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Optimisation of XAD extraction methodology for the assessment of biodegradation potential of ^{14}C-phenanthene in soil

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Abstract

This study investigated the use of a hydrophobic resin, amberlite XAD, as a tool for assessing the biodegradation potential of ^{14}C-phenanthrene in soil. The method was optimised in terms of soil/XAD ratio, shaking, extraction time and eluting solvent. The most effective method was then tested on selected XADs, and the performance compared with cyclodextrin (HP-β-CD) and dichloromethane (DCM) extractions suitability to predict phenanthrene biodegradation in soil over 100 d. Results showed that the optimum conditions for the XAD extraction technique are a 2:1 soil/XAD ratio, 100 rpm mixing for 22 h and elution using a DCM:methanol solution (1:1). Mineralisation of ^{14}C-phenanthrene was accurately predicted by HP-β-CD (r^2 = 0.990, slope = 0.953, intercept = 1.374) and XAD-4 extractions (r^2 = 0.989, slope = 0.820, intercept = 6.567), while DCM overestimated the bioaccessibility of ^{14}C-phenanthrene (r^2 = 0.999, slope = 1.328, intercept = -49.507). This investigation showed that XAD extraction can be considered a suitable non-exhaustive technique for estimating biodegradability of phenanthrene in soil.

Keywords: Hydrophobic resins, XAD, HP-β-CD, bioaccessibility

1. Introduction

The need to measure the bioaccessibility of anthropogenic chemicals is fast becoming an important step in the risk assessment of contaminated soil and sediment. Bioaccessibility refers to the fraction of contaminant “as that which is available to cross an organism’s cellular membrane from the environment, if the organism has access to the chemical. However, the chemical may be either physically removed from the organism or only bioavailable after a period of time” (Semple et al., 2004). This has also been referred to as the labile and rapidly desorbing fractions (Semple et al., 2007; Riding et al., 2013; Ortega-Calvo et al., 2015) and has also found an application in assessing the biodegradation potential of hydrocarbons in soil (Semple et al., 2006; 2007), where a number of methods have been tested for their ability to predict contaminant accessibility in soil, including Soxhlet extraction using organic solvents (Kelsey and Alexander, 1997), mild solvents extraction (Kelsey et al., 1997; Reid et al., 2000a; Liste and Alexander, 2002), persulfate oxidation (Cuypers et al., 1999; Dandie et al., 2010), surfactant extraction and solid-phase extraction (Cornelissen et al., 1998; van der Wal et al., 2004). Others include aqueous based extraction using hydropropyl-β-cyclodextrin (HP-β-CD) (Reid et al., 2000b; Papadopoulos et al., 2007; Rhodes et al., 2008) and sub-critical water extraction (Hawthorne et al., 2002; Latawiec et al., 2008). Aqueous based HP-β-CD extraction has received significant attention in the literature and has been found to accurately predict the accessibility of polycyclic aromatic hydrocarbons (PAHs) to microorganisms across a wide range of soil types and conditions (Cuypers et al., 2002; Reid et al., 2000b; Doick et al., 2006; Hickman et al., 2008; Rhodes et al., 2008; 2012; Bernhardt et al., 2013; Yu et al., 2016).

Amberlite extra adsorbent material (XAD) is a macrospherical styrene-divinyl non-ionic resin with numerous macropores and large surface area, exhibiting a high affinity to sorb organic compounds that are either dissolved in pore-water or readily desorbable from soil particles (Tan, 2014). The adsorptive capacity of this resin has been proposed to be potentially applicable as a non-exhaustive extraction to estimate the bioaccessibility of organic contaminants in soil and sediment (Puglisi et al., 2003; Lei et al., 2004; Vázquez-Cuevas and Semple, 2016). The hydrophobic nature of the sorption sites located throughout XAD macro spherical structure, represent a chemically attractive sink in which organic contaminants can partition (Lamoureux and Brownawell, 2004). This property has been harnessed to facilitate the detection of trace contaminants concentration in different environmental matrices including pore water (Care et al., 1982; Burnham et al., 1972) and air sampling (Chuang et al., 1999; Wania et al., 2003; Araki et al., 2009). In addition, it has been used to study the desorption and partition behaviour of PAHs (Northcott and Jones, 2000) and polychlorinated biphenyls (PCBs) (Carroll et al., 1994) in soil and sediment, respectively. XAD extraction process can best be described as a passive sampler, where the sorbed HOCs can be eluted with an appropriate organic solvent and then measured appropriately (Wania et al., 2003). Previous reports have described bioaccessibility measurements of HOCs in soils and sediments (Lei et al., 2004; Patterson et al., 2004; Doick et al., 2005; Simpson et al., 2006; Rhodes et al., 2008; 2012), albeit with mixed success.

The major factors affecting XAD sorption are thought to include physical and chemical properties of the matrix including pH, temperature, amount and type of humic materials, flow rates and resin sorption capacity (Daignault et al., 1988). However, it is not clear how different physical parameters such as surface area and the resin pore size, might affect the extraction of organic contaminants onto XAD from complex matrices such as soil. In this present study, XAD extraction was optimised in terms of the soil/XAD ratio, the degree of mixing, the choice of eluting solvent and the duration of extraction. Therefore, the aim of this study was to develop an optimised methodology for the use of XAD extractions as a predictor of the bioaccessible fraction of ^{14}C-phenanthrene. More specifically, the duration of extraction, which corresponds to the time required for the desorption/adsorption process to achieve equilibrium. Further, the influence of XAD physical properties on the sorption capacity was tested using four polymeric types of the resin. Finally, the optimised method was validated and compared with DCM and HP-β-CD extractions and the ability to predict the mineralisation of ^{14}C-phenanthrene.

2. Materials and methods

2.1 Materials

^{12}C-Phenantherene was obtained from Sigma Aldrich Co, Ltd., UK. $^{9\text{-}14}$C-Phenanthrene was purchased from American Radiochemical Company, (ARC- USA). Acetone, hexane, and methanol were obtained from Fischer Scientific UK. Dichloromethane (DCM) and toluene were obtained from Rathburn Chemicals and Merck UK, respectively. XAD-2, XAD-4, XAD-16 and XAD-1180N were obtained from Sigma Aldrich and they differed in physic-chemical properties (Table 1), UK and HP-β-CD from Acros Organics UK. Sample oxidiser cocktails (Carbon-Trap and Carbon-Count); Combust-aid; and the Ultima Gold scintillation cocktails were obtained from Perkin Elmer, UK.

2.2 Soil preparation

Uncontaminated soil (organic matter = 2.1; pH = 6.5, clay =19.5%, silt = 20%, sand = 60%) was collected from A horizon (0 - 15 cm) at Myerscough College, Preston, U.K. Field moist soil was air-dried and passed through a 2.308 mm sieve to remove stones and other plant debris. Standard for soil spiking with $^{12/14}$C-phenanthrene was prepared in acetone to deliver a final concentration of 10 mg kg^{-1} and an activity of 83.3 Bq g^{-1}. Soil spiking was performed following a single-step spiking/rehydration technique described by Doick et al. (2003). Soil was stored in amber glass jars and incubated at 21 ± 1 ºC in a controlled temperature room until needed.

2.3 Determination of total ^{14}C-phenanthrene in soil

The total amount of ^{14}C-phenanthrene activity in soil was determined by combustion (Packard 307 Sample oxidiser) at each sampling period (0, 25, 50 100 d). Soil samples (1 g dw soil; $n = 3$) were weighed into cellulose combustion cones and combusted for three minutes and facilitated by the addition of 200 µl of Combust-aid. $^{14}CO_2$ released from the combustion was trapped by Carbon-Trap (10 mL) and Carbon-Count (10 mL). Sample oxidiser trapping efficiency was determined before combustion and found to be greater than 92 % in all cases. The $^{14}CO_2$ in the scintillant was quantified by liquid scintillation counting (10 min, LSC- Canberra Packard Tri Carb 2300 TR, UK) after 24 h storage in the dark to correct for chemolumniscence.

2.4 Optimisation of extraction parameters

2.4.1 Soil/XAD ratio and degree of mixing

Soils (2 g dw ± 0.2; $n = 3$) were weighed into 50 mL Teflon centrifuge tubes. Different amounts (0.2, 0.5, 1 and 2 g) of damp XAD-2 were added to the soils and extracted with a solution of 0.01 M $CaCl_2$ for 24 h onto an orbital shaker (100 rpm). Following 45 minutes centrifugation at 4000 *g*, XAD was separated from the slurry and quantitatively removed by decantation through a 50 mL glass funnel plucked with glass wool. The resin was allowed to dry in a fume cabinet and transferred to 40 mL Wheaton glass vials and mixed with 15 mL of DCM:methanol (1:1). Following a 5 min sonication, the vials were transferred to an orbital shaker at 100 rpm to elute the adsorbed chemical from XAD. An aliquot of eluate was sampled into 15 mL Ultima Gold liquid scintillation cocktail and analysed by LSC as previously described.

Optimisation of the degree of mixing soil extractions (2 ± 0.25 g dw; $n = 3$) using 1 g ± 0.05 XAD-2 were carried out. Sets of triplicates were placed onto an orbital shaker at 20, 40, 60, 80, 100 or 120 rpm for 24 h and processed as described above.

2.4.2 Elution solvent

Extraction was performed as described previously (Section 2.4.1). Triplicate samples of the recovered XAD were eluted with 15 mL of either DCM:methanol (1:1), hexane:acetone (1:1), hexane:acetone (3:1), or toluene:methanol (1:1) as recommended by Northcott and Jones (2000).

2.4.3 Optimisation of extraction time for selected hydrocarbons

The optimisation of the extraction time was performed to ensure system equilibration before termination of extraction process. XAD extractions were performed as described earlier (Section 2.4.1), except that triplicate samples were removed for further analysis at 3, 6, 12, 24, 36, 48 or 96 h shaking.

2.5 Comparing the adsorption capacities of selected XADs

The physical properties of the selected XAD are presented on Table 1. Spiked soils ($n = 3$) were aged in sealed amber glass jars for 100 d. After 0, 25, 50 and 100 d, samples were removed for XAD extraction. The extraction, recovery and elution of the sorbed chemical were performed based on the results from the optimisation procedure (Section 3.1). Quantification of the ^{14}C-phenanthrene was done by LSC as described previously.

2.6 Chemical extractability of ^{14}C-activity from soil

2.6.1 DCM-shake extraction

^{14}C-Phenanthrene was exhaustively extracted from the soil using DCM as a benchmark for individual XAD and HP-β-CD extractions. Soils (1.5 ± 0.05 g dw, n = 3) were ground to a fine powder with the addition of equal mass of sodium sulphate as described by Papadopoulos et al. (2007). Ground samples were transferred to 50 mL polycarbonate centrifuge tubes and extracted with 20 mL DCM by mixing onto an orbital shaker at 100 rpm in a controlled temperature room (21 ± 1 ºC) for 20 h. An aliquot of eluate was sampled into 15 mL Ultima Gold liquid scintillation cocktail and analysed by LSC as previously described.

2.6.2 Hydroxypropyl-β-cyclodextrin (HP-β-CD) extraction

HP-β-CD extractability was determined by aqueous based cyclodextrin extraction following the protocol developed by Reid et al. (2000a). In short, 25 mL of a 50 mM HP-β-CD solution was added to a 50 mL Teflon centrifuge tubes (n = 3) containing 1.25 ± 0.05 g (dw) soil (20:1 solution to soil ratio). Tubes were then horizontally placed onto an orbital shaker at 100 rpm and mixed for 22 h. Phase separation was achieved by a 60 min centrifugation at 4000 *x g*. An aliquot of the supernatant (5 mL) was mixed with liquid scintillation cocktail and quantified by LSC as previously described.

2.7 Mineralisation assay

Mineralisation of ^{14}C-phenanhrene was assessed over time through a respirometry assay using modified 250 mL Schott bottles (Reid et al., 2001; Semple et al., 2006). Respirometry was performed following the addition of a phenanthrene degrading bacterial inoculum (*Pseudomonas* sp.). Soils (10 g dw, *n* = 3) were placed into the respirometers and mixed with inorganic mineral basal salts solution (MBS) to form a soil:liquid ratio of 1:3 (Doick and Semple, 2003). The bacterial inoculum (*Pseudomonas* sp.), previously isolated from a petroleum contaminated soil capable of utilising phenanthrene as the sole carbon source for growth, was cultured at 0.1 g l^{-1} phenanthrene in 300 ml sterile minimal basal salt (MBS) solution at 20 ^{o}C and at 100 rpm on an orbital shaker (Ogbonnaya et al., 2014). The bacterial inoculum (*Pseudomonas* sp. - 5 ml; ca. 10.28×10^7 - 12.36×10^7 cell g^{-1} dw) was prepared following the procedure detailed by Ogbonnaya et al. (2014) and added to the respirometers. Bottles were placed onto an orbital shaker (100 rpm) and incubated in a controlled environment room at 21 ± 1 ºC. The evolution of $^{14}CO_2$ following mineralisation was assessed by using a suspended scintillation glass vial containing 1 M NaOH (1 ml) solution. Sampling of the trapped $^{14}CO_2$ was performed by the periodic replacement of the trap over 14 days. Incubated traps were mixed with 5 mL liquid scintillation cocktail and analysed by LSC as previously described.

2.8 Data presentation and statistical analysis

Following blank correction, statistical analyses of data were conducted by ANOVA - post hoc Tukey test, and comparison of relationships between groups was performed using the student's t-test or paired *t* test, where appropriate (SigmaStat 3.5). Statistical significance of data was assumed at 95% confidence interval ($p < 0.05$). Graphical representation of the data and linear regressions were plotted on SigmaPlot (SPSS version 12.0 for Windows).

3. Results

3.1 Optimisation of extraction conditions

3.1.1 Loss of ^{14}C-phenanthrene associated activity in soil

Following increases in contact time (0, 25, 50 and 100 d), the total amount of ^{14}C-phenanthrene-associated activity decreased from approximately 100% to a mean of 34%. The significant loss (p < 0.01) was recorded between 0 and 25 days contact time, after which there was no further loss (p > 0.05) of ^{14}C-phenanthrene activity of the soils during incubation.

3.1.2 Soil/XAD ratio

The total amount of soil associated ^{14}C-phenantherene extracted by XAD-2 negligibly increased with increasing soil/XAD ratio up to 1 g (Table 2). Although maximum ^{14}C-phenanthrene extractability was achieved using soil/XAD ratio 2:1 (20.73 %), there was no statistical significant difference among ratios ($p > 0.05$). There was a small decrease in the total extractability moving from 2:1 to 1:1, which can be explained by the difficulty in handling 2 g of XAD-2 (20-60 mesh). From this observation, soil/XAD (2:1) was selected for optimum performance. Further analysis of the supernatant for residual ^{14}C-phenanthrene showed that the desorbed chemical was effectively trapped by the XAD, with total ^{14}C-phenanthrene in the supernatant been negligible (< 1 %) (Table 2). In addition, residual soil associated ^{14}C-phenanthrene determined by sample oxidation following the removal of the labile fraction showed a mass balance of between 97 % - 103 %, indicating that all the contaminant introduced can be accounted for.

3.1.3 Degree of mixing

The mean total ^{14}C-phenanthrene extractability assessed using 0, 20, 40, 80, 100 and 120 rpm was 22.29 %, 50.33 %, 57.47 %, 68.21 %, 75.08 %, 74.87 %, respectively (Figure 1). ^{14}C-phenanthrene extractability significantly ($p < 0.05$) increased with increasing shaking up to 100 rpm. However, ^{14}C-phenanthrene extractability amongst 80, 100, and 120 rpm appeared to be statistically similar ($p > 0.05$). Nonetheless, maximum extractability was obtained using 100 rpm (75.08 %). The slight decline in total extractability by increasing the shaking from 100 to 120 rpm may be due to increased agitation in the system, which might possibly affect the sorption process. From this observation, a standard agitation of 100 rpm was employed for the rest of this study.

3.1.4 Eluting solvent

Total ^{14}C-phenanthrene recovered from XAD using DCM:methanol (1:1), toluene:methanol (1:1), hexane:acetone (1:1), and hexane:acetone (3:1) were 95.61 ± 5.3 %, 88.45 ± 3.2 %, 106.02 ± 4.8 % and 85.54 ± 3.7 %, respectively (Figure 2). The lowest recovery was obtained by using hexane:acetone (3:1), while the maximum recovery (106.02%) was observed with hexane:acetone (1:1). These two solutions were observed to promote the formation of phases in combination with the soil and the resins and therefore not considered as suitable eluting solvents. No significant difference was found between the fraction eluted using DCM:methanol and toluene:methanol ($p > 0.05$). DCM:methanol produced a better recovery (95.61 %) and had less than 2 % residual ^{14}C-phenanthrene detected in the aqueous phase, hence the choice of DCM:methanol (1:1) for the remaining part of this study.

3.1.5 Extraction time profiles for ^{14}C-phenanthrene

To determine the optimum duration for extraction, the kinetics of extractions were investigated. Extraction was performed for 3, 6, 12, 24, 48, 72 or 96 h (Figure 3). The ^{14}C-phenanthrene (K_{oc} = 4.15) showed 90 % extractability after 24 h shaking showing equilibration of the desorption/adsorption process in the system. There were no significant differences in extractability at successive time points ($p > 0.05$). Based on these results, the optimum extraction time for ^{14}C-phenanthene was defined as 22 h.

3.2 Comparison of adsorption capacities of for different XAD resins with increased soil-phenanthrene contact time.

Total ^{14}C-phenanthrene extractability was compared amongst four hydrophobic resins (XAD-2, XAD-4, XAD-16 and XAD-1180N, Table 1), after up to 100 d soil-phenanthrene contact time. Prior to experimental setup, elution efficiencies of DCM:methanol (1:1) on various XADs were determined and found to be approximately 85 %, 95 %, 87 % and 92 %, respectively. Thus, these recovery efficiencies were factored into the final calculations. The amounts of ^{14}C-phenanthrene extracted from freshly spiked soil (0 d) and subsequent ageing times are presented on Table 3. Following increasing soil:phenanthrene contact time, the total ^{14}C-phenanthrene extractability declined significantly ($p < 0.05$) across the four investigated XADs (Table 3). While there were significant differences ($p < 0.05$) between extractabilities after 25, 50, and 100 d in comparison to extractability at 0 d, there was however no significant difference ($p > 0.05$) in the total extractability by the four XADs at each time point. This showed that the sorption capacity of the four XADs was not affected by the differences in the resins pore size and surface area under the conditions by which the present study was conducted.

3.3 Chemical extractability of ^{14}C-phenanthrene and its prediction of bioaccessibility with increased soil-PAH contact time

The extractability of ^{14}C-phenanthrene by DCM, HP-β-CD and XAD-4 was tested at each soil-phenanthrene contact time (0, 25, 50, 100 d). In freshly spiked soil, no significant differences were observed between the amount of ^{14}C-phenanthrene extracted from the soil by HP-β-CD, DCM and the amount quantified through XAD-4 extractability ($p > 0.05$).

Increasing soil-phenanthrene contact time (25, 50 and 100 d) led to a consistent decrease in the amount of ^{14}C-phenanthrene extraction. However, the extracted ^{14}C-phenanthrene varied with chemical extractant (Table 3). There was insignificant difference between total ^{14}C-phenanthrene extracted by individual extractants (DCM, HP-β-CD, XAD-4) after 50 and 100 d ($p > 0.05$). However, when the amount of ^{14}C-phenanthrene extracted by each chemical extractant was compared, it was observed that DCM extracted larger amounts of ^{14}C-phenanthrene when compared against either HP-β-CD or XAD-4 after increasing contact times (25, 50, 100 d).

3.4 Correlation between chemical extraction and extent of ^{14}C-phenanthrene mineralisation with increased soil-PAH contact time

The mineralisation of ^{14}C-phenanthrene was monitored over 14 days at each contact time (0, 25, 50 and 100 d). It was observed that the extent of mineralisation significantly decreased with increasing soil:phenanthrene contact time. The total amount of ^{14}C-phenanthrene mineralised after spiking (0 d contact time) was significantly higher ($p < 0.05$) when compared to other time points (25, 50, 100 d) (Table 3). Similarly, the amount mineralised after 25 d contact time was significantly higher ($p < 0.05$) than subsequent time points.

In order to test the ability of chemical extractions to predict the extent of ^{14}C-phenanthrene mineralisation, individual extractions were compared against the extent of ^{14}C-phenanthrene mineralisation through linear regression (Figure 4). Linear correlation between ^{14}C-phenanthrene mineralisation and DCM-shake extraction across the four contact time points produced a good regression ($r^2 = 0.999$). However, the slope (1.328) and intercept (-49.51) showed that DCM-shake extraction as employed in the current study overestimated bioaccessibility and hence biodegradability of phenanthrene in soil. On the contrary, linear correlation between ^{14}C-phenanthrene mineralisation and HP-β-CD extractions across the four time points produced a good regression was observed to present a close 1:1 relationship ($r^2 = 0.990$, slope = 0.953, intercept = 1.374). Similar trends were also observed when comparing the amount of ^{14}C-phenanthrene extracted from XAD-4 ($r^2 = 0.989$, slope = 0.820, intercept = 6.567).

4. Discussion

4.1 Optimisation of XAD extraction

Prior extraction regime at each contact time (0, 25, 50, 100 d), the remaining ^{14}C-phenanthrene associated activity was determined through soil sample oxidation and the results showed that there was initial significant loss of activity. The initial loss of ^{14}C-phenanthrene activity between 0 and 25 days contact time of incubation was apparently mainly due to microbial degradation and to a minimal extent, volatilisation (Allan et al., 2006; Ogbonnaya et al., 2016). After the 25 days contact time, there was apparently no further loss of ^{14}C-phenanthrene activity through microbial degradation due to unavailability of the contaminant to biodegradation.

The condition for optimum XAD extractability of phenanthrene established in the present study indicated that soil:XAD (2:1), extracted for >20 h at 100 rpm using DCM:methanol (1:1) as the eluting solvent was adequate for predicting bioaccessibility of phenanthrene to catabolically active microbial inoculum. Previous studies have been conducted using different conditions. For instance, Patterson et al. (2004) employed 20 h extraction with soil:XAD-4 (2.5:1) and sequential elution first with 10 mL DCM, then 4.95 mL of butanol then 4.95 mL of acetonitrile. The authors reported mixed results, with XAD-4 extraction accurately predicting the biodegradation of naphthalene in one out of four different soils (Patterson et al., 2004). One of the reasons for this behaviour was suggested to be the formation of a coating around XAD grains with fine soil particles (Patterson et al., 2004). However, another reason for the inconsistent performance of the XAD as used in that study could be due to the incorporation of a buffering step by Patterson et al. (2004); where K_2CO_3 was added to promote flocculation, thus facilitating the removal of XAD from the soil slurry. In a preliminary study carried out in the present investigation, K_2CO_3 was shown to negatively influence the adsorption process as it enhanced desorption of the sorbed chemical on the XAD resin back into the slurry and promoted the extraction of dissolved organic matter (DOM) from soil (Patterson et al., 2004). These, together with the coating of the XAD surface reported by the authors, might account for the results obtained in that study (Patterson et al., 2004).

In another study, Simpson et al. (2006) reported that a 6 h extraction with sediment:XAD-2 (1:1) was a poor predictor of the bioavailability of a mixture of low and high molecular weight PAHs when compared against the bioaccumulation of these compounds by the benthic bivalve *Tellina deltaidalis*. Although XAD-2 was observed to overestimate bioaccumulation of most low molecular weight (LMW) PAHs to the sediment organism, Simpson et al. (2006) attributed this behaviour to the toxicity of the hydrocarbons which resulted in the reduction of soil ingestion by the organisms, as well as increasing their mortality. In contrast, Lei et al. (2004) reported that among different sediment:XAD-2 ratios and mixing times, a one week extraction procedure with a 1:1 sediment:XAD-2 ratio was sufficient to predict the microbial degradation of LMW PAHs (2-4 aromatic rings). However, XAD-2 extractions overestimated the biodegradability of higher molecular weight PAHs such as benzo[a]anthracene, chrysene, benzo[b,k]fluoranthene and benzo[a]pyrene (Lei et al., 2004). The current study only examined the applicability of XAD-2, which has a larger pore diameter (100 Å) than XAD-4 (100 Å), capable of accommodating significant amounts of PAHs relative to the biodegradable portions. Hence, this current study showed that the effectiveness of applying XAD as a chemical extractant in soil to predict bioaccessibility is inherently governed by the physic-chemical properties of the adsorbent resin (Juan-Peiro et al., 2012). Furthermore, this study brings to light the plausible applicability of utilising XAD-4 as a preferable mild chemical extractant compared to other XAD resins. Considering the extraction parameters reported by previous authors, the methodology in this current study produced valuable results. The soil:XAD (2:1) for 22 h to predict bioaccessibility of phenanthrene to catabolically active microbial inoculum represents an advancement of previous methodologies, based on the soil:XAD ratio and extraction timing perspectives. The 22 h time period was enough to measure the rapidly desorbable portion of ^{14}C-phenanthrene to optimally locate and sorb onto the accessible XAD adsorption sites. Measuring labile and rapidly desorbable fractions of organic contaminants in soil has been well defined as that which is bioavailable and potentially bioavailable for microbial degradation and represents a sensible descriptor for the biodegradation endpoint of the contaminant in soil (Semple et al., 2004; 2007; Ogbonnaya and Semple, 2013; Ortega-Calvo et al., 2015). The 22 h XAD-4 extraction correlated strongly with the already established time duration for extracting low molecular weight (LMW) PAHs using HP-β-CD in soil.

4.2 Comparative prediction of ^{14}C-phenanthrene biodegradability in soil

The mineralisation assay is used to describe the amount of a contaminant that can be degraded by microorganisms in soil (Semple et al., 2006). Given the conditions of the assay, it is the bioaccessible fraction of ^{14}C-phenanthrene within the soil is mineralised to $^{14}CO_2$, hence, the non-mineralised fraction represent the non-bioaccessible fraction of ^{14}C-phenanthrene to the degrading inoculum (Semple et al., 2006; Ogbonnaya et al., 2014). Semple et al. (2006) showed that after mineralisation had ended in a respirometry assay set-up that the addition of more phenanthrene degraders did not result in significant increase in mineralisation, in that the initial degraders had utilised the bioaccessible fraction.

Both the HP-β-CD and the XAD-4 extraction accurately represented the mineralisation of ^{14}C-phenanthrene while the DCM-shake extraction was observed to overestimate it. This result is in line with previous studies on the prediction of microbial biodegradation of phenanthrene using HP-β-CD shake extractions reported by different authors (Reid et al., 2000b; Swindell and Reid, 2006; Rhodes et al., 2008; Hickman et al., 2008; Rhodes et al., 2012; Ogbonnaya et al., 2014), where close 1:1 linear relationships were typically observed. Single HP-β-CD extraction (20-22 h) successfully removes a combination of the rapidly desorbing and aqueously soluble (labile) fractions of LMW PAHs in soils by forming inclusion complexes within the hydrophobic cavity of HP-β-CD. This measurement can be valuable in predicting biodegradation endpoints in the assessment of contaminated land (Rhodes et al., 2012; Sanchez et al., 2013; Ortega-Calvo et al., 2015; Ogbonnaya et al., 2016). However, prediction of bioaccessibility has been less successful in larger soil organisms, such as earthworms, high molecular weight (HMW) PAHs contaminated soils and soils with relatively high amounts of black carbon (Hickman and Reid, 2005; Barther and

Pelletier, 2007; Papadopoulos et al., 2007; Rhodes et al., 2012; Cui et al., 2013; Oyelami et al., 2014). Thus, identifying a supporting technique that can overcome these barriers with similar efficiency would be valuable for the assessment of contaminated land.

HP-β-CD works as a complexing agent, whilst XAD works as a mild chemical adsorbent (Cui et al., 2013). The application of XAD extraction for predicting phenanthrene bioaccessibility in soil has not been reported before, though other studies have reported similar trends. Lei et al. (2004) observed the capacity of XAD-2 extraction to predict 2-, 3-, and 4-ring hydrocarbons in sediments, thereby supporting the present findings. Similarly, data from an study carried by Xia et al. (2010) allowed the authors to conclude that residual amounts of ^{14}C-phenanthrene in soil after XAD-2 assisted desorption and biodegradation were similar. Although not considered within the objectives of Xia et al. (2010), these results support the observed in the present investigation suggesting that it is possible to quantify similar amounts of ^{14}C-phenanthrene through XAD assisted extractions and microbial biodegradation. Moreover, other XAD has also been reported to accurately represent the fraction of *p,p´*-dichlorodiphenyl dichloroethylene (*p,p´*-DDE), a by-product of the organochlorine pesticide DDT, available to earthworms (Škulcová et al., 2016). Further, Škulcová et al. (2016) also reported that XAD consistently produced a closer relationship with the bioaccessible fraction than predictors such as HP-β-CD.

5. Conclusions

The optimum conditions for the XAD extraction technique are a 2:1 soil/XAD ration, 100 rpm mixing for 22 h and elution using a DCM:methanol solution (1:1). Furthermore, this methodology was highly comparable with HP-β-CD extraction technique in terms of its accuracy predicting the microbial degradation of aromatic hydrocarbons in soil. Therefore, it can be concluded that XAD extraction closely mimics the mass transfer mechanisms that govern the bioaccessibility of hydrophobic organic contaminants in soil and sediment. Further testing and validation is however required to establish its place as a reliable tool for assessing biodegradation potential of a wide range of soil and sediment-bound organic contaminants, and hence its application in the assessment of contaminated land bioremediation potential as well as in the risk assessment of non-polar organic chemicals.

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Tables

Table 1. General physical and chemical properties of selected Amberlite XADs according to the manufacturer

Properties	XAD-2	XAD-4	XAD-16	XAD-1180N
Appearance	White solid	Opaque solid	Translucent solid	White solid
Type	Polymeric	Polymeric	Polymeric	Polymeric
Mesh size (mm)	20 - 60	20 - 60	20 – 60	20 – 50
Density (g mL^{-1})	1.02	1.02	1.03	1.03
Surface area (mm^2)	300	600	750	900
Pore diameter (Å)	100	50	300	600

Table 2. Total ^{14}C-phenanthrene extracted by different soil/XAD-2 ratios. The residual ^{14}C-activity in the supernatant and soil pellets is also presented. Values represent the mean ± standard error of the mean (*n* = 3). Data with the same lower case letter down the column indicates no statistical significant difference ($p > 0.05$).

Soil/XAD ratio	**Resin added (g)**	**Amount of ^{14}C-phenanthrene (%)**			
		XAD-2	Supernatant	Soil residue	Mass balance*
10/1	0.2	16.63 ± 0.4^{a}	0.57 ± 0.1^{a}	84.11 ± 2.8^{a}	101.31 ± 3.2
4/1	0.5	18.68 ± 2.3^{a}	0.51 ± 0.0^{a}	79.58 ± 3.5^{a}	98.77 ± 4.3
2/1	1.0	20.73 ± 1.2^{a}	0.66 ± 0.1^{a}	80.64 ± 3.5^{a}	102.03 ± 2.6
1/1	2.0	18.85 ± 1.5^{a}	0.41 ± 0.1^{a}	82.17 ± 4.0^{a}	101.43 ± 2.8

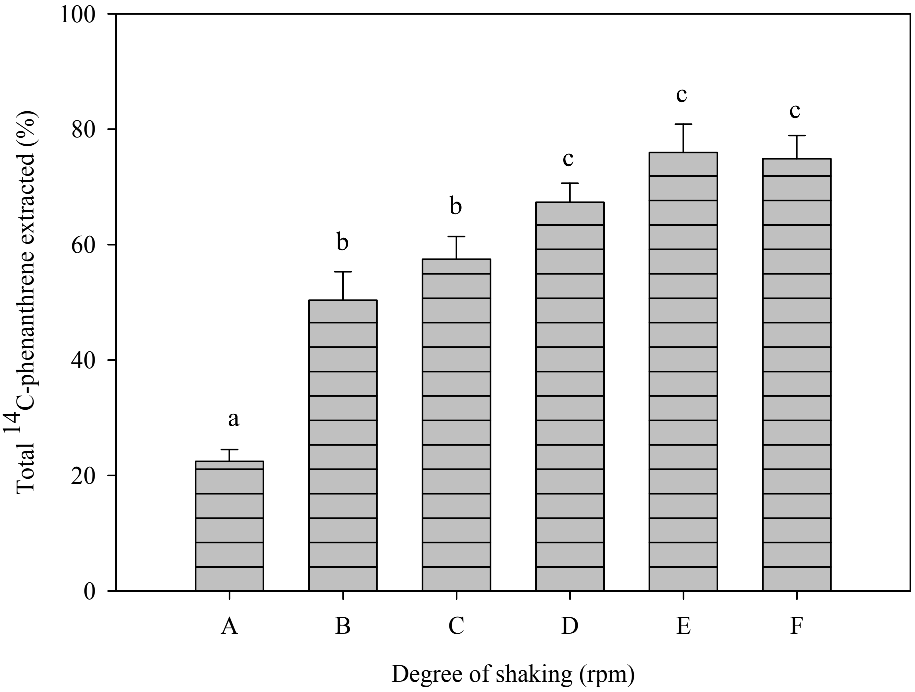
*Equals total XAD-2 extracted + residual ^{14}C-in the supernatant + residual ^{14}C in soil.

Table 3. ^{14}C-phenanthrene extracted by four XADs: XAD-2, XAD-4, XAD-16, and XAD-1180N; in compar extent of mineralisation at 0, 25, 50 and 100 d soil-PAH contact time. Values represent the mean ± standard e the same lower case letter down the column indicates no statistical significant difference ($p > 0.05$).

Soil-PAH contact time (d)	**XAD-extractable (%)**				**HP-β-CD (%)**	**DCM (%)**
	XAD-2	XAD-4	XAD-16	XAD-1180N		
0	85.82 ± 3.5^{bA}	85.72 ± 5.0^{bA}	83.76 ± 3.2^{bA}	89.48 ± 4.5^{bA}	78.98 ± 4.5^{aA}	94.91 ± 5.92^{cA}
25	27.74 ± 1.2^{aB}	30.22 ± 2.1^{bB}	25.83 ± 1.9^{aB}	26.06 ± 1.8^{aB}	32.25 ± 3.9^{bB}	63.99 ± 3.21^{cB}
50	16.99 ± 1.0^{aC}	18.05 ± 1.8^{aC}	15.78 ± 1.1^{aC}	15.19 ± 1.0^{aC}	23.35 ± 1.7^{bC}	53.08 ± 3.48^{cC}
100	16.82 ± 2.4^{aC}	19.15 ± 2.3^{aC}	17.53 ± 2.1^{aC}	16.11 ± 1.1^{aC}	18.97 ± 2.9^{aD}	51.82 ± 3.80^{bC}

a: No statistical significant difference ($p > 0.05$) amongst extractions and mineralisation in soil within each contact time; A: No within each extraction or during mineralisation across different contact times; b or c: Statistically higher ($p < 0.05$) extents of extrac contact time; B or C: Statistically higher ($p < 0.05$) extents of extraction or mineralisation in soil across different contact times.

651 **Figures**



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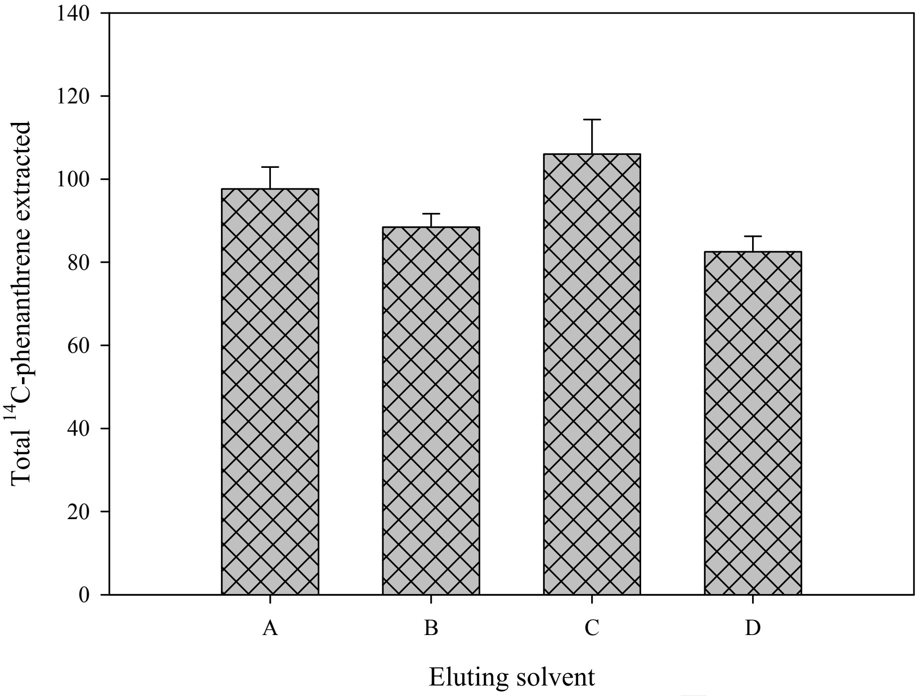

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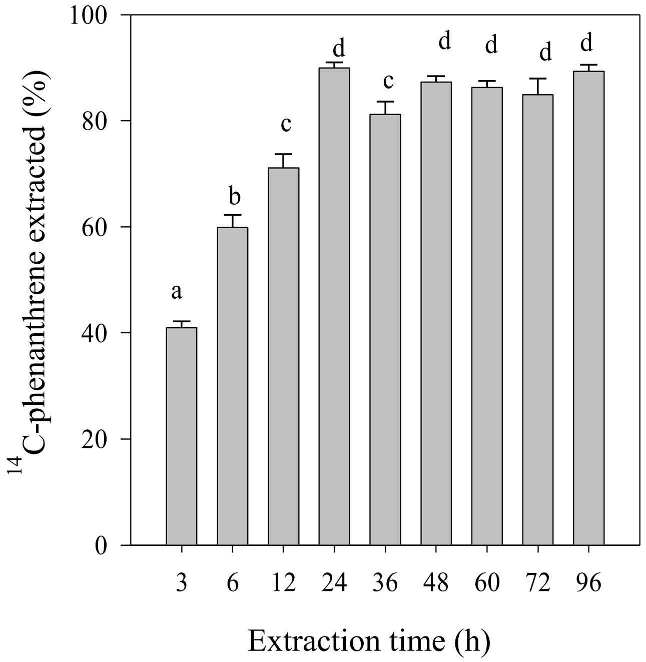

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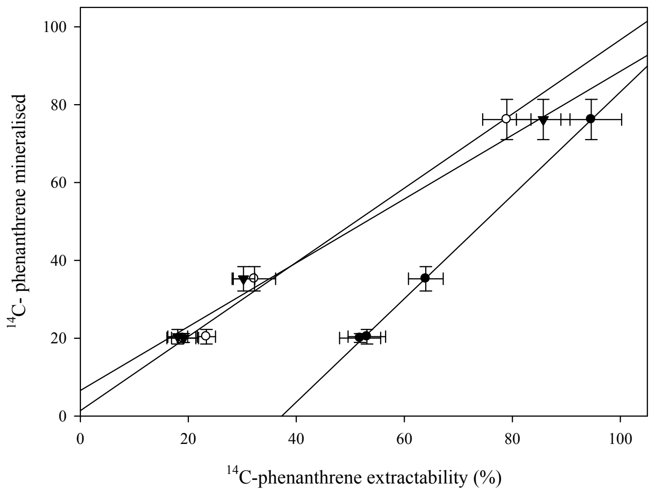

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Highlights

- Organic solvent extraction was more effective than XAD at removing phenanthrene from soil
- The extraction of phenanthrene using XAD was comparable to the of HPCD from soil
- Mineralisation of ^{14}C-phenanthrene was predicted by HPCD and XAD-4 extractions