA.

94 In fact, two solvent extraction methods, including one mild solvent extraction using butanol
95 (BuOH, vortex for 50 seconds), and another using harsh mix-solvent dichloromethane/acetone
96 (DCM/Ace, v:v = 1:1) facilitated by sonication were previously found to have significant
97 correlations ($R^2 = 0.67$ and 0.75 respectively) with oral bioavailability of B[a]P using a swine
98 model (Duan et al., 2014). This was despite a slope value over 1 for both extraction methods,
99 indicating that they underestimated the RB of B[a]P in soils (Duan et al., 2014). Therefore, a
100 slight modification was made for both methods to increase their extraction capacity. For BuOH
101 extraction, the 50 seconds vortex extraction was extended to shaking over 24 hours in an end-
102 over-end shaker following Luo et al. (2012). Increased extraction time had significantly
103 increased the extractability of PAHs using BuOH (Gomez-Eyles et al., 2010). And following
104 DCM/Ace (1:1) extraction, a subsequent saponification process using NaOH was included to
105 release B[a]P sequestered in soil organic matter (SOM). It has been reported that such alkaline
106 hydrolysis reactions could cleave ester-linked bound residues from the non-extractable
107 macromolecular soil matrix (Richnow et al., 2000).

108 Our previous study has also demonstrated that the ageing process varies amongst different
109 soils (Duan et al., 2015). Thus, the major task of this study was to investigate whether increasing

110 pH and EC values and ageing will change B[a]P extractability in two contrasting soils.
111 Particular attention was paid to the form of non-extractable residue over time and to comparison
112 between the extraction efficacies of the two extraction methods.

113 **2. Materials and methods**

114 *2.1 Soils*

115 The two selected contrasting soils were a sandy Sodosol soil (GIA) and a clayey black
116 Vertisol soil (BDA). Pertinent soil properties of the soils are shown in Table 1. They have
117 similar pH and EC values but varied largely in terms of texture and clay mineralogy as well as
118 organic carbon content. For both soils, the pH and EC values were altered either separately or
119 simultaneously to designated levels. This generated a series of four soils, including the original
120 soils (GIA and BDA), pH modified soils (GIA/BDA-pH), EC modified soils (GIA/BDA-EC),
121 and soils modified by both pH and EC (GIA/BDA-pH&EC). Diluted sodium hydroxide (0.2
122 mol L⁻¹) and sodium chloride (0.1 mol L⁻¹) were used to alter pH and EC values, respectively.
123 A preliminary experiment was carried out to determine the amounts of both solutions required
124 for each soil. After modification, the soils were air dried, gently ground and stored. Their pH
125 and EC values were checked again before spiking with B[a]P.

126 *2.2 Soil spiking and ageing*

127 The soils were spiked with B[a]P at 10 mg kg⁻¹ on a dry weight basis following Duan et al.
128 (2014). To ensure that the same amount of B[a]P was delivered to each soil, a 10 mL air-tight
129 glass syringe was used to distribute B[a]P stock solution (1000 mg L⁻¹, in n-hexane) into eight
130 4 mL glass vials (each 1.2 mL). The vials were sealed with PTFE-lined caps. When the soils
131 were ready for spiking, a glass pipette was used to deliver all the stock solution in the 4 mL vial

132 to each soil. Briefly, 120 g of each soil (dry weight) was placed into a 250 mL amber glass jar.
133 Then the stock solution (1.2 mL) was transferred to each soil using a glass pipette dropwise in
134 a fume hood. An additional 0.6 mL Hexane was used to rinse the glass vial to ensure the
135 complete transfer of the B[a]P. This step was repeated twice. In total < 2% solvent (v/w) was
136 used for spiking. The jars were left open in the fume hood overnight to allow the solvent to
137 evaporate. The bottles were then capped (caps were PTFE-lined) and placed on an end-over-
138 end shaker for 24 h to homogenise the sample. The B[a]P spike recovery and sample
139 homogeneity was checked by taking triplicate 1.0 g samples for DCM/Ace extraction before
140 adding Milli-Q water to reach about 60% of soil water holding capacity for ageing at room
141 temperature. The jars were opened every week and subsamples were taken after 7, 21, 49, 84
142 and 119 days and subjected to the different extraction methods described below.

143 *2.3 Extraction of B[a]P from soil*

144 Three methods were used to extract B[a]P from soil: a mild solvent extraction with BuOH,
145 an exhaustive solvent extraction using DCM/Ace and a subsequent saponification extraction
146 after DCM/Ace extraction using NaOH. The extraction methods are explained below. B[a]P
147 extractability at each ageing time was calculated from:

$$148 \quad \text{Extractability (\%)} = \frac{m_{\text{extracted}}/m_{\text{dry soil}}}{10 \text{ mg kg}^{-1}} \times 100\% \quad (1)$$

149 where $m_{\text{extracted}}$ was the mass of B[a]P extracted from soil (ug), $m_{\text{dry soil}}$ was the soil dry weight
150 (g). All extractions were performed using 22 mL glass centrifuge vials with PTFE-lined caps
151 in triplicate.

152 For BuOH extraction, 1.0 g of soil sample was taken and 10 mL of BuOH was added. The
153 glass centrifuge vials were properly sealed and placed in a box to shade them from light on a

154 flat-bed shaker for 24 h set at 120 rpm. The vials were then centrifuged at 2000 g for 30 min to
155 separate the solid phase. An aliquot of the BuOH extract was filtered through a 0.45 μm PTFE
156 syringe filter and stored in a 2 mL amber HPLC vial.

157 The DCM/Ace extraction method followed (Duan et al. 2015). In brief, 1.0 g of soil sample
158 was mixed with an adequate amount of anhydrous Na_2SO_4 to form a free flow sample. Then,
159 10 mL of premixed solvent DCM/Ace (1:1, v/v) was added. The extraction was facilitated by
160 sonication (40 KHz for 15 min) twice, in between the samples were vortexed to resuspend the
161 soil particles. The solvent extract was separated by centrifugation at 3000 g for 20 min and
162 decanted into another 40 mL glass vial. The whole extraction procedure was repeated for further
163 two times. The solvent extracts were combined (~ 30 mL) and evaporated under a gentle N_2 gas
164 flow, following which 5 mL of ACN was added to redissolve the extract. An aliquot of the
165 sample (~ 2 mL) was then filtered through a 0.45 μm PTFE syringe filter into a 2 mL vial.

166 The soil samples after DCM/Ace extraction were allowed to dry (solvent evaporation) in a
167 fume hood. Then 5 mL of 2 mol L^{-1} NaOH solution was added (Ma et al., 2012). The vials were
168 then capped tightly, and placed in an oven set at 100 $^\circ\text{C}$ for 2 hours. The samples were allowed
169 to cool down after removal from the oven. The samples were then acidified to pH 1~2 with 6
170 mol L^{-1} HCl. The mixtures were then extracted with 5 mL Hex three times. The combined Hex
171 extracts were evaporated under a gentle N_2 gas stream and re-dissolved in 5 mL of ACN,
172 followed by filtering through 0.45 μm PTFE filters into 2 mL HPLC vials for HPLC analyses.
173 All samples were stored at -20 $^\circ\text{C}$ until analysed.

174 B[a]P concentrations were determined using an Agilent 1260 HPLC system coupled with a
175 diode array detector (HPLC-DAD) and a fluorescence detector (HPLC-FLD). Two ranges of

176 calibration curves were made based on the sample concentration, using DAD (at a wavelength
177 of 267 nm) and/or an FLD detector (with an excitation wavelength of 297 nm and an emission
178 wavelength of 405 nm) to encompass the wide concentration range of B[a]P from 25 $\mu\text{g L}^{-1}$ to
179 5 mg L^{-1} .

180 *2.4 Model fitting of B[a]P ageing kinetics*

181 The ageing kinetics of B[a]P in soils was described by a first-order kinetic model (Eq. (2))
182 (Duan et al., 2015).

$$183 \quad y_t = y_0 \times e^{-k \times t} \quad (2)$$

184 where y_0 and y_t are the modelled extractability of B[a]P (%) at day 0 and day t , k is the
185 decreasing rate constant (d^{-1}).

186 *2.5 Quality assurance and quality control*

187 Laboratory glassware was soaked in alkaline for 24 h, washed under continuous water flow,
188 oven-dried at 120 °C for 4 h, and rinsed twice with acetone prior to use. Background
189 concentrations in both soils were checked before use. No detectable B[a]P concentration was
190 found in both study soils. Spike recovery of B[a]P using same procedure in spiked silica sand
191 (at 50 mg kg^{-1}) had shown a complete recovery ($100 \pm 0.5\%$, $n=5$) previously (Duan et al.,
192 2015).

193 In this study, B[a]P spiked at 10 mg kg^{-1} was examined in each of the eight soils ($n=3$)
194 before adding water for ageing (Day 0). In brief, spike recovery of B[a]P ranged from $36 \pm 0.8\%$
195 to $102 \pm 3\%$ in GIA, from $85 \pm 3\%$ to $101 \pm 5\%$ in BDA and their modified soils. Details of
196 these results and discussion are shown in 3.1.

197 B[a]P calibration standards were analysed along with the different batches of samples at
198 different ageing time. The slope of standard curve showed good consistency over the whole
199 study ($SD < 1.5$, $n=6$).

200 *2.6 Statistical analysis*

201 Model fitting of the B[a]P ageing process was carried out using Microsoft Excel. One-way
202 ANOVA was used to test the between-group differences of B[a]P extractability estimated by
203 each method as influenced by ageing time for each soil and effect of pH and/or EC modification
204 for both soils at the same ageing time. If the variance of B[a]P extractability was homogeneous
205 among different ageing times or different modified soils, the least-squares mean separation with
206 Duncan's correction was used to test the differences. Otherwise, if the variance was
207 heterogeneous, Tamhane's T3 test was used to test the differences. Variance analysis was used
208 to calculate the contributions of ageing time, pH and/or EC to the changes in B[a]P
209 extractability. Data analyses were conducted in R. 3.4.3. Significance level was set at $p < 0.05$.

210 **3. Results and discussion**

211 *3.1 Influence of pH and EC on B[a]P spike recovery*

212 Spike recovery of B[a]P in both GIA and BDA and their modified soils is presented in Table
213 S1. They are generally high ($> 85\%$), which is in accordance with previous studies and
214 demonstrated the high extraction capacity of DCM/Ace (Song et al., 2002; Duan et al., 2014;
215 Duan et al., 2015). There were two exceptions to this general observation, the unmodified soil
216 GIA and its EC modified soil GIA-EC, for which B[a]P recovery was only $36 \pm 0.8\%$ and 49
217 $\pm 0.7\%$, respectively. Subsequent NaOH saponification could not extract more B[a]P from these
218 two soils as well ($< 1.5\%$). However, in comparison, B[a]P recovery in GIA-pH and GIA-

219 pH&EC was as high as $88 \pm 0.6\%$ and $102 \pm 3\%$, respectively. These results suggest that
220 increasing pH has a significant impact on the B[a]P recovery in GIA, immediately after spiking.
221 It was also noted previously that extractability of B[a]P in GIA was much lower than its
222 subsurface soil (GIB) that has higher pH and EC values (Duan et al., 2014). Compared to the
223 marked influence of pH on B[a]P recovery in soil GIA, the influence of pH and EC on the B[a]P
224 recovery in BDA was very limited. The spike recovery of B[a]P in BDA and its pH and/or EC
225 modified soils were all similar, ranging from $85 \pm 3\%$ to $101 \pm 5\%$.

226 *3.2 Influence of pH and EC on B[a]P ageing process*

227 Fig. 1 exhibits the change in B[a]P extractability estimated by BuOH and DCM/Ace in both
228 soils and their pH and/or EC modified treatment over time. More details of the extracted
229 fractions by each method, including that released by NaOH saponification, are presented in
230 Table S1. The fitted first-order kinetic model parameters (i.e., y_0 and k) as well as R^2 and p
231 values are presented in Table S2. The R^2 values for all soils ranged from 0.71 to 0.99 ($p < 0.001$),
232 indicating a general good fit of the data. The general decreasing trend of B[a]P extractability in
233 all soils estimated by both extraction methods indicated that B[a]P went through a sorptive
234 diffusion/sequestration process (ageing process) in soil (Reid et al., 2000; Duan et al., 2015).
235 However, the ageing effect was clearly more significant in the clayey soil BDA than in the
236 sandy soil GIA (indicated by higher k values). In the sandy soil GIA, the extractability of B[a]P
237 estimated by both BuOH and DCM/Ace was similar and followed the same order constantly
238 over the investigated ageing period with the unmodified soil having the lowest extractability
239 (pH&EC > pH > EC > GIA). Nevertheless, the decreasing rate constant k also indicated that
240 the ageing effect was most pronounced in the unmodified soil GIA compared with its modified

241 soils, with the second being the pH modified soil. The influence of pH and/or EC modification
242 was much less significant in the clayey soil BDA, indicated by the smaller difference in the k
243 value among treatments. Changes in B[a]P also followed the same order as in GIA but the
244 difference between different treatments became insignificant as ageing time increased.

245 Further variance analysis (Fig. 2) of relative contributions of ageing time, pH, EC and
246 pH&EC on B[a]P extractability indicated that for GIA, all these four factors had a significant
247 impact on the B[a]P extractability estimated by both BuOH and DCM/Ace extractions ($p <$
248 0.001), with a major contribution from pH&EC, for BuOH and DCM/Ace extraction methods
249 (at 58% and 57% respectively). While for soil BDA, there were slight differences between using
250 the different extraction methods. However, ageing time was the dominant influencing factor,
251 that contributed to $> 85\%$ of the changes in the B[a]P extractability (at 85% and 93% for BuOH
252 extraction and DCM/Ace extraction, respectively). These results indicated that the sandy soil
253 GIA was much more vulnerable to changes in pH and EC than the clayey soil BDA.

254 It is worth noting that pH and/or EC adjustments significantly increased the B[a]P
255 extractability in GIA immediately after spiking, and the modelled extractability of B[a]P at day
256 0 (y_0) was close to (slight lower) the estimated spike recovery (Table S1 and S2). While in BDA,
257 despite following more or less the same sequence in the different treatments, the modelled
258 intercept (y_0) values were much lower than the actual estimated spike recovery. This indicated
259 that the ageing process between adding water for ageing to estimation at day 7, the ageing
260 process was much faster than model prediction for this type of soil (clayey and had higher TOC
261 than GIA).

262 The influence of pH and EC on the ageing process of B[a]P in soil is susceptible to changes

263 in the soil surface charge, resulting from both mineral phase, especially the clay minerals, and
264 the soil organic matter phase (SOM). Lower pH and EC have been reported to favour sorption
265 of PAHs on humic acid and mineral-bound humic substances (Schlautman and Morgan, 1993;
266 Murphy et al., 1994; Laor et al., 1998; Feng et al., 2006). These phenomena were mainly
267 attributed to changes in the humic acid surface charge. With increasing pH, organic matter
268 deprotonated and became more negatively charged. These polar sites have lower affinity for
269 PAHs, resulting in lower sorption. Also, more favourable sorption sites become available at
270 soil organic matter for the sorption of HOC as organic matter adopts elongated configuration at
271 low ionic strength (Na^+ electrolyte) as a result of charge repulsion between ionised functional
272 groups (Murphy et al., 1994). With much lower TOC (0.78%) and clay content (5.6%) in GIA,
273 it would have much less buffering capacity than BDA. This explained why GIA was more
274 vulnerable to pH and/or EC changes. Also, increasing pH may promote SOM dissolution
275 (Andersson and Nilsson, 2001). However, the much lower B[a]P extractability in the
276 unmodified soil GIA compared to unmodified BDA may be attributed to other critical soil
277 properties, such as its much higher $\text{PF} < 6 \text{ nm}$ (Table 1).

278 *3.3 Non-extractable residue of B[a]P in soils*

279 In this study, a NaOH saponification method was used to extract the remaining extractable
280 B[a]P fraction after DCM/Ace extraction in soils (Ma et al., 2012; Gao et al., 2017). Data is
281 presented in Table S1. The amount of B[a]P extracted by NaOH saponification was not large
282 (ranged from 0.3%~13%) as it was performed following the exhaustive DCM/Ace extraction.

283 The HOC remaining in soil after exhaustive extraction is considered as a non-extractable
284 residue (NER) (Gao et al., 2017). In this study, the B[a]P NER was defined as the fraction of

285 B[a]P spiked into the soil that cannot be extracted by the exhaustive DCM/Ace extraction
286 followed by the subsequent NaOH saponification. It was calculated as 100% - DCM/Ace
287 extraction - NaOH saponification. A large amount of NER was formed over time in both GIA
288 and BDA and their modified soils (Fig. 3, and data with significance presented in Table S3).
289 NER varied widely from 9%~95% in the GIA and its pH and/or EC modified soils, while in
290 BDA, NER showed relatively small variance among the modified treatments but dramatically
291 increased with ageing (ranging from 35% to 79%). Detailed variance analysis on contribution
292 of each factor indicated pH&EC accounted for 61% of the variability in NER in GIA, following
293 by pH and EC individually, accounted for about 30% and 7% of the variance in NER. Whereas
294 in BDA, ageing accounted for 85% of the variability of NER, followed by pH&EC, which
295 accounted for about 10% of the variabilities, leaving contributions from pH, and EC almost
296 negligible (Fig. 2).

297 Ageing of HOCs in soil was related to partitioning into particulate SOM or mineral particles
298 through slow diffusion and entrapment in soil micropores (Kaestner et al., 2016; Gao et al.,
299 2017). This is evidenced by the $PF < 6$ nm and the higher NER in the unmodified soil GIA
300 compared to the unmodified soil BDA (70%~95% versus 46%~79%, Fig. 3 and Table S3). It
301 was noted that the surface area in GIA was two times greater than that in BDA, and the average
302 pore diameter of GIA was also smaller than that of soil BDA. However, GIA has a low TOC,
303 which indicated that the higher content of TOC in BDA may have prevented B[a]P access to
304 the mesopores at the beginning of the ageing time. The gradual increase in NER over time in
305 BDA also exhibited its capacity to sequester B[a]P. The entrapment or sequestration of B[a]P
306 by $PF < 6$ nm plays a significant role in the B[a]P ageing process (Duan et al., 2015). It was

307 also noted from the previous swine study, that B[a]P oral bioavailability in BDA (~40%) was
308 higher than that in GIA (~20%) (Duan et al., 2014).

309 A strong significant correlation was identified between extractability of B[a]P using BuOH
310 and DCM/Ace (Fig. S1, $R^2 = 0.934$, $p < 0.001$) with a slope coefficient close to 1. Detailed
311 analysis of data found this correlation was further improved with the inclusion of B[a]P released
312 by NaOH saponification (Fig. 4). The correlation between the BuOH extractability of B[a]P
313 and total extractable B[a]P however, was much better for GIA than for BDA, with $R^2 = 0.995$
314 and $R^2 = 0.849$ respectively. The close to 1 slope coefficients (varied from 0.93 to 1.02)
315 suggested the extraction capacity of this 24 h BuOH extraction method was comparable to that
316 using the complex sequential extraction using DCM/Ace and NaOH saponification for these
317 soils. Further investigation on field contaminated soils which contain PAH mixtures and
318 inorganic component may need to prove these correlations in soils and the capability of BuOH
319 extraction to replace the exhaustive extraction method.

320 **4. Conclusion**

321 B[a]P extractability declined with increasing ageing time in both GIA and BDA and their
322 modified soils. The variation of B[a]P extractability over ageing time in all soils fitted well with
323 the first-order kinetic model. Increased pH and/or EC enhanced B[a]P extractability for both
324 soils, and followed the order of pH&EC > pH > EC. The enhanced increments of B[a]P
325 extractability after pH and/or EC adjustments was significantly higher in GIA than in BDA,
326 demonstrating that sandy soil GIA with lower TOC was more vulnerable to changes by pH and
327 EC than the clayey soil BDA containing expandable clay minerals and relatively higher TOC.

328 A mild solvent extraction using BuOH and an exhaustive mix-solvent DCM/Ace extraction

329 followed by NaOH saponification were used to extract B[a]P fractions in each soil over ageing.
330 The BuOH extraction was found to be equivalent to the sequential DCM/Ace extraction and
331 NaOH saponification since the slope coefficients were close to 1 (varied from 0.93 to 1.02),
332 indicating this much simpler method could replace the complex sequential extractions in this
333 study. This could be due to B[a]P was spiked into the soils in a simple matrix which is solvent.
334 However, in reality, field contaminated soils will contain PAH mixtures and other organic and
335 inorganic component as co-contaminants. For which, further examination of this correlation in
336 order to validate the capability of BuOH extraction is needed.

337 Large amounts of NER were formed over the 119-day ageing period. It varied widely in
338 GIA and its modified soils (ranging from 9% to 95%) and was heavily influenced by pH and
339 EC. The extent of influences of these modifications on form of NER in soil was much less in
340 BDA and the difference was mainly attributed to ageing (35%~79%). Pore size fraction with
341 diameter < 6 nm (PF < 6 nm), TOC and clay content contributed to the differences in the B[a]P
342 extractability over time. This study further demonstrated the importance of soil properties on
343 the extractability of HOC such as B[a]P. It also provided direct evidence supporting that
344 increased pH and EC might have contributed to higher oral bioavailability of B[a]P using a
345 swine model.

346

347 **Acknowledgements**

348 The authors are grateful to the Cooperative Research Centre for Contamination Assessment
349 and Remediation of the Environment (CRC CARE) and the China Scholarship Council for
350 financial support.

351

352 **Declarations of interest: none**

353

354 **References**

355 Andersson, S., Nilsson, S.I., 2001. Influence of pH and temperature on microbial activity,
356 substrate availability of soil-solution bacteria and leaching of dissolved organic carbon in
357 a mor humus. *Soil Biology & Biochemistry* 33, 1181-1191.

358 Cipullo, S., Prpich, G., Campo, P., Coulon, F., 2018. Assessing bioavailability of complex
359 chemical mixtures in contaminated soils: Progress made and research needs. *Sci. Total*
360 *Environ.* 615, 708-723.

361 Duan, L., Naidu, R., Liu, Y., Palanisami, T., Dong, Z., Mallavarapu, M., Semple, K.T., 2015.
362 Effect of ageing on benzo[a]pyrene extractability in contrasting soils. *J. Hazard. Mater.*
363 296, 175-184.

364 Duan, L., Palanisami, T., Liu, Y., Dong, Z., Mallavarapu, M., Kuchel, T., Semple, K.T., Naidu,
365 R., 2014. Effects of ageing and soil properties on the oral bioavailability of
366 benzo[a]pyrene using a swine model. *Environ. Int.* 70, 192-202.

367 Feng, X., Simpson, A.J., Simpson, M.J., 2006. Investigating the role of mineral-bound humic
368 acid in phenanthrene sorption. *Environ. Sci. Technol.* 40, 3260-3266.

369 Gao, Y., Hu, X., Zhou, Z., Zhang, W., Wang, Y., Sun, B., 2017. Phytoavailability and
370 mechanism of bound PAH residues in filed contaminated soils. *Environ. Pollut.* 222, 465-
371 476.

372 Gomez-Eyles, J.L., Collins, C.D., Hodson, M.E., 2010. Relative proportions of polycyclic

373 aromatic hydrocarbons differ between accumulation bioassays and chemical methods to
374 predict bioavailability. *Environ. Pollut.* 158, 278-284.

375 Kaestner, M., Nowak, K.M., Miltner, A., Schaeffer, A., 2016. (Multiple) Isotope probing
376 approaches to trace the fate of environmental chemicals and the formation of non-
377 extractable 'bound' residues. *Curr. Opin. Biotechnol.* 41, 73-82.

378 Kramer, B.K., Ryan, P.B., 2000. Soxhlet and microwave extraction in determining the
379 bioaccessibility of pesticides from soil and model solids. *Proceedings of the 2000*
380 *Conference on Hazardous Waste Research* 196-210.

381 Laor, Y., Farmer, W.J., Aochi, Y., Strom, P.F., 1998. Phenanthrene binding and sorption to
382 dissolved and to mineral-associated humic acid. *Water Res.* 32, 1923-1931.

383 Li, X., Shuang, S., Li, X., Kong, L., Xu, L., Tai, P., Lin, X., Jia, C., Gong, Z., 2013. The effect
384 of concentrations and properties of phenanthrene, pyrene, and benzo(a)pyrene on
385 desorption in contaminated soil aged for 1 year. *J. Soils Sed.* 13, 375-382.

386 Luo, L., Lin, S., Huang, H., Zhang, S., 2012. Relationships between aging of PAHs and soil
387 properties. *Environ. Pollut.* 170, 177-182.

388 Ma, B., Wang, J., Xu, M., He, Y., Wang, H., Wu, L., Xu, J., 2012. Evaluation of dissipation
389 gradients of polycyclic aromatic hydrocarbons in rice rhizosphere utilizing a sequential
390 extraction procedure. *Environ. Pollut.* 162, 413-421.

391 Maliszewska-Kordybach, B., 2005. Dissipation of polycyclic aromatic hydrocarbons in freshly
392 contaminated soils - The effect of soil physicochemical properties and aging. *Water Air*
393 *and Soil Pollution* 168, 113-128.

394 Meng, F., Chi, J., 2017. Effect of *Potamogeton crispus* L. on bioavailability and biodegradation

395 activity of pyrene in aged and unaged sediments. *J. Hazard. Mater.* 324, 391-397.

396 Murphy, E.M., Zachara, J.M., Smith, S.C., Phillips, J.L., Wietsma, T.W., 1994. Interaction of
397 hydrophobic organic compounds with mineral-bound humic substances. *Environ. Sci.*
398 *Technol.* 28, 1291-1299.

399 Ortega-Calvo, J.J., Tejada-Agredano, M.C., Jimenez-Sanchez, C., Congiu, E., Sungthong, R.,
400 Niqui-Arroyo, J.L., Cantos, M., 2013. Is it possible to increase bioavailability but not
401 environmental risk of PAHs in bioremediation? *Journal of Hazardous Materials* 261, 733-
402 745.

403 Pardo, F., Santos, A., Romero, A., 2016. Fate of iron and polycyclic aromatic hydrocarbons
404 during the remediation of a contaminated soil using iron-activated persulfate: A column
405 study. *Sci. Total Environ.* 566, 480-488.

406 Pufulete, M., Battershill, J., Boobis, A., Fielder, R., 2004. Approaches to carcinogenic risk
407 assessment for polycyclic aromatic hydrocarbons: a UK perspective. *Environ. Pollut.* 40,
408 54-66.

409 Reid, B.J., Jones, K.C., Semple, K.T., 2000. Bioavailability of persistent organic pollutants in
410 soils and sediments-a perspective on mechanisms, consequences and assessment. *Environ.*
411 *Pollut.* 108, 103-112.

412 Richnow, H.H., Annweiler, E., Koning, M., Luth, J.C., Stegmann, R., Garms, C., Francke, W.,
413 Michaelis, W., 2000. Tracing the transformation of labelled [1-¹³C] phenanthrene in a soil
414 bioreactor. *Environ. Pollut.* 108, 91-101.

415 Riding, M.J., Doick, K.J., Martin, F.L., Jones, K.C., Semple, K.T., 2013. Chemical measures of
416 bioavailability/bioaccessibility of PAHs in soil: Fundamentals to application. *J. Hazard.*

417 Mater. 261, 687-700.

418 Ruby, M.V., Davis, A., Schoof, R., Eberle, S., Sellstone, C.M., 1996. Estimation of lead and
419 arsenic bioavailability using a physiologically based extraction test. *Environ. Sci. Technol.*
420 30, 422-430.

421 Schlautman, M.A., Morgan, J.J., 1993. Effects of aqueous chemistry on the binding of
422 polycyclic aromatic hydrocarbons by dissolved humic materials. *Environ. Sci. Technol.*
423 27, 961-969.

424 Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., Harms, H., 2004. Defining
425 bioavailability and bioaccessibility of contaminated soil and sediment is complicated.
426 *Environ. Sci. Technol.* 38, 228A-231A.

427 Song, Y.F., Jing, X., Fleischmann, S., Wilke, B.M., 2002. Comparative study of extraction
428 methods for the determination of PAHs from contaminated soils and sediments.
429 *Chemosphere* 48, 993-1001.

430 Ye, X., Ma, J., Wei, J., Sun, K., Xiong, Q., 2019. Comparison of the bioavailability of
431 benzo[a]pyrene (B[a]P) in a B[a]P-contaminated soil using the different addition
432 approaches. *Scientific Reports* 9, 1-9.

433 Yu, L., Duan, L., Naidu, R., Semple, K.T., 2018. Abiotic factors controlling bioavailability and
434 bioaccessibility of polycyclic aromatic hydrocarbons in soil: Putting together a bigger
435 picture. *Sci. Total Environ.* 613, 1140-1153.

436

Table 1

Selected physicochemical properties of the soils used in this study.

Soil ID	pH ^a	EC ($\mu\text{S cm}^{-1}$)	TOC (%)	Particle size fraction (%)			Surface area ($\text{m}^2 \text{g}^{-1}$)	Average pore diameter (\AA) ^b	PF ^c <6 nm	Classification	Soil mineralogy analysed by XRD
				Sand	Silt	Clay					
GIA	6.87	64.7	0.78	78.1	16.2	5.6	9.91	49.0	46.7	Sodosol	Quartz, illite, montmorillonite-kaolinite interstratifications
GIA-pH	8.57	88.8									
GIA-EC	6.67	436									
GIA-pH&EC	8.63	444									
BDA	6.92	86.5	3.27	53.0	16.1	30.9	4.01	81.3	22.8	Black Vertisol	Montmorillonite, kaolinite, montmorillonite-kaolinite interstratifications, feldspar
BDA-pH	8.25	176									
BDA-EC	6.47	439									
BDA-pH&EC	8.23	483									

^a pH measured in water at soil: water ratio=1:5.^b Adsorption average pore width (4 V/A by BET).^c PF < 6 nm: proportion of pores less than 6 nm (%).

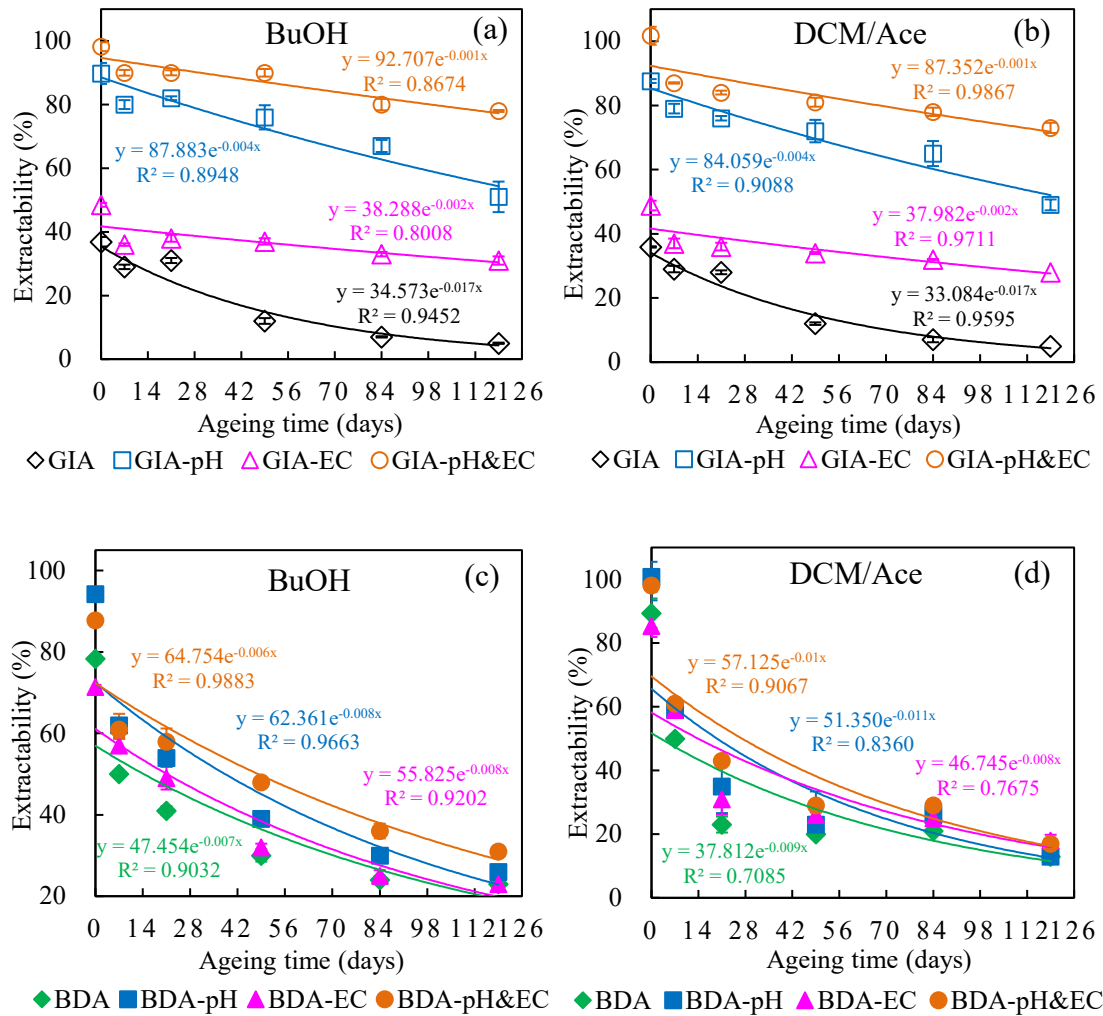


Fig. 1 B[a]P extractability in GIA and its modified soils extracted by BuOH (a) and DCM/Ace (b) and in BDA and its modified soils extracted by BuOH (c) and DCM/Ace (d) over ageing time. Data at Day 0 indicated B[a]P spike recovery before adding water for ageing. Data from Day 7 to 119 was fitted by a first-order kinetic model. Each fitting was presented along with R^2 and indicated by colour.

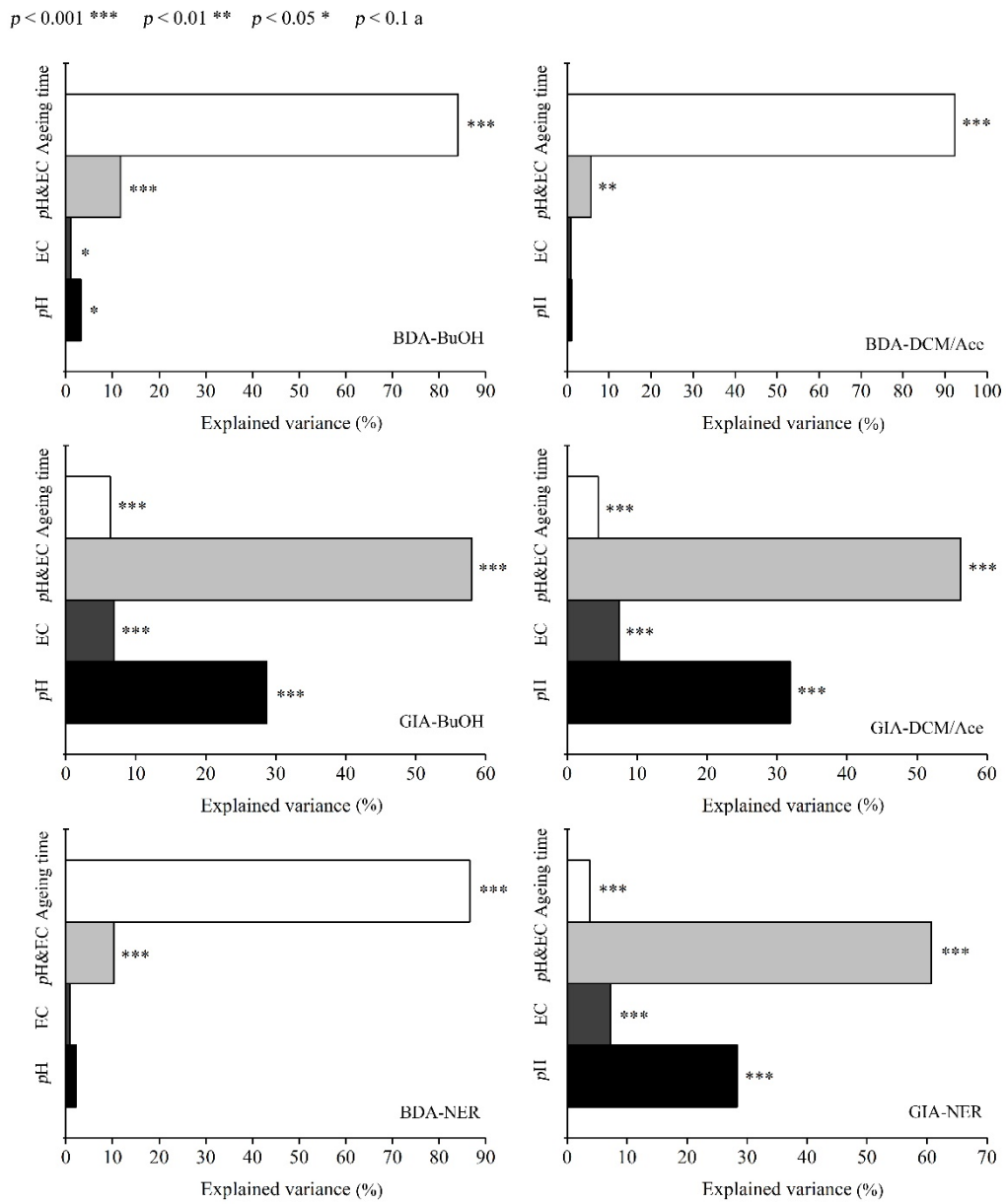


Fig. 2 The contributions of ageing time, pH and/or EC to B[a]P extractability and non-extractable residue (NER) in BDA and GIA calculated using variance analysis.

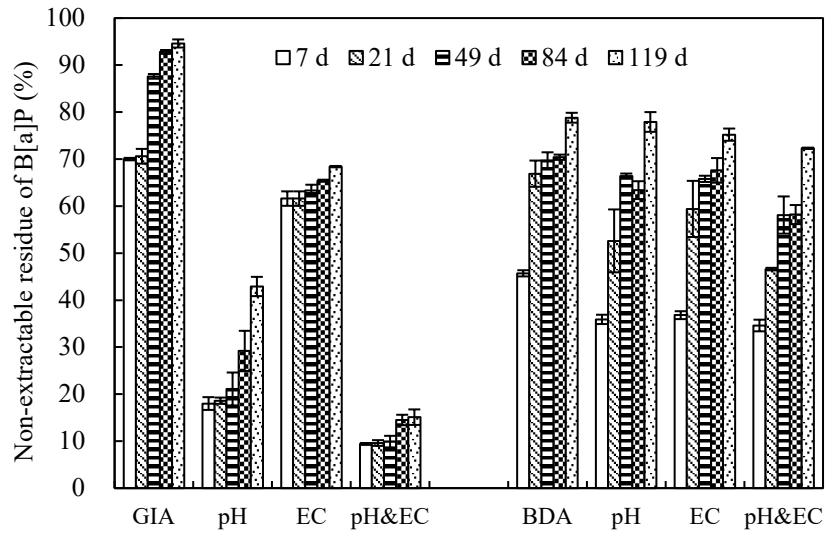


Fig. 3 Non-extractable residue of B[a]P (100-DCM/Ace-NaOH, %) in GIA, BDA and their modified soils over ageing time.

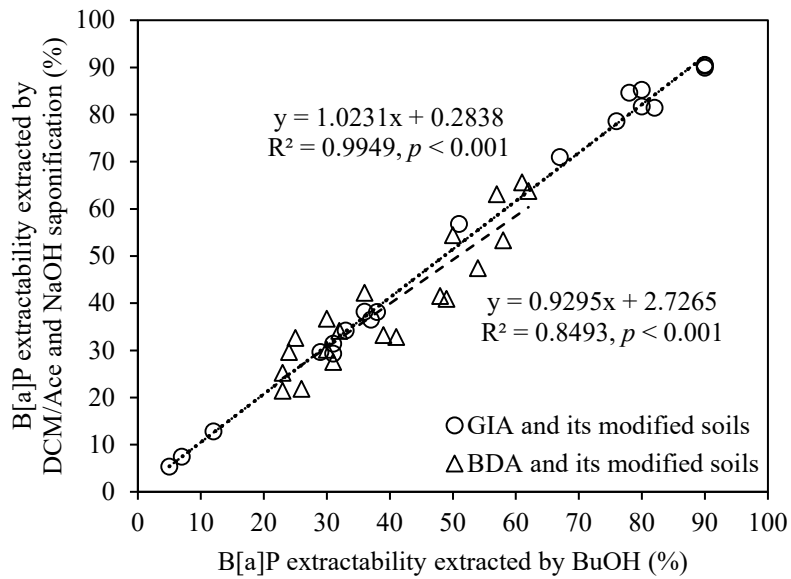


Fig. 4 Correlations between BuOH extractability and total extractable B[a]P estimated by DCM/Ace + NaOH saponification sequential extraction for both GIA and BDA and their modified soils.

Influence of pH and EC on the ageing process of benzo[a]pyrene in two contrasting soils

Fanbo Meng^{a,b}, Xiaodong Yang^{b,c}, Luchun Duan^{b,d*}, Ravi Naidu^{b,d}, Md Nuruzzaman^b, Kirk T.

Semple^e

^a Institute of Soil, Jinan Environmental Research Academy, Jinan 250102, China

^b Global Centre for Environmental Remediation (GCER), ATC Building, the University of Newcastle, Callaghan Campus, NSW 2308, Australia

^c Department of Geography & Spatial Information Technology, Ningbo University, Ningbo 315211, China

^d Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), the University of Newcastle, Callaghan Campus, NSW 2308, Australia

^e Lancaster Environmental Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom

* **Corresponding author:** Luchun Duan, Global Centre for Environmental Remediation (GCER), ATC Building, the University of Newcastle, Callaghan Campus, NSW 2308, Australia; e-mail: luchun.duan@newcastle.edu.au

Table S1

B[a]P extractability (%) in GIA, BDA and their modified soils extracted by BuOH, DCM/Ace and NaOH saponification over ageing.

Ageing time/d	BuOH				DCM/Ace				NaOH saponification			
	GIA	pH	EC	pH&EC	GIA	pH	EC	pH&EC	GIA	pH	EC	pH&EC
0	36.9±1.5Ad	89.8±3.3Ab	48.5±0.6Ac	98.2±1.4Aa	35.9±0.8Ad	87.6±0.6Ab	48.8±0.7Ac	101.7±2.8Aa	0.5±0.0Dc	1.0±0.1Fb	1.2±0.1Db	1.5±0.0Fa
7	29.1±0.7Cd	79.8±1.4BCb	36.1±0.4Bc	90.0±0.9Ba	29.4±0.2Bd	79.3±1.5Bb	37.2±1.5Bc	87.1±0.2Ba	0.6±0.0Cd	2.7±0.1Eb	1.2±0.1Dc	3.5±0.0Ea
21	30.9±0.8Bd	81.9±0.6Bb	37.7±1.1Bc	90.2±0.8Ba	28.1±1.0Bd	76.0±0.7Cb	36.3±1.5BCc	84.1±0.6Ca	1.3±0.2Ad	5.4±0.0Db	2.1±0.1Cc	6.3±0.0Da
49	12.1±0.9Dd	75.9±3.8Cb	36.8±1.0Bc	90.0±1.2Ba	11.6±0.6Cd	72.3±3.5Cb	34.2±1.2BCc	81.2±1.4Ca	0.8±0.1Bd	6.6±0.0Bb	2.5±0.1Bc	8.9±0.2Ba
84	6.9±0.2Ed	66.9±1.9Db	33.0±0.7Cc	79.5±1.8Ca	6.8±0.4Dd	64.8±3.9Db	32.4±0.3Cc	78.3±1.3Da	0.4±0.0Ed	6.0±0.4Cb	2.2±0.0Cc	7.2±0.3Ca
119	4.6±0.2Fd	50.8±4.8Eb	31.3±1.3Cc	77.5±0.3Da	5.1±0.8Ed	49.3±1.7Eb	28.2±0.1Dc	73.3±1.6Ea	0.3±0.0Fd	7.8±0.7Ab	3.4±0.1Ac	11.6±0.4Aa
	BDA	pH	EC	pH&EC	BDA	pH	EC	pH&EC	BDA	pH	EC	pH&EC
0	78.3±1.1Ac	94.2±1.5Aa	71.4±0.5Ad	87.8±1.1Ab	89.4±4.6Ab	100.8±4.7Aa	85.3±3.4Ab	98.1±4.7Aa	2.1±0.3Dd	3.6±0.1Ec	5.4±0.6Ca	3.0±0.2Db
7	49.7±0.4Bc	61.9±2.8Ba	56.6±1.8Bb	61.1±2.4Ba	49.9±1.2Bb	59.3±0.9Ba	59.1±0.8Ba	60.8±1.2Ba	4.4±0.6Cab	4.8±0.3Da	4.1±0.1Db	4.6±0.4Cab
21	41.1±1.1Cc	54.4±2.2Cb	48.7±2.8Cc	58.2±3.2Ba	23.3±2.5Cc	35.0±8.4BCb	30.7±4.9Cb	43.1±0.5Ca	9.8±0.2Ab	12.4±1.3Aa	9.9±1.2Ab	10.3±0.3Bb
49	30.0±1.5Dd	39.0±0.9Db	32.1±0.9Dc	48.2±0.4Ca	20.2±1.3Cb	23.4±1.2Ca	26.1±0.8CDa	29.4±4.4Da	10.1±0.7Ab	10.2±0.7Bb	8.1±0.2Bc	12.5±0.4Aa
84	23.9±1.0Ec	30.2±1.1Eb	25.4±1.4Ec	36.4±1.8Da	21.0±0.8Cb	25.9±1.5Ca	24.8±2.4Da	28.6±1.8Da	8.6±0.3Bc	10.7±0.4Bb	7.6±0.3Bd	13.2±0.8Aa
119	22.6±0.8Ec	26.0±1.1Fb	23.0±1.3Ec	31.3±1.3Ea	12.8±1.2Db	13.3±1.2Db	17.6±1.8Ea	17.2±0.3Ea	8.4±0.2Bb	8.8±0.9Cb	7.2±0.6Bc	10.5±0.4Ba

Different capital letters indicate significant differences among ageing time ($p < 0.05$). Different lowercase letters indicate significant differences among GIA/BDA and its modified soils at the same ageing time ($p < 0.05$).

Table S2

First-order kinetic model fitting parameters for B[a]P ageing in GIA, BDA and their modified soils

from 7 days to 119 days.

Extraction	Soil ID	y_0	k (d ⁻¹)	R^2	P -value
BuOH	GIA	34.5	0.017	0.94	< 0.001
	GIA-pH	87.8	0.004	0.89	< 0.001
	GIA-EC	38.2	0.002	0.80	< 0.001
	GIA-pH&EC	92.7	0.001	0.86	< 0.001
DCM/Ace	GIA	33.1	0.017	0.96	< 0.001
	GIA-pH	84.0	0.004	0.91	< 0.001
	GIA-EC	37.9	0.002	0.97	< 0.001
	GIA-pH&EC	87.3	0.001	0.98	< 0.001
BuOH	BDA	47.4	0.007	0.90	< 0.001
	BDA-pH	62.4	0.008	0.96	< 0.001
	BDA-EC	55.8	0.008	0.92	< 0.001
	BDA-pH&EC	64.7	0.006	0.99	< 0.001
DCM/Ace	BDA	37.8	0.009	0.71	< 0.001
	BDA-pH	51.3	0.011	0.84	< 0.001
	BDA-EC	46.7	0.008	0.76	< 0.001
	BDA-pH&EC	56.1	0.010	0.90	< 0.001

y_0 is modelled extractability of B[a]P (%) at day 0 and k is the decreasing rate constant (d⁻¹).

Table S3

NER of B[a]P (%) in GIA, BDA and their modified soils over ageing.

Ageing time/d	GIA	pH	EC	pH&EC
7	70.0 ± 0.3Da	18.0 ± 1.4Cc	61.7 ± 1.5Cb	9.4 ± 0.2Bd
21	70.5 ± 1.6Da	18.7 ± 0.7Cc	61.6 ± 1.5Cb	9.6 ± 0.6Bd
49	87.6 ± 0.5Ca	21.1 ± 3.5Cc	63.3 ± 1.3Cb	9.9 ± 1.2Bd
84	92.7 ± 0.4Ba	29.2 ± 4.3Bc	65.5 ± 0.2Bb	14.5 ± 1.1Ad
119	94.6 ± 0.9Aa	42.9 ± 2.1Ac	68.5 ± 0.1Ab	15.1 ± 1.7Ad
	BDA	pH	EC	pH&EC
7	45.7 ± 0.7Da	35.8 ± 1.0Dbc	36.7 ± 0.8Dab	34.6 ± 1.2Dc
21	66.9 ± 2.8Ca	52.6 ± 6.7Cbc	59.4 ± 6.0Cab	46.6 ± 0.3Cc
49	69.7 ± 1.8BCa	66.4 ± 0.5Ba	65.8 ± 0.7Ba	58.1 ± 4.0Bb
84	70.4 ± 0.6Ba	63.4 ± 1.9Bb	67.7 ± 2.6Ba	58.2 ± 2.0Bc
119	78.8 ± 1.1Aa	77.9 ± 2.1Aa	75.2 ± 1.3Ab	72.2 ± 0.1Ac

Different capital letters indicate significant differences among ageing time ($p < 0.05$). Different lowercase letters indicate significant differences among GIA/BDA and its modified soils ($p < 0.05$).

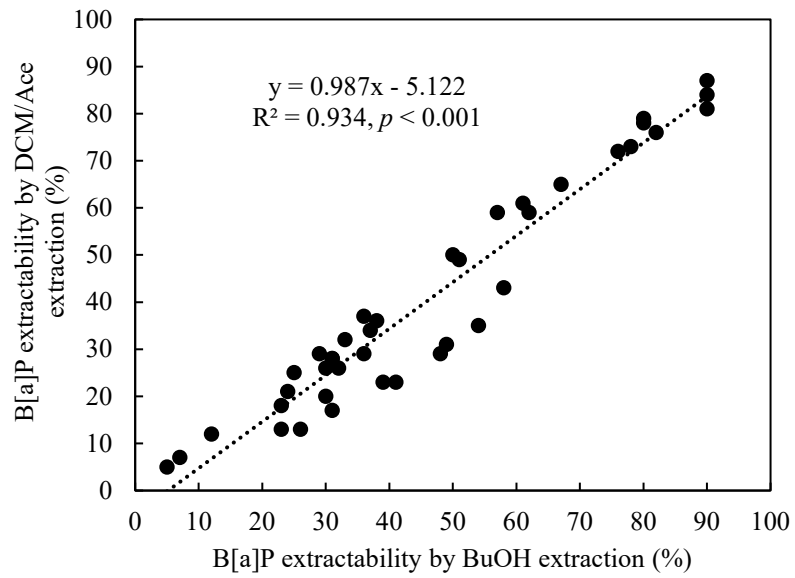


Fig. S1 Correlation between B[a]P BuOH extractability and DCM/Ace extractability in GIA, BDA and their modified soils.