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

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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 contracting 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

1. Introduction

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

The contaminant bioavailability is defined as the fraction of the total amount that is 'freely available' in a medium for uptake i.e., able to cross the cellular membrane of an organism at a given point of time (Semple et al., 2004). Therefore, it is the bioavailable fraction, rather than the total contaminant in soils, that is critical for defining exposure, uptake and the consequent risk to the environmental receptors, and could be used to assess the effectiveness or feasibility of bioremediation technologies (Li et al., 2013). Many researchers have observed an 'ageing effect' of hydrophobic organic contaminants (HOCs), such as PAHs in soil (Duan et al., 2015; Meng and Chi, 2017; Ye et al., 2019). Such process are often determined by increased sorption 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               $$\text{Extractability } (\%) = \frac{m_{extracted}/m_{dry\ soil}}{10\ \text{mg kg}^{-1}} \times 100\% \qquad (1)$$

149   where $m_{extracted}$ was the mass of B[a]P extracted from soil (ug), $m_{dry\ soil}$ 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 °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 °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

calibration curves were made based on the sample concentration, using DAD (at a wavelength

of 267 nm) and/or an FLD detector (with an excitation wavelength of 297 nm and an emission

wavelength of 405 nm) to encompass the wide concentration range of B[a]P from 25 µg L^{-1} to

5 mg L^{-1}.

2.4 Model fitting of B[a]P ageing kinetics

The ageing kinetics of B[a]P in soils was described by a first-order kinetic model (Eq. (2))

(Duan et al., 2015).

$$y_t = y_0 \times e^{-k \times t} \tag{2}$$

where y_0 and y_t are the modelled extractability of B[a]P (%) at day 0 and day t, k is the

decreasing rate constant (d^{-1}).

2.5 Quality assurance and quality control

Laboratory glassware was soaked in alkaline for 24 h, washed under continuous water flow,

oven-dried at 120 °C for 4 h, and rinsed twice with acetone prior to use. Background

concentrations in both soils were checked before use. No detectable B[a]P concentration was

found in both study soils. Spike recovery of B[a]P using same procedure in spiked silica sand

(at 50 mg kg^{-1}) had shown a complete recovery (100 ± 0.5%, n=5) previously (Duan et al.,

2015).

In this study, B[a]P spiked at 10 mg kg^{-1} was examined in each of the eight soils (n=3)

before adding water for ageing (Day 0). In brief, spike recovery of B[a]P ranged from 36 ± 0.8%

to 102 ± 3% in GIA, from 85 ± 3% to 101 ± 5% in BDA and their modified soils. Details of

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 ± 0.8% and 49

217   ± 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 ± 0.6% and 102 ± 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 ± 3% to 101 ± 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

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

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

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

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 PF< 6 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

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

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

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Declarations of interest: none

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436

Table 1

Selected physicochemical properties of the soils used in this study.

Soil ID	pH[a]	EC (μS cm^{-1})	TOC (%)	Particle size fraction (%)			Surface area (m^2 g^{-1})	Average pore diameter (Å)[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 (%).
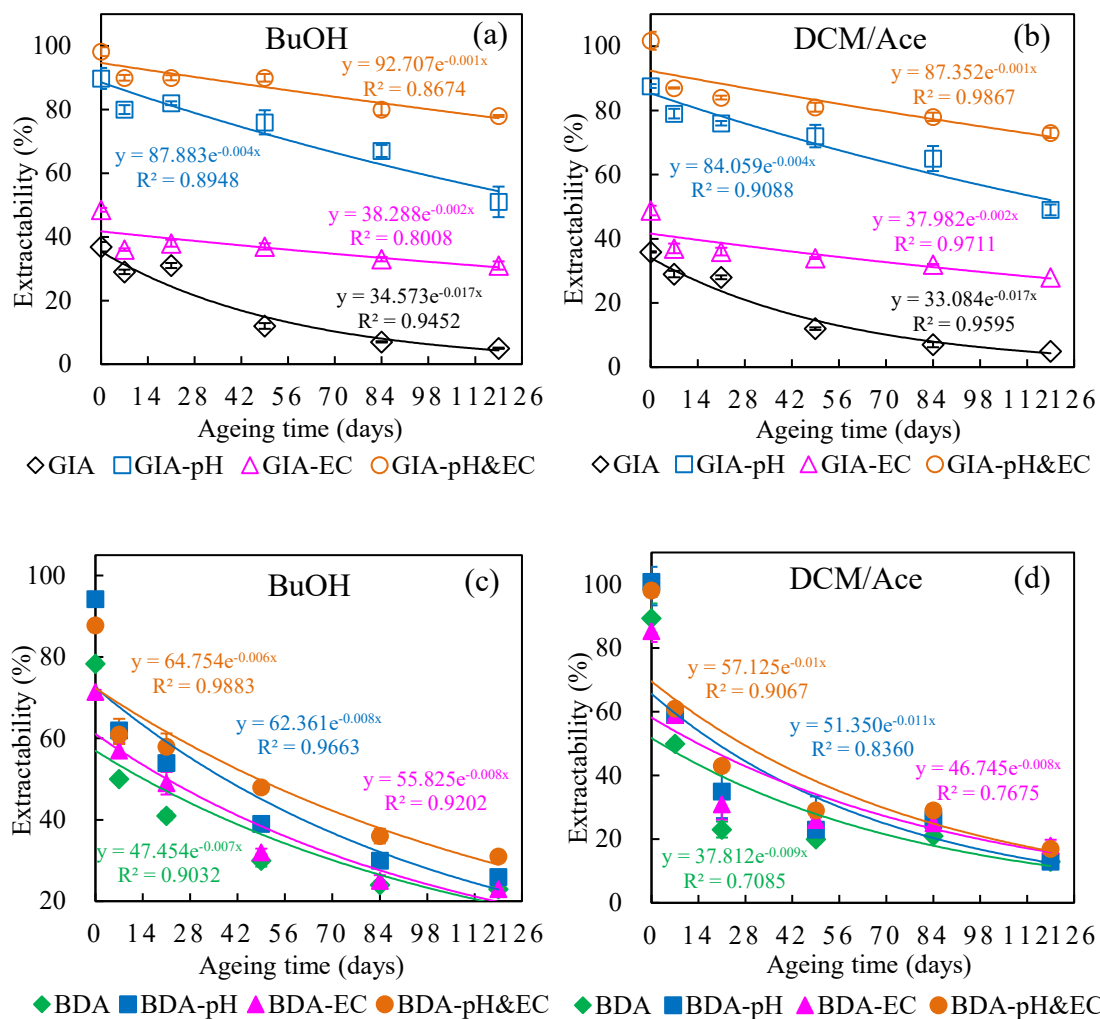
Fig. 1 B[a]P extractability in GIA and its modified soils extracted by BuOH (a) and DCM/Ace (b) and in BDA and its modified soils extracted by BuOH (c) and DCM/Ace (d) over ageing time. Data at Day 0 indicated B[a]P spike recovery before adding water for ageing. Data from Day 7 to 119 was fitted by a first-order kinetic model. Each fitting was presented along with R^2 and indicated by colour.
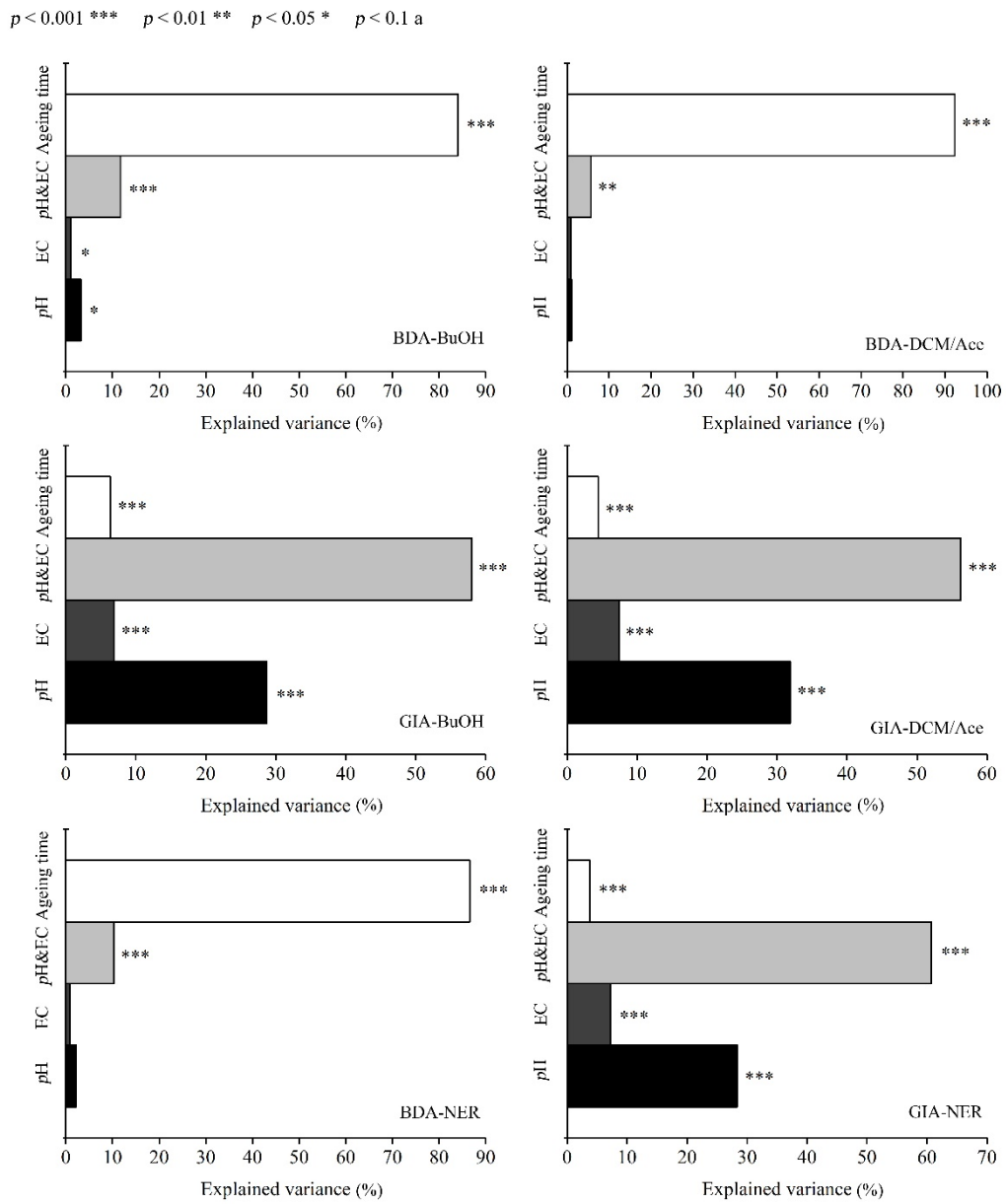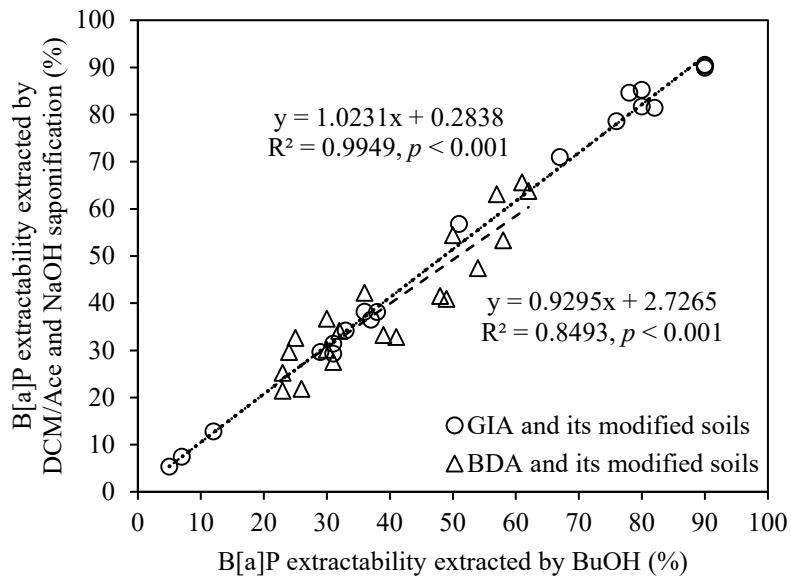
Fig. 2 The contributions of ageing time, pH and/or EC to B[a]P extractability and non-extractable residue

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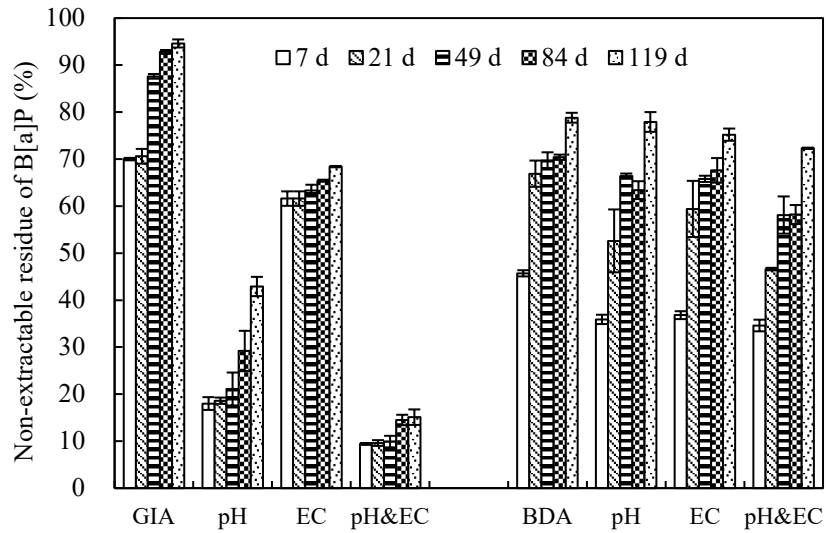
Fig. 3 Non-extractable residue of B[a]P (100-DCM/Ace-NaOH, %) in GIA, BDA and their modified soils over ageing time.

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

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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 ($p < 0.05$).
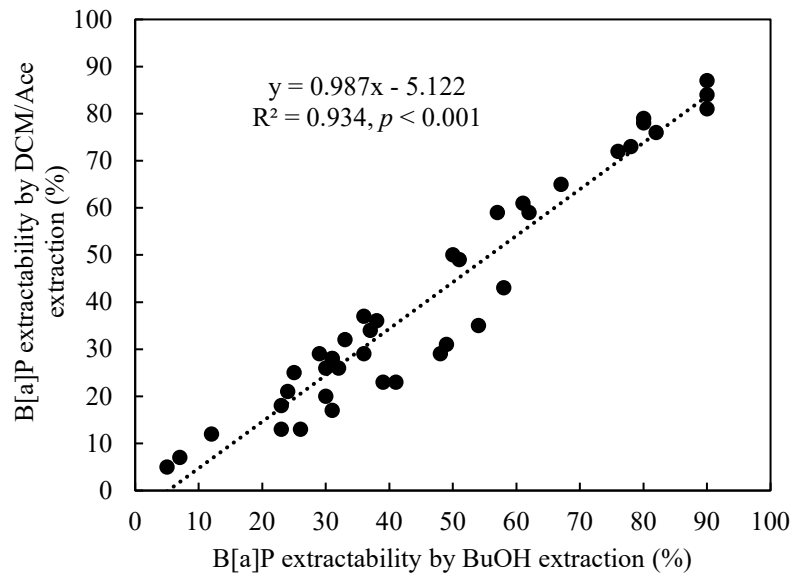
Fig. S1 Correlation between B[a]P BuOH extractability and DCM/Ace extractability in GIA, BDA and their modified soils.