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

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

\textsuperscript{a} Institute of Soil, Jinan Environmental Research Academy, Jinan 250102, China
\textsuperscript{b} Global Centre for Environmental Remediation (GCER), ATC Building, the University of Newcastle, Callaghan Campus, NSW 2308, Australia
\textsuperscript{c} Department of Geography & Spatial Information Technology, Ningbo University, Ningbo 315211, China
\textsuperscript{d} Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), the University of Newcastle, Callaghan Campus, NSW 2308, Australia
\textsuperscript{e} Lancaster Environmental Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom

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

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

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

From a human health risk assessment perspective, bioavailability of HOCs is the amount of compound that is desorbed from soil through desorption processes under physiological conditions, which is available for uptake into the circulatory system (Ruby et al., 1996; Kramer and Ryan, 2000), which needs to be estimated using in vivo animal studies, such as rat or swine models. However, animal studies are expensive and time-consuming, sometimes are not even possible due to ethics issues, hence these type of data are scarce. Among the limited studies, our previous research investigated the influence of soil properties on the oral bioavailability (BA) of B[a]P in soils, in which eight soils with significantly varied soil properties were investigated using a swine model (Duan et al., 2014). Despite being spiked at the same concentration (at 50 mg kg⁻¹), BA of B[a]P in most soils estimated after 90 days of ageing ranged from 20% to 60%, which was significantly lower than that estimated in freshly spiked silica sand. Significant negative correlations were identified between relative bioavailability of B[a]P in soil (RB, compared to that in freshly spiked silica sand used as reference material that assists comparison between different soils) and two specific soil properties, namely FPAC (fine particle associated carbon) and PF < 6 nm (meso-pore size less than 6 nm fraction) in most soils. In addition, there were two obvious outlier soils, both with elevated pH and EC. These soils
showed much higher oral bioavailability compared to the general correlation(s) (Duan et al., 2014). One of the outlier soils was a subsurface soil (GIB, sampled from 20 cm to 40 cm depth from the surface). Its surface soil (GIA), which had lower pH and EC values, however, was part of the set of soils that showed strong significant correlation with FPAC and meso pores < 6 nm. These results strongly indicated that higher soil pH and EC may result in the higher B[a]P bioavailability in soil GIB compared to GIA.

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

Our previous study has also demonstrated that the ageing process varies amongst different soils (Duan et al., 2015). Thus, the major task of this study was to investigate whether increasing
pH and EC values and ageing will change B[a]P extractability in two contrasting soils. Particular attention was paid to the form of non-extractable residue over time and to comparison between the extraction efficacies of the two extraction methods.

2. Materials and methods

2.1 Soils

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

2.2 Soil spiking and ageing

The soils were spiked with B[a]P at 10 mg kg⁻¹ on a dry weight basis following Duan et al. (2014). To ensure that the same amount of B[a]P was delivered to each soil, a 10 mL air-tight glass syringe was used to distribute B[a]P stock solution (1000 mg L⁻¹, in n-hexane) into eight 4 mL glass vials (each 1.2 mL). The vials were sealed with PTFE-lined caps. When the soils were ready for spiking, a glass pipette was used to deliver all the stock solution in the 4 mL vial
to each soil. Briefly, 120 g of each soil (dry weight) was placed into a 250 mL amber glass jar.

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

2.3 Extraction of B[a]P from soil

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

\[
\text{Extractability (\%)} = \frac{m_{\text{extracted}}}{m_{\text{dry soil}}} \times 100\% \\
\text{(1)}
\]

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

For BuOH extraction, 1.0 g of soil sample was taken and 10 mL of BuOH was added. The glass centrifuge vials were properly sealed and placed in a box to shade them from light on a
flat-bed shaker for 24 h set at 120 rpm. The vials were then centrifuged at 2000 g for 30 min to separate the solid phase. An aliquot of the BuOH extract was filtered through a 0.45 µm PTFE syringe filter and stored in a 2 mL amber HPLC vial.

The DCM/Ace extraction method followed (Duan et al. 2015). In brief, 1.0 g of soil sample was mixed with an adequate amount of anhydrous Na₂SO₄ to form a free flow sample. Then, 10 mL of premixed solvent DCM/Ace (1:1, v/v) was added. The extraction was facilitated by sonication (40 KHz for 15 min) twice, in between the samples were vortexed to resuspend the soil particles. The solvent extract was separated by centrifugation at 3000 g for 20 min and decanted into another 40 mL glass vial. The whole extraction procedure was repeated for further two times. The solvent extracts were combined (~30 mL) and evaporated under a gentle N₂ gas flow, following which 5 mL of ACN was added to redissolve the extract. An aliquot of the sample (~2 mL) was then filtered through a 0.45 µm PTFE syringe filter into a 2 mL vial.

The soil samples after DCM/Ace extraction were allowed to dry (solvent evaporation) in a fume hood. Then 5 mL of 2 mol L⁻¹ NaOH solution was added (Ma et al., 2012). The vials were then capped tightly, and placed in an oven set at 100 ºC for 2 hours. The samples were allowed to cool down after removal from the oven. The samples were then acidified to pH 1~2 with 6 mol L⁻¹ HCl. The mixtures were then extracted with 5 mL Hex three times. The combined Hex extracts were evaporated under a gentle N₂ gas stream and re-dissolved in 5 mL of ACN, followed by filtering through 0.45 µm PTFE filters into 2 mL HPLC vials for HPLC analyses. All samples were stored at -20 ºC until analysed.

B[a]P concentrations were determined using an Agilent 1260 HPLC system coupled with a 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.
B[a]P calibration standards were analysed along with the different batches of samples at different ageing time. The slope of standard curve showed good consistency over the whole study (SD < 1.5, n=6).

2.6 Statistical analysis

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

3. Results and discussion

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

Spike recovery of B[a]P in both GIA and BDA and their modified soils is presented in Table S1. They are generally high (> 85%), which is in accordance with previous studies and demonstrated the high extraction capacity of DCM/Ace (Song et al., 2002; Duan et al., 2014; Duan et al., 2015). There were two exceptions to this general observation, the unmodified soil GIA and its EC modified soil GIA-EC, for which B[a]P recovery was only $36 \pm 0.8\%$ and $49 \pm 0.7\%$, respectively. Subsequent NaOH saponification could not extract more B[a]P from these two soils as well (< 1.5%). However, in comparison, B[a]P recovery in GIA-pH and GIA-
pH&EC was as high as 88 ± 0.6% and 102 ± 3%, respectively. These results suggest that increasing pH has a significant impact on the B[a]P recovery in GIA, immediately after spiking. It was also noted previously that extractability of B[a]P in GIA was much lower than its subsurface soil (GIB) that has higher pH and EC values (Duan et al., 2014). Compared to the marked influence of pH on B[a]P recovery in soil GIA, the influence of pH and EC on the B[a]P recovery in BDA was very limited. The spike recovery of B[a]P in BDA and its pH and/or EC modified soils were all similar, ranging from 85 ± 3% to 101 ± 5%.

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

Fig. 1 exhibits the change in B[a]P extractability estimated by BuOH and DCM/Ace in both soils and their pH and/or EC modified treatment over time. More details of the extracted fractions by each method, including that released by NaOH saponification, are presented in Table S1. The fitted first-order kinetic model parameters (i.e., $y_0$ and $k$) as well as $R^2$ and $p$ values are presented in Table S2. The $R^2$ values for all soils ranged from 0.71 to 0.99 ($p < 0.001$), indicating a general good fit of the data. The general decreasing trend of B[a]P extractability in all soils estimated by both extraction methods indicated that B[a]P went through a sorptive diffusion/sequestration process (ageing process) in soil (Reid et al., 2000; Duan et al., 2015). However, the ageing effect was clearly more significant in the clayey soil BDA than in the sandy soil GIA (indicated by higher $k$ values). In the sandy soil GIA, the extractability of B[a]P estimated by both BuOH and DCM/Ace was similar and followed the same order constantly over the investigated ageing period with the unmodified soil having the lowest extractability (pH&EC > pH > EC > GIA). Nevertheless, the decreasing rate constant $k$ also indicated that 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
in the soil surface charge, resulting from both mineral phase, especially the clay minerals, and
the soil organic matter phase (SOM). Lower pH and EC have been reported to favour sorption
of PAHs on humic acid and mineral-bound humic substances (Schlautman and Morgan, 1993;
Murphy et al., 1994; Laor et al., 1998; Feng et al., 2006). These phenomena were mainly
attributed to changes in the humic acid surface charge. With increasing pH, organic matter
deprotonated and became more negatively charged. These polar sites have lower affinity for
PAHs, resulting in lower sorption. Also, more favourable sorption sites become available at
soil organic matter for the sorption of HOC as organic matter adopts elongated configuration at
low ionic strength (Na+ electrolyte) as a result of charge repulsion between ionised functional
groups (Murphy et al., 1994). With much lower TOC (0.78%) and clay content (5.6%) in GIA,
it would have much less buffering capacity than BDA. This explained why GIA was more
vulnerable to pH and/or EC changes. Also, increasing pH may promote SOM dissolution
(Andersson and Nilsson, 2001). However, the much lower B[a]P extractability in the
unmodified soil GIA compared to unmodified BDA may be attributed to other critical soil
properties, such as its much higher PF<6 nm (Table 1).

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

In this study, a NaOH saponification method was used to extract the remaining extractable
B[a]P fraction after DCM/Ace extraction in soils (Ma et al., 2012; Gao et al., 2017). Data is
presented in Table S1. The amount of B[a]P extracted by NaOH saponification was not large
(ranged from 0.3%~13%) as it was performed following the exhaustive DCM/Ace extraction.
The HOC remaining in soil after exhaustive extraction is considered as a non-extractable
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
also noted from the previous swine study, that B[a]P oral bioavailability in BDA (~40%) was
higher than that in GIA (~20%) (Duan et al., 2014).

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

4. Conclusion

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

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

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

Acknowledgements

The authors are grateful to the Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) and the China Scholarship Council for financial support.
Declarations of interest: none

References


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


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


Mater. 261, 687-700.


Table 1

Selected physicochemical properties of the soils used in this study.

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>pH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EC (µS cm&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>TOC (%)</th>
<th>Particle size fraction (%)</th>
<th>Surface area (m&lt;sup&gt;2&lt;/sup&gt; g&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Average pore diameter (Å)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PF&lt;sub&gt;c&lt;/sub&gt;&lt;6 nm</th>
<th>Classification</th>
<th>Soil mineralogy analysed by XRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIA</td>
<td>6.87</td>
<td>64.7</td>
<td>0.78</td>
<td>78.1</td>
<td>16.2</td>
<td>5.6</td>
<td>9.91</td>
<td>49.0</td>
<td>46.7</td>
</tr>
<tr>
<td>GIA-pH</td>
<td>8.57</td>
<td>88.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIA-EC</td>
<td>6.67</td>
<td>436</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIA-pH&amp;EC</td>
<td>8.63</td>
<td>444</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDA</td>
<td>6.92</td>
<td>86.5</td>
<td>3.27</td>
<td>53.0</td>
<td>16.1</td>
<td>30.9</td>
<td>4.01</td>
<td>81.3</td>
<td>22.8</td>
</tr>
<tr>
<td>BDA-pH</td>
<td>8.25</td>
<td>176</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDA-EC</td>
<td>6.47</td>
<td>439</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDA-pH&amp;EC</td>
<td>8.23</td>
<td>483</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> pH measured in water at soil: water ratio=1:5.

<sup>b</sup> Adsorption average pore width (4 V/A by BET).

<sup>c</sup> 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\textsuperscript{a,b}, Xiaodong Yang\textsuperscript{b,c}, Luchun Duan\textsuperscript{b,d,*}, Ravi Naidu\textsuperscript{b,d}, Md Nuruzzaman\textsuperscript{b}, Kirk T. Semple\textsuperscript{e}

\textsuperscript{a} Institute of Soil, Jinan Environmental Research Academy, Jinan 250102, China

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

\textsuperscript{c} Department of Geography & Spatial Information Technology, Ningbo University, Ningbo 315211, China

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

\textsuperscript{e} Lancaster Environmental Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom

\textbf{* 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.

<table>
<thead>
<tr>
<th>Ageing time/d</th>
<th>BuOH</th>
<th>DCM/Ace</th>
<th>NaOH saponification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GIA</td>
<td>pH</td>
<td>EC</td>
</tr>
<tr>
<td>0</td>
<td>36.9±1.5Ad</td>
<td>89.8±3.3Ab</td>
<td>48.5±0.6Ac</td>
</tr>
<tr>
<td>7</td>
<td>29.1±0.7Cd</td>
<td>79.8±1.4BCb</td>
<td>36.1±0.4Bc</td>
</tr>
<tr>
<td>21</td>
<td>30.9±0.8Bd</td>
<td>81.9±0.6Bb</td>
<td>37.7±1.1Bc</td>
</tr>
<tr>
<td>49</td>
<td>12.1±0.9Dd</td>
<td>75.9±3.8Cb</td>
<td>36.8±1.0Bc</td>
</tr>
<tr>
<td>84</td>
<td>6.9±0.2Ed</td>
<td>66.9±1.9Db</td>
<td>33.0±0.7Cc</td>
</tr>
<tr>
<td>119</td>
<td>4.6±0.2Fd</td>
<td>50.8±4.8Eb</td>
<td>31.3±1.3Cc</td>
</tr>
</tbody>
</table>

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.

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

$y_0$ 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.

<table>
<thead>
<tr>
<th>Ageing time/d</th>
<th>GIA</th>
<th>pH</th>
<th>EC</th>
<th>pH&amp;EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>70.0 ± 0.3Da</td>
<td>18.0 ± 1.4Cc</td>
<td>61.7 ± 1.5Cb</td>
<td>9.4 ± 0.2Bd</td>
</tr>
<tr>
<td>21</td>
<td>70.5 ± 1.6Da</td>
<td>18.7 ± 0.7Cc</td>
<td>61.6 ± 1.5Cb</td>
<td>9.6 ± 0.6Bd</td>
</tr>
<tr>
<td>49</td>
<td>87.6 ± 0.5Ca</td>
<td>21.1 ± 3.5Cc</td>
<td>63.3 ± 1.3Cb</td>
<td>9.9 ± 1.2Bd</td>
</tr>
<tr>
<td>84</td>
<td>92.7 ± 0.4Ba</td>
<td>29.2 ± 4.3Bc</td>
<td>65.5 ± 0.2Bb</td>
<td>14.5 ± 1.1Ad</td>
</tr>
<tr>
<td>119</td>
<td>94.6 ± 0.9Aa</td>
<td>42.9 ± 2.1Ac</td>
<td>68.5 ± 0.1Ab</td>
<td>15.1 ± 1.7Ad</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BDA</th>
<th>pH</th>
<th>EC</th>
<th>pH&amp;EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>45.7 ± 0.7Da</td>
<td>35.8 ± 1.0Dbc</td>
<td>36.7 ± 0.8Dab</td>
</tr>
<tr>
<td>21</td>
<td>66.9 ± 2.8Ca</td>
<td>52.6 ± 6.7Cbc</td>
<td>59.4 ± 6.0Cab</td>
</tr>
<tr>
<td>49</td>
<td>69.7 ± 1.8BCa</td>
<td>66.4 ± 0.5Ba</td>
<td>65.8 ± 0.7Ba</td>
</tr>
<tr>
<td>84</td>
<td>70.4 ± 0.6Ba</td>
<td>63.4 ± 1.9Bb</td>
<td>67.7 ± 2.6Ba</td>
</tr>
<tr>
<td>119</td>
<td>78.8 ± 1.1Aa</td>
<td>77.9 ± 2.1Aa</td>
<td>75.2 ± 1.3Ab</td>
</tr>
</tbody>
</table>

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.