Enhanced Recovery of Non-extractable Benzo[a]pyrene Residues in Contrasting Soils using Exhaustive Methanolic and Non-methanolic Alkaline Treatments

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ABSTRACT

The fate, impacts and significance of polycyclic aromatic hydrocarbon (PAH) non-extractable residues (NERs) in soils remain largely unexplored in risk-based contaminated land management. In this study, 7 different methanolic and non-methanolic alkaline treatments, and the conventional methanolic saponification, were used to extract benzo[a]pyrene (B[a]P) NERs that had been aged for 180 d from four contrasting soils. Up to 16% and 55% of the amount of B[a]P spiked (50 mg/kg) into soils was non-extractable after 2 d and 180 of aging, respectively; indicating rapid and progressive B[a]P sequestration in soils over time. The recovery of B[a]P from soils after 180 d of aging was increased by up to 48% by the 7 different alkaline extractions, although the extraction efficiencies of the different alkaline treatments did not differ significantly ($p > 0.05$). Approximately 40% of B[a]P NERs in the sandy-clay-loam organic matter-rich soil was recovered by the exhaustive alkaline extractions after 180 d of aging, compared to only 10% using conventional methanolic saponification. However, the amounts of B[a]P NERs recovered depend on soil properties and the amounts of NERs in soils. A significant correlation ($R^2 = 0.69$, $p < 0.001$) was also observed between the amounts of B[a]P recovered by each of the 7 alkaline extractions in the contrasting soils, and corresponding NERs at 180 d of aging, indicating a potential association warranting further investigations. Extraction techniques that estimate the amounts of PAH NERs recoverable in soil can help give a better understanding of the fate of NERs in soil.

INTRODUCTION

The fate and behaviour of hydrophobic organic contaminants (HOCs), such as polycyclic aromatic hydrocarbons (PAHs), in soil have been investigated for decades and are still being researched. A range of solvent extractions schemes have been used to investigate PAH extractability to elucidate their fate in soil. Non-exhaustive extractants including, hydroxylpropyl-β-cyclodextrin (HPCD), TENAX, XAD, and *n*-butanol, are used to extract weakly sequestered PAHs in soil, whereas, exhaustive solvents or solvent mixtures (e.g. dichloromethane, acetone, hexane) are used to extract PAHs that are more strongly sequestered in soils. These conventional solvent extractions are limited by their inability to extract 100% of PAHs from soil, as there are strong interactions between PAHs and soil organic matter (SOM), especially in long-term contaminated soils [1-4]. After successive exhaustive solvent extractions, residual PAHs, termed 'non-extractable residues (NERs)', may remain in soils as has been shown in studies using radio- or stable-isotope tracers [5-7]. Different terms have been used interchangeably in the literature to describe NERs, including: non-bioavailable, non-labile, residual, resistant, highly sequestered, slowly-desorbing, non-desorbing, desorption-resistant, recalcitrant, (ad)-sorbed, strongly sorbed, and bound among others [8].

The formation of NERs results from progressive contaminant sequestration in soil [9,10]. Sequestration as used describes the tortuous diffusion of HOCs into micro- and/or mesopores, and/or physical and chemical sorption of HOCs to soil matrices [1,11]. The interactions between PAHs with SOM, clay minerals, and carbon-rich materials such as black carbon also promote PAH sequestration [3,10,12-14]. According to Kastner et al. [2,7], non-covalently bonded NERs of HOCs which are entrapped and strongly adsorbed in soils are referred to as Type I NERs, whereas covalently-bonded NERs are Type II NERs. Microbial mineralisation of biodegradable parent compounds into non-toxic natural compounds may result in the formation of biogenic or Type III NERs which are incorporated into the SOM and undergo natural turnover processes [2,15,16]. Total NERs in soil is therefore a summation of the three types. While Type I to II have low to high stability in soil, Type III NER bears no environmental risks or relevance [2]. Because of their stability, the amounts of NERs in soils are difficult to measure, thereby, impacting thorough understanding of their fate in soils. In soils where there are stronger soil-PAH interactions, there is likely to be larger amounts of PAH NERs. Associated sequestration mechanisms include partitioning of PAHs into the complex macromolecular structures of humic materials in soil, such as humic and fulvic acid, humin, and SOM-mineral complexes within different particle size fractions or aggregates, particularly silt and clay [2,3,17,18]. Of the humic materials in soil, humin is reported to exhibit the greatest PAH sequestration ability due to its larger organic carbon content and embedded micropores [3,6,19]. When labile ester or amide or ether bonds of SOM are disrupted, SOM may be partially dissolved resulting in the release of humic materials, as well as PAHs incorporated within the humic matter matrix [2,20]. As a result, PAH recoveries from soils can be substantially increased.

Techniques to measure the amounts of NERs in soil would benefit risk-based approaches to contaminated land risk assessment. This is because, changes in the amount of NERs in soil due to the effects of dynamic processes, such as biodegradation, bioaccumulation, or remobilisation, could be monitored reliably. This should result in better decision making regarding NER stability in soil during contaminated land assessment and management. In addition, efficient extraction of the so called ‘NERs’ would result in a better understanding of total contaminant loads in soil which is useful for mass balance calculation purposes.

Based on the understanding of soil humic matter-HOCs interactions, methanolic saponification of soils employing a 2 h or 5 h sample heating time has been used to increase recovery of PAHs in soil [6,20,21]. Methyl isobutyl ketone extraction has also been used to disaggregate humin to release substantial amounts of associated PAHs, as well as PAHs associated with mineral fractions in soils [3]. Other techniques involving ^{14}C-sample combustion and scintillation counting have been used for complete or near-complete mass balancing of PAHs spiked in soil [6,19]; particularly, when complemented by chromatography techniques [5,18]. The costs associated with ^{14}C-sample combustion and scintillation counting techniques mean that these techniques are not easily accessible. Various exhaustive alkaline treatments have been used for routine soil humic matter extraction, including sodium hydroxide, sodium pyrophosphate, sodium fluoride, potassium hydroxide, and a combination of these chemicals [22-24]. Specifically, modifying these exhaustive alkaline extractions for the purpose of improving recovery of PAH NERs in soil may also allow better understanding of PAH partitioning, mobility, and availability in soils.

The impact and significance of NERs in soils are uncertain [8,25]. The 256th American Chemical Society (ACS) national meeting in Boston (August 19-23, 2018) with one of the themes: "Non-Extractable Residue (NER) Bio-accessibility and Potential Risks" further illustrates the paucity of information on the fate of NERs in soil [26]. Considering that the fate of PAH NERs in soil has been largely unexplored, this study evaluates whether 7 different exhaustive alkaline extractions, with or without methanol, access similar B[a]P NER fractions in soils, and whether they increase recovery of B[a]P NERs from soils compared to conventional methanolic saponification.

EXPERIMENTAL METHODS

Chemicals. Analytical grade B[a]P (> 96% purity), analytical grade acetone (Ace), acetonitrile (ACN), dichloromethane (DCM), ethanol (Analytical Grade), methanol (MeOH, HPLC Grade), toluene (Tol, 99.8%), potassium hydroxide (KOH) and silica sand were obtained from Sigma Aldrich Pty Ltd., Sydney, Australia. Sodium hydroxide (NaOH) and sodium fluoride (NaF) were sourced from the same suppliers. Hexane (Hex, HPLC grade) was purchased from Fisher Scientific, Loughborough, UK.

Soils. Four soils (I, M, B, and N) were utilised in this study and their properties were reported previously [27]. Soils I, M, B, and N were sandy-loam, sandy-clay-loam, sandy-clay-loam and loamy-sand, respectively, based on USDA textural classification. Soil organic matter contents of soils I (13.3), M (21.4%), B (11.0%), and N (4.8%) were estimated by loss on ignition [27]. The total organic carbon content (TOC) of acid-hydrolysed soils I, M, B and N were 4.3, 7.4, 3.5 and 1.2%, respectively. Prior to combustion of acid-hydrolysed soils at 1350 °C using LECO CNS analyser to determine the contents of hard organic carbon (hard OC), soft OC fractions were removed by wet oxidation with persulphate according to a previously described method [28]. The difference between TOC and hard OC contents estimated soft OC contents in the soils [28]. A thermogravimetric analysis (TGA) was also used to determine the contents of nonpyrogenic OC (weight losses at 200–470 °C) and black carbon (BC)-rich fractions (weight losses at 470–600 °C) in soils based on the thermal stability of these fractions [29].

Experimental Design. Previously documented quality assurance and quality control procedures were followed throughout the experiment [27]**.** Air-dried soils were sieved to less than 2 mm , spiked with 50 mg/kg B[a]P, rehydrated to field moisture contents (25% to 40% on dry weight basis), and kept for 180 d in the dark as described previously [27]. Dried soils were treated according to the design in Figure 1. Briefly, duplicates (1 g) of 7 subsamples were each extracted exhaustively with DCM/Ace in an ultrasonication bath and prepared for HPLC analysis as described previously [27]. The amount (µg/g) of B[a]P in soils that were extracted by DCM/Ace was referred to as total-extractable B[a]P, whereas the NER was estimated as the difference between the spiked concentration (50 µg/g) and the total-extractable concentration. Percentage extractability calculations were generally based on the amounts of B[a]P spiked (50 mg/kg) into the soil as described below:

$$\text{Extractability (\%)} = \left(\frac{\text{Amount of B[a]P Extracted by Solvent (µg)}}{\text{Amount of B[a]P Spiked into Soil (µg)}}\right) \text{X } 100\%$$

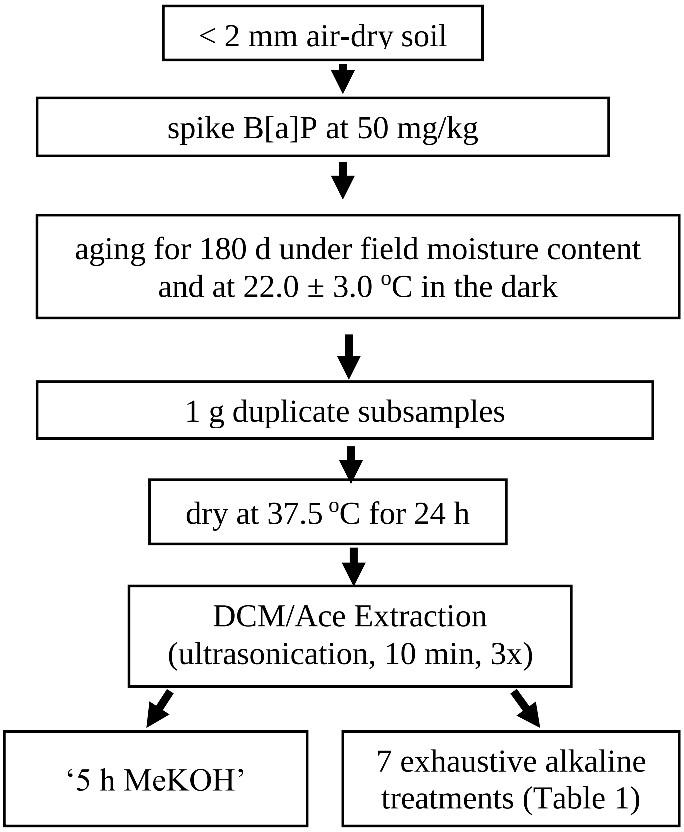

Figure 1. Experimental design.

Each of the extracted soils (n = 2) were then subjected to one of each of 7 exhaustive alkaline treatments (Table 1). A fresh subsample (1 g, n = 2) of each soil was then exhaustively extracted again and the extracted soil was subjected to methanolic saponification for 5 h. The resulting extracts were liquid-liquid extracted using Hex and prepared for HPLC analysis [27]. Extractability was then determined as previously described.

Table 1. Exhaustive methanolic and non-methanolic alkaline treatments used

ID	alkaline treatment	ratio (*v/v*)
1	2 M sodium hydroxide (NaOH) solution, 2 M NaOH	n/a
2	‘1’ + methanol (MeOH), ‘1’ + MeOH	1:14
3	‘1’ + 0.4 M sodium fluoride (NaF) mixture, ‘1’ + 0.4 M NaF	1:1
4	‘3’ + MeOH	1:14
5	0.1 M NaOH + 0.4 M NaF	1:1
6	‘5’ + MeOH	1:14
7	2 M potassium hydroxide (KOH) + MeOH, MeKOH	1:14

n/a: not applicable.

Methanolic Saponification and Exhaustive Alkaline Treatment of Soils. Soil containing B[a]P NERs (i.e. pre-extracted soil) was hydrolysed using different methanolic and non-methanolic alkaline treatments. The conventional methanolic saponification procedure (mixture of pre-extracted soil with 10 mL MeOH/2 M KOH (14:1, *v/v*)), thereafter referred to as ‘5 h MeKOH’, has been described previously [27]. For the more exhaustive alkaline treatments, soils were first mixed with 10 ml of one of the alkaline solutions (Table 1). The mixtures were vortexed for 10 s and end-over-end rotated (100 rpm) for 24 h. The soil mixtures were then heat-treated in a temperature-controlled oven at 100 ºC for 5 h and allowed to cool. The heat-treated samples were first liquid-liquid extracted with 5 ml ethanol:Hex (1:1, *v/v*) and vortexed briefly. Ethanol was added in all sample bottles to minimise the impacts of lipid emulsions especially observed in the sole alkaline treatments

(1, 3, and 5 in Table 1). Soil mixtures were then ultrasonicated for 10 min. The mixture was centrifuged, and the hexane layer collected as described previously. The liquid-liquid extraction was conducted 2 more times with only 5 ml Hex. The combined extracts from each of the '5 h MeKOH' and exhaustive alkaline treatments were then prepared for HPLC analysis [27]. The fractions of B[a]P NERs recovered by the '5 h MeKOH' and each of the exhaustive alkaline treatments were calculated relative to the estimated amounts of B[a]P NERs in the soils.

HPLC Analysis of B[a]P. The concentrations of B[a]P in the extracts were analysed with an Agilent 1100 Series HPLC equipped with a fluorescence detector (excitation wavelength = 230 nm and emission wavelength = 460 nm), as in our previous study [27] .

Data Analysis. Extractability data were analysed statistically with SPSS (IBM Corp, Version 24), and graphing was by both Origin (Microcal Software Inc. USA, version 6) and SPSS, without data transformation. There were 2 independent, and 2 or more outcome variables. The independent variables included 4 soil types and up to 8 different methanolic and non-methanolic alkaline treatments. The outcome variables were total extractability and extractabilities by the different alkaline treatments (μg/g or %). The levels of significance adopted was $p < 0.05$. A Student's t test was used to compare B[a]P extractabilities between each of the 7 exhaustive alkaline treatments and '5 h MeKOH'. One-way ANOVA was used to test between-group differences, such as effects of soil types on B[a]P extractability, using Games Howell's test for post hoc analysis [30]. Where data were not normally-distributed (Shapiro-Wilk Normality Test, $p < 0.05$), the Student's t test and one-way ANOVA were replaced by Mann-Whitney U and Kruskal Wallis tests, respectively.

RESULTS AND DISCUSSION

Total Extractability and Mass Balance of B[a]P in Soils. Total extractability 2 d after spiking in all four soils ranged from 84% (42.1 ± 1.7 µg/g, soil M) to 97% (48.7 ± 4.0 µg/g, soil N), indicating good recovery. That 16% or less B[a]P (i.e. NER) could not be recovered by the DCM/Ace extraction at the initial soil-B[a]P contact time (2 d) indicates that B[a]P sequestration occurred rapidly. Considering B[a]P's hydrophobicity (Log Kow = 6.3) [31] and the soils' physico-chemical properties, the amounts of B[a]P NERs formed at the initial soil-contact time could be expected. Rapid sequestration of PAHs have been noted in other studies [16,19]. An additional 4% to 13% of the 50 µg/g B[a]P spiked, corresponding to 1.9 ± 0.02 µg/g to 6.3 ± 0.04 µg/g B[a]P, were recovered after methanolic saponification ('5 h MeKOH') of pre-extracted soils 2 d after spiking and this confirmed the rapid B[a]P sequestration in the soils only 2 d after spiking. Hence, mass balance (sum of total-extractable B[a]P and B[a]P extracted by alkaline treatments) achieved after 2 d of spiking ranged from 87.9 ± 3.4% to 108.3% ± 6.3%, indicating near-complete or complete recovery of B[a]P spiked in soils.

Spike recovery was > 95% from silica sand after 180 d of aging indicating only minimal procedural losses in the laboratory. However, total B[a]P extractability in soils after 180 d of aging ranged from 45% (22.7 ± 1.5 µg/g, soil B) to 67% (33.7 ± 1.1 µg/g, soil I) (Figure 2). This suggests that approximately 33% to 55% of the amounts of B[a]P (50 µg/g) spiked in soils were non-extractable after 180 d of aging. Sequestration of PAHs is known to increase with aging time as a result of increased soil-PAH interactions [1,19]. Therefore, the fractions of PAHs which are extractable will be expected to decrease with increasing aging time, whereas fractions of PAH NERs will be expected to increase, particularly for hydrophobic HMW PAHs [32,33]. The NERs may include fractions which are occluded in meso- or micro-pores

[18,28], or adsorbed to surfaces of soil matrices by physical (Van der Waals) or weak non-covalent interactions (π-π) [2,28], as well as fractions strongly sequestered to hard OC or BC-rich fractions in soils [11,13,14,28,34].

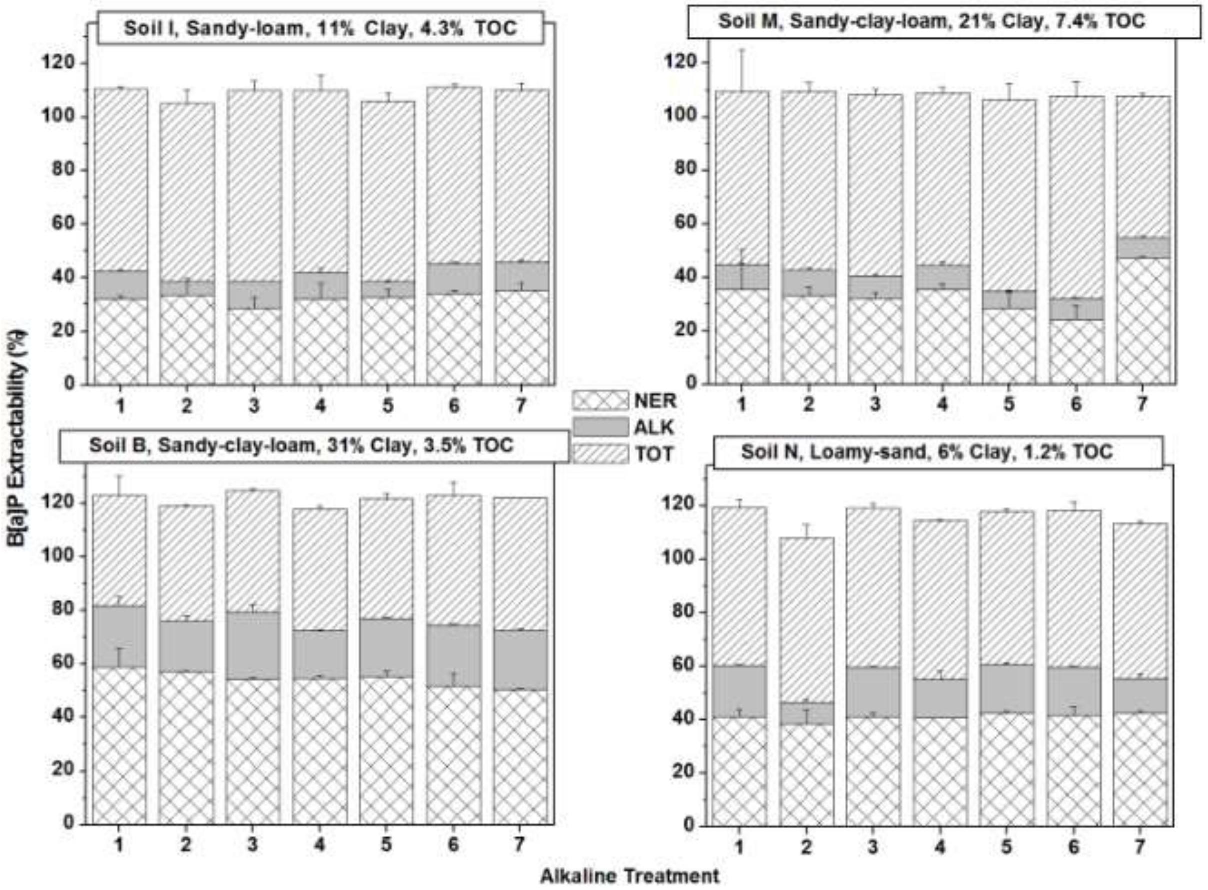

Figure 2. Extractability of B[a]P in soils subjected to 7 different alkaline treatments after 180 d of aging. NER is non-extractable residue; ALK is amounts recovered by the 7 different alkaline treatments ranging from 8% (4.2 ± 0.6 µg/g, soil M) to 22% (10.8 ± 1.2 µg/g, soil I; and TOT is total extractability ranging from 45% (22.7 ± 1.5 µg/g, soil B) to 67% (33.7 ± 1.1 µg/g, soil I). 1 g soil was extracted with 3 mL DCM/Ace by ultrasonication (40 KHz, 10 min, 3x). After extraction, combined supernatant was prepared for HPLC analysis. Pre-extracted soils containing B[a]P NERs were extracted with 10 mL alkaline solutions (Table 1). Values are means of duplicates ± standard deviations.

In this study, a positive relationship was observed between hard OC and BC-rich fractions (r = 0.98, *p* = 0.02), as well as between soft OC and nonpyrogenic OC fractions (r = 0.92, *p* = 0.08) (Figure 3). As a result of the recalcitrant nature of hard OC, PAHs in soils with large amounts of hard OC or BC-rich fractions can be strongly sequestered. Desorption of PAHs from these sites are likely to be slow [13,14,28]. Extensive descriptions of the mechanisms of PAH sequestration in soils have been documented elsewhere [1,11].

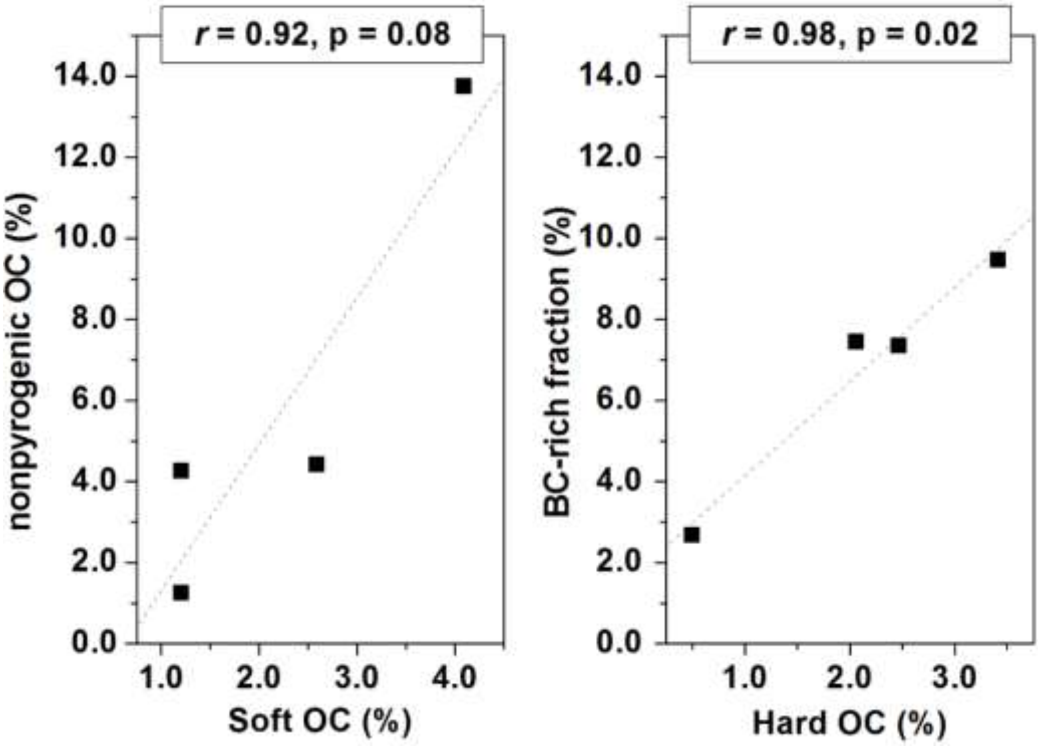

Figure 3. Relationship between organic carbon fractions in soils. The contents of total organic carbon (TOC) of acid-hydrolysed soils were determined by combustion at 1350 °C using LECO CNS analyser. Prior to combustion of acid-hydrolysed soils to determine the contents of hard organic carbon (hard OC), soft OC fractions were removed by wet oxidation with persulphate. The difference between TOC and hard OC contents estimated soft OC contents in the soils. A thermogravimetric analysis was used to determine the contents of nonpyrogenic OC (weight losses at 200–470 °C) and black carbon (BC)-rich fractions (weight losses at 470–600 °C) in soils based on the thermal stability of these fractions.

The capacity for PAH sequestration also differs with the quantity and quality of hard OC; hence, the amounts of extractable and sequestered PAHs in soils with contrasting physico-chemical characteristics will be expected to vary [11,12]. The fraction of hard OC to TOC was largest in the sandy-clay-loam soil B, which may explain the decreased extractability and stronger sequestration of B[a]P in soil B than in the other soils. It has also been reported that ^{14}C-phenanthrene extractability by HPCD and the total extents of ^{14}C-phenanthrene mineralisation decreased significantly ($p < 0.001$) by up to 50% with the increasing addition of 0.1 to 5% of activated carbon to 4 soils aged for 100 d, especially in clayey-loam soils [13]. In addition, soils with larger amounts of fine-sized particles associated with OC fractions in soil have been reported to possess substantial sequestration capacity for B[a]P compared to soils with smaller amounts of these fine-sized fractions [18]. In this study, a positive relationship was also observed between the amounts of clay and the fractions of hard OC relative to TOC content (r = 0.87, p = 0.13), and relative to soft OC fractions in soils (r = 0.89, p = 0.11) (Figure 4).

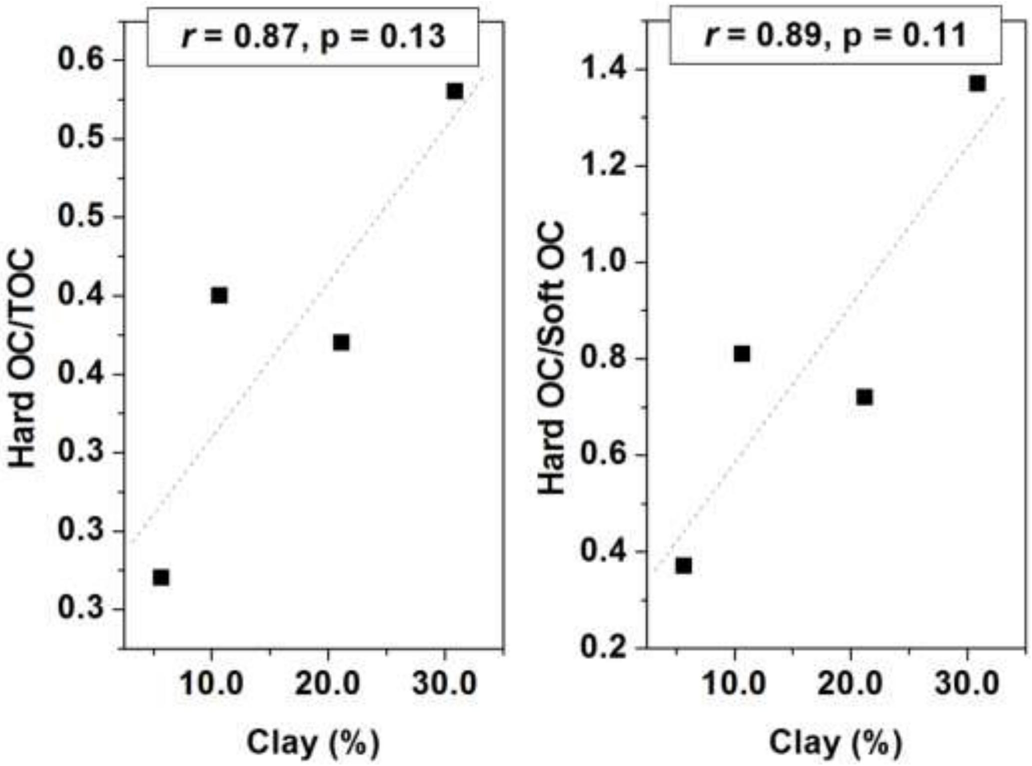

Figure 4. Relationship between clay and recalcitrant organic carbon fractions in soils. The contents of total organic carbon (TOC) of acid-hydrolysed soils were determined by combustion at 1350 °C using LECO CNS analyser. Prior to combustion of acid-hydrolysed soils to determine the contents of hard organic carbon (hard OC), soft OC fractions were removed by wet oxidation with persulphate. The difference between TOC and hard OC contents estimated soft OC contents in the soils.

This relationship may indicate the association between recalcitrant OC fractions and fine-sized soil particles which serve as sites for PAH sequestration in soils, thereby favouring greater sequestration of B[a]P in soil B. A wider range of soils may be needed to validate the observed relationships. Methanolic or non-methanolic alkaline treatments may partially release occluded or sequestered fractions following hydrolysis of SOM [6,35], thereby increasing mass recovery of PAHs spiked in soils [20]. Hence, after the 7 different methanolic and non-methanolic treatments (excluding '5 h MeKOH'), mass balance (%) of B[a]P after 180 d of aging in this study ranged from 71.8 ± 6.2 to 81.3 ± 3.7, 60.7 ± 1.4 to 83.7 ± 4.9, 62.0 ± 1.7 to 71.6 ± 0.9, and 69.3 ± 6.4 to 78.3 ± 3.3 for soils I, M, B, and N, respectively (Figure 5). This showed that there was an increase of approximately 13 (soil I) to 48% (soil B) of extractable B[a]P in pre-extracted soils following the alkaline treatments.

Overall, B[a]P mass balance in the soils after 180 d of aging were much reduced than after fresh spiking and ranged from 61 to 84%. The fractions of the spiked B[a]P (50 µg/g) that were not recovered (16 to 39%) after 180 d of aging were attributed to B[a]P NERs that were highly sequestered in soils and non-extractable by the different alkaline treatments utilised. In previous work using same soils to those used in this study, B[a]P recoveries after fresh spiking ranged from 85% to 93%, and ^{14}C-radioactivity in 160 d aged soils ranged from 88% to 93% in the soils, except in soil B (77%) [5,18]. This suggested that mass balance of B[a]P spiked in soils decreased further by approximately 10 to 31% from 160 d to 180 d of aging. The variations in B[a]P recovery may be attributed to the influence of different soil properties. In a different study where Soxhlet extraction was used [28], total extractability of B[a]P in 7 contrasting soils that were spiked at 10 µg/g of B[a]P also ranged from 98 to 103% after fresh spiking of B[a]P, and from 75 to 82 % after 200 d aging . Another study that used a DCM-soxtec extraction technique and analysed ^{14}C-B[a]P activity in extracts reported 83% and 73% total extractability in sewage-sludge amended arable crop soil (10 µg/g B[a]P) after

10 and 170 d of aging, respectively; the reported mass balances at 10 d and 259 d of aging were 94% and 77%, respectively [6]. Furthermore, complete recovery of ^{14}C-B[a]P was not achieved even after combusting spiked sterile soils that had been aged for 525 d [6].

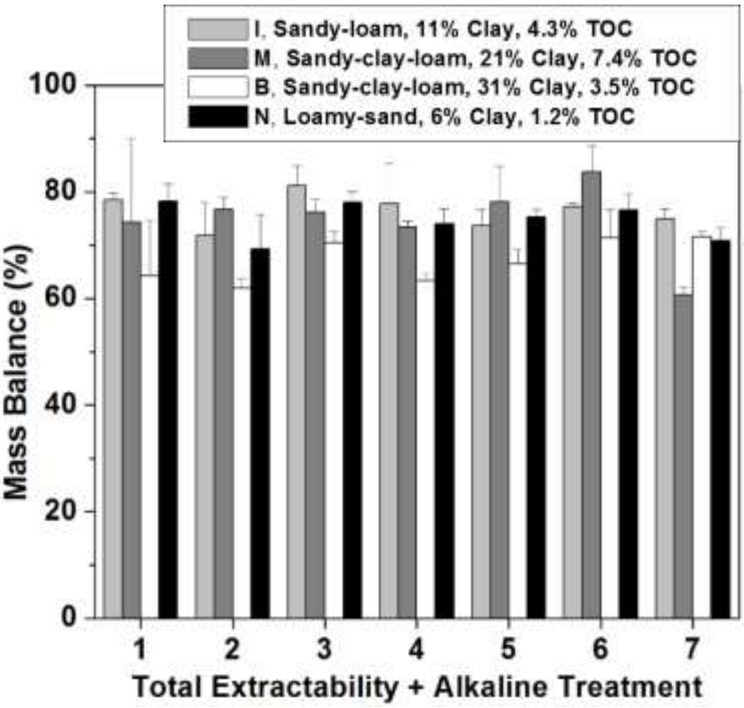

Figure 5. Mass balance of B[a]P in soils after 180 d of aging. The different alkaline treatments exclude '5 h MeKOH'. 1 g soil was extracted with 3 mL DCM/Ace by ultrasonication (40 KHz, 10 min, 3x). After extraction, combined supernatant was prepared for HPLC analysis. Pre-extracted soils containing B[a]P NERs were extracted with 10 mL alkaline solutions (Table 1). Mass balance is the sum of total-extractable B[a]P and B[a]P extractability by the different alkaline treatments. Values are means of duplicates ± standard deviations.

Overall, the total B[a]P extractability and mass balance as measured in this study were generally similar to the other cited studies where a range of solvents and extraction techniques were used. The differences in B[a]P extractability may be attributable to the influence of the contrasting properties of the soils utilised and has been discussed more extensively in our previous studies [27,34] .

Comparing B[a]P Recoveries in Soils between the 7 Exhaustive Methanolic and Non-methanolic Alkaline Treatments. Alkaline treatments '1' (2 M NaOH), '3' (2 M NaOH +

0.4 M NaF), and '6' (0.1 M NaOH + 0.4 M NaF + MeOH) showed relatively greater recoveries (%) based on the amounts of B[a]P spiked in soils (50 mg/kg) (Figure 6). Specifically, the fractions of B[a]P NERs recovered among the 7 treatments in each of soils I, M, B, and N ranged from 29.3 ± 0.3 – 34.7 ± 3.9%, 23.8 ± 6.5 – 33.1 ± 5.7%, 33.0 ± 1.0 - 45.6 ± 4.6%, and 20.8 ± 6.0 – 47.3 ± 4.1%, respectively (Figure 6). The key result was that up to 50% of B[a]P NERs in the sandy-clay-loam and hard OC-rich soil B can be recovered by the exhaustive treatments after 180 d of aging.

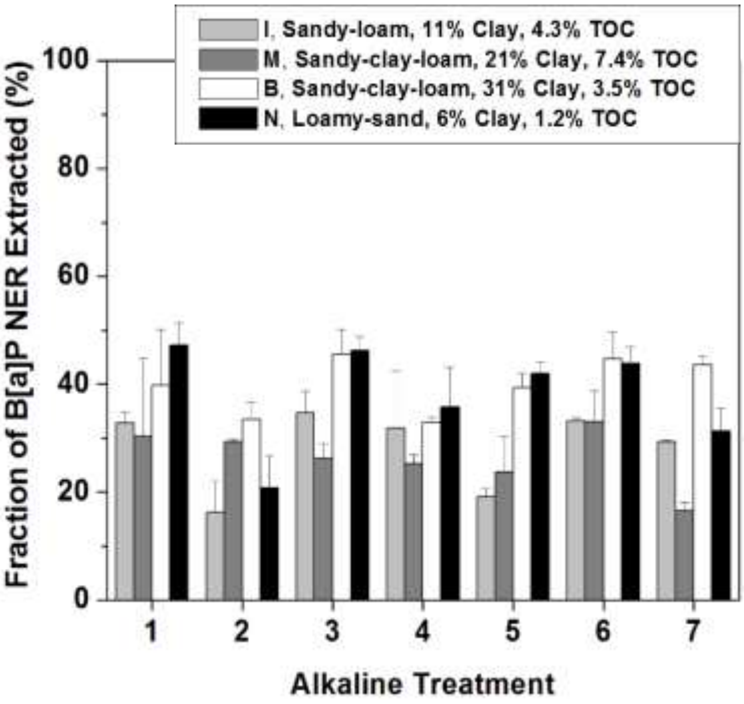

Figure 6. Fraction (%) of B[a]P NERs recovered by the 7 different alkaline treatments. The different alkaline treatments exclude '5 h MeKOH'. 1 g soil was extracted with 3 mL DCM/Ace by ultrasonication (40 KHz, 10 min, 3x). After extraction, combined supernatant was prepared for HPLC analysis. Pre-extracted soils containing B[a]P NERs were extracted with 10 mL alkaline solutions (Table 1). The fraction of B[a]P NER recovered is the percentage of B[a]P extracted by the different alkaline treatments relative to the amounts of B[a]P NER after DCM/Ace extraction. Values are means of duplicates ± standard deviations.

Generally, recoveries of B[a]P NERs (%) in each soil did not differ ($p > 0.05$) between the 7 different alkaline treatments (Figures 2 and 6). Mass balances of B[a]P were also not significantly different ($p > 0.05$) between the 7 different alkaline treatments ($p > 0.05$) (Figure 5). These observations generally reveal similar extraction capacities of the 7 different alkaline treatments and suggest that the treatments access similar B[a]P NER fractions in each soil. Therefore, the B[a]P recoveries by the 7 different alkaline treatments in each soil were averaged (Figure 7), and further referred to as 'exhaustive methanolic and non-methanolic alkaline treatments' for subsequent discussions.

Exhaustive Methanolic and Mon-methanolic Alkaline Treatments versus Conventional Methanolic Saponification. A key observation after 180 d of aging in this study was that recoveries of B[a]P in soilsfollowing exhaustive methanolic and non-methanolic alkaline treatments were generally greater ($p < 0.05$) compared to those in soils subjected to '5 h MeKOH' (Figure 7), particularly in the sandy-clay-loam soil B. For instance, B[a]P extractability in soil B following the exhaustive methanolic and non-methanolic alkaline treatments was approximately 22% (10.8 ± 1.2 µg/g); this corresponded to39.9 ± 5.2% of B[a]P NER after 180 d of aging. However, B[a]P extractability in soil B following '5 h MeKOH' was approximately 7% (3.7 ± 0.3 µg/g); this corresponded to only 10.1 ± 0.9% of B[a]P NERs after 180 d of aging.

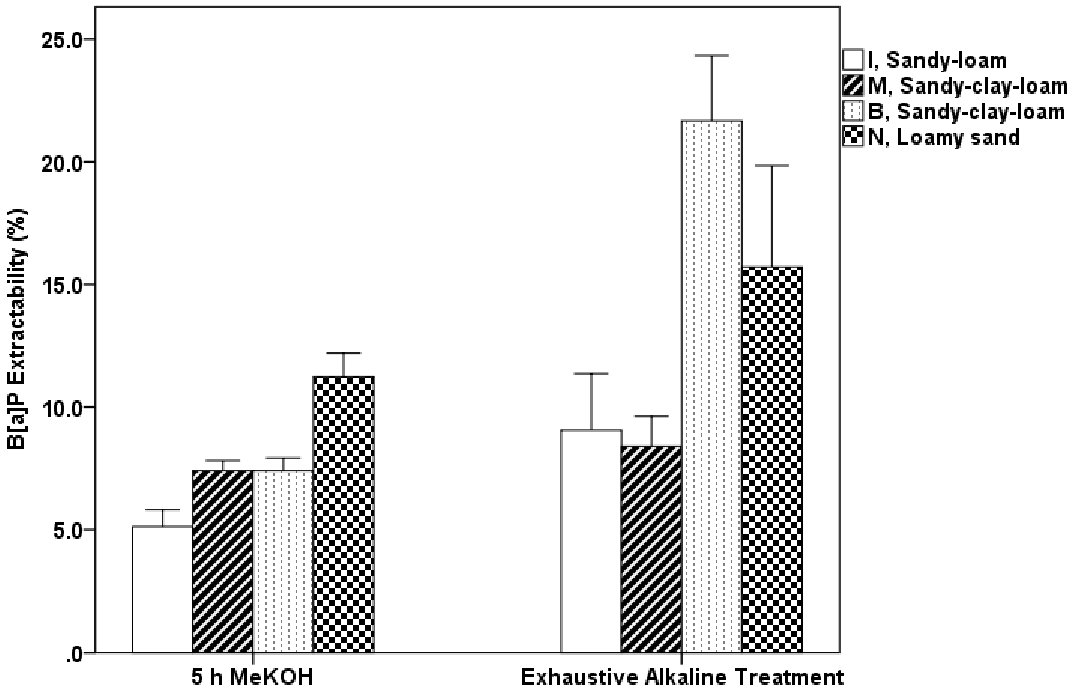

Figure 7. Comparison of B[a]P extractability between exhaustive alkaline treatments and methanolic saponification ('5 h MeKOH') of pre-extracted soils. Amounts of B[a]P recovered by the exhaustive alkaline treatments range from 8% (4.2 ± 0.6 µg/g, soil M) to 22% (10.8 ± 1.2 µg/g, soil B), whereas amounts of B[a]P recovered by 5 h MeKOH range from 5% (2.6 ± 0.4 µg/g, soil I) to 11% (5.6 ± 0.5 µg/g, soil B). 1 g soil was extracted with 3 mL DCM/Ace by ultrasonication (40 KHz, 10 min, 3x). After extraction, combined supernatant was prepared for HPLC analysis. Pre-extracted soils containing B[a]P NERs were extracted with 10 mL alkaline solutions (Table 1), or by conventional methanolic saponification. Values are means of duplicates ± standard deviations.

Our previous investigations using the same soils showed that B[a]P NERs increased with aging [34] . Another study in which the same soils were aged for 160 d showed similar trends of increasing NER over time [5]. Also, B[a]P recovered by methanolic saponification of pre-extracted soils tended to decrease over time [6]. It was therefore expected that the amounts (%) of B[a]P NERs recoverable in soils after 180 d of aging would decrease or remain unchanged when compared to recoveries after shorter aging periods, using the same extraction techniques. Indeed, there was generally no significant difference ($p > 0.05$) between B[a]P NERs recovered in the same soils subjected to '5 h MeKOH' after 180 d of aging in this study, and after 33 d of aging in our previous investigations [27](. The extraction efficiency of '5 h MeKOH' did not change substantially between 33 d and 180 d of aging; this showed that similar fractions of B[a]P NERs in soils were still being accessed.

The observation that the exhaustive methanolic and non-methanolic alkaline treatments generally had greater B[a]P NER recoveries (%) at 180 d of aging than the often used '5 h MeKOH' after the same aging period was interesting. This implied that additional B[a]P NER fractions in soils were still accessible. The recoveries of B[a]P NERs in soil may therefore be limited by the operational design of the methanolic or non-methanolic alkaline treatment used which may affect understanding of B[a]P fate in soil. Based on the results of this study, the conventional methanolic saponification of pre-extracted soils may be made more exhaustive to increase recovery of B[a]P NERs, as well as for better estimation of total B[a]P concentrations in soils. This may allow a better understanding of B[a]P fate in soils. The differences in recoveries of B[a]P NERs from the 4 soils investigated indicate that soil properties and the amounts of NERs in soils with similar contamination history may influence the amounts of NERs than can be recovered by the alkaline treatments.

A significant correlation ($R^2 = 0.69$, $p < 0.001$, $n = 4$) was observed between the amounts of B[a]P recovered by each of the 7 alkaline treatments in the pre-extracted soils, and the corresponding amounts of estimated NERs in the soils at 180 d of aging (Figure 8). The observed correlation suggests a potential relationship between B[a]P NERs in pre-extracted soils with similar contamination history and amounts of B[a]P extractable by the alkaline treatments. The quantification of the exact amounts of PAH NERs in soils is difficult. Very useful but complex techniques involving ^{14}C- and ^{13}C- tracers have been used to quantify and classify NERs in soil [7]. However, the information obtained usually relates to ^{14}C- or ^{13}C-activities from non-specific origins in soil, rather than non-extractable parent PAHs residues or their metabolites. The position of the radio- or stable isotope- tracer on the PAH molecule also influences the amount of activity attributed to NERs [36].

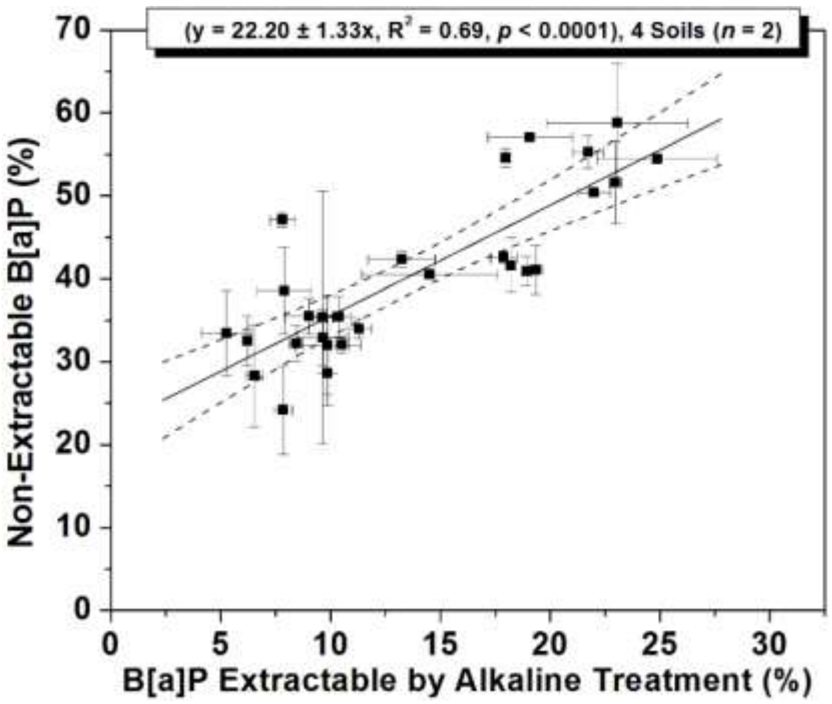

Figure 8. Relationship between B[a]P NERs in soils and the amounts recovered by methanolic and non-methanolic alkaline treatments. The dotted lines represent 95% confidence limits.

In this study, quantification of B[a]P in soil relates only to extractable parent compounds. The potential relationship described earlier warrants further investigations using a wider range of soils, as it implies a potential to estimate the amounts of NERs in soils based on extractability by methanolic or non-methanolic alkaline treatments. In this regard, a combination of the alkaline treatments used in this study and ^{14}C- or ^{13}C-tracer approaches are suggested.

Does the Enhanced Recovery of B[a]P NERs in Soils have Implications for Contaminated Land Risk Assessment and Decision-Making?

Much of the current work on the risk assessments of PAHs in soils, as well as risk-based approaches to contaminated land management, focus on the bioavailable and/or bioaccessible fractions [37-39]. Bioavailable and bioaccessible fractions have been defined as fractions that are readily available and potentially available, respectively, to pose risks to human and environmental health following exposure [40]. The measurement of these fractions may vary depending on the extraction techniques and conditions used, and the impact and significance of the extracted fractions also vary depending on whether human or ecological health risk assessment is the focus [8,12].

However, little emphasis is placed on the impact and significance of PAH NERs in soils, despite their uncertainty for long-term stability and potential to pose risks to human and environmental health following exposure [8,11,41]. Our recent work in this regard provided empirical data that showed that the amounts of B[a]P NERs that were potentially remobilisable, after re-equilibrating long-term aged soils for 30 d, were small (less than 5% of the amount of B[a]P spiked, 10 or 50 mg/kg) [34]. According to the study [34], it was documented that the amounts of B[a]P remobilised in the 4 soils utilised were generally below the B[a]P health investigation levels (3 µg/g) from an Australian risk assessment perspective, and that the potential risks that may be posed from exposure to the remobilised B[a]P were acceptable. The small amounts of remobilised B[a]P may result from slow repartitioning of fractions that were entrapped, occluded, adsorbed, or sequestered into readily available compartments in soils during re-equilibration [34]. Further, B[a]P remobilisation was supported by the decrease in the fractions, and absolute amounts, of B[a]P NERs in soils recovered by methanolic saponification after the re-equilibration periods [34]. Therefore, fractions of B[a]P NERs in soils that are extractable by methanolic saponification may indicate fractions that may be potentially remobilised.

Although the potential risks associated with remobilisable B[a]P fractions in the soils utilised were generally acceptable from a risk assessment perspective, the use of extraction techniques that do not completely recover B[a]P fractions that are potentially remobilisable may underestimate the risks that may be associated with B[a]P NERs in soils. Since there was generally no significant difference ($p > 0.05$) in B[a]P extractability from soils between the 7 different alkaline treatments in this study, it is proposed that any of the exhaustive alkaline extraction would enhance the recovery of highly sequestered B[a]P in soils than the conventional methanolic saponification, particularly alkaline treatments 1, 3, and 6. The enhanced recovery is important for a better understanding of the fate of B[a]P NERs in soils which is very useful for B[a]P risk assessments.

CONCLUSION

Non-extractable B[a]P residues in soils are currently not considered as important in risk assessments as they are highly sequestered in soil and have very minimal potential to pose risks or cause harm to human and environmental health. However, whether NERs have potential to be remobilised in soils in the long-term, and the amounts that may be remobilised are of concern, remain areas of interest in contaminated land risk assessment. A cost-effective technique that could reliably estimate the amounts of NERs recoverable or remobilisable in soils may be very useful for risk-based approaches to managing long-term PAH contaminated soils. Our results demonstrate that 7 exhaustive alkaline extractions access similar B[a]P NER fractions, and that their use resulted in enhanced recovery of B[a]P NERs in soils compared to conventional methanolic saponification. The results may be indicative for other PAHs, as B[a]P is usually the model PAH at most PAH-contaminated sites. Such exhaustive alkaline treatments can provide more realistic estimations of total B[a]P concentrations based on mass balance in spiked soils, and contribute to a better understanding of PAH fate in soils.

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ACKNOWLEDGMENT

The authors acknowledge the Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) and Global Centre for Environmental Remediation (GCER), University of Newcastle Australia (UoN) for providing support. AU is grateful to UoN and CRC CARE for the UoN International Postgraduate Research and CRC CARE scholarships, respectively. AU also acknowledges the kind assistance from Oluyoye Michael Idowu of GCER during laboratory experiments.

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