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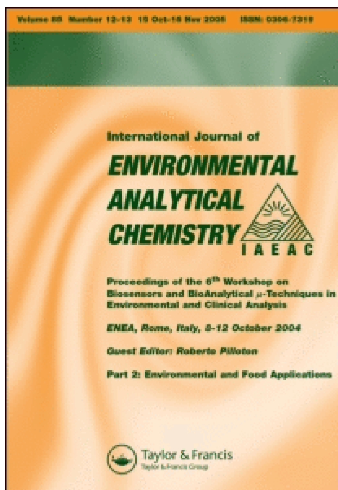

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FIELD-BASED SUPERCRITICAL FLUID EXTRACTION AND IMMUNOASSAY FOR DETERMINATION OF PAHs IN SOILS

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A field-compatible supercritical-fluid extraction (SFE) device and method for extraction of organic contaminants from soil has been developed and combined with field-based immunoassay for on-site PAH (polycyclic aromatic hydrocarbon) determination. The optimised extraction method was tested in field experiments on natural samples with varying water content (0–32% w/w) without any sample pretreatment, yielding an average PAH recovery of 80% versus Soxhlet. The immunoassay functioned in buffer-diluted 10% v/v MeOH SFE extracts, allowing direct determination of PAHs with minimum sample manipulation. Immunoassay served as a reliable semi-quantitative technique for rapid screening of PAHs in SFE preparations of natural samples extracted in the field. Poor performance of the commercial solvent-shake extraction (SSE) method further supported the on-site SFE/immunoassay method.

Keywords: Field-based technology; Environmental risk assessment; Soil; Organic contaminants; Supercritical-fluid extraction; ELISA

INTRODUCTION

The contamination of soils, particularly by organic pollutants at industrial sites, demands the development of appropriate methods of extraction and analysis to determine the nature and concentration of the pollutants and to allow implementation of appropriate risk assessments and remedial strategies. Conventional methods for site assessment entail extensive site-wide sampling, with subsequent sample despatch to a centralised laboratory, where complex procedures such as Soxhlet extraction and GC-MS analyses, are performed [1]. Since such processes are laborious, expensive, solvent intensive and introduce a significant time-delay into the programme, there is an increasing demand for rapid and reliable field-based analytical methods for the low-cost and efficient extraction and analysis of organic pollutants [2]. More field-amenable methods include simple solvent-shake extraction (SSE), but recover

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only those species weakly bound to the sample matrix, providing an incomplete picture of the extent of contamination [3,4].

Supercritical fluid extraction (SFE) is a well-established United States EPA (Environmental Protection Agency) approved method for the hitherto laboratory-based extraction of analytes from soils. SFE yields quantitative recoveries for a range of analytes with minimum organic solvent consumption, typically ~10 mL [2,5,6]. SFE is essentially simple and rapid, based on the compression and heating of a gas above its critical pressure and temperature, the resulting supercritical fluid (SF) being swept through the sample [7]. SFE yields extracts that generally do not require additional concentration or fractionation steps [7–9]. With appropriate hardware, SFE is a robust technique and is therefore a logical choice for evaluation as a potential field-based extraction method.

There are no reports regarding the development of dedicated field-based SFE devices, although Bowadt *et al.* [2] describe a laboratory system within an all-terrain vehicle. The aim of this study was to source, optimise and validate a field-transportable SFE system and method for the on-site extraction of PAH contaminants from soils, with comparable extraction performance to laboratory-based Soxhlet extraction. Optimisation and validation were performed against the only commercially available field-based solvent extraction method and to the well-validated Soxhlet method. PAHs were chosen since they are widespread in the environment and exhibit a broad range of volatilities and physico-chemical properties. The principle of back-pressure regulation (BPR) was assessed as a means of eliminating restrictor blockage issues, a source of significant problems to SFE practitioners [2,5,10].

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There are two main methods of controlling fluid flow in SFE devices, regulation and restriction. Restrictors control flow rate by mechanical adjustment of an aperture comprising a needle valve and valve seat, thereby controlling or "restricting" fluid flow. This method is prone to restrictor plugging, due to solute precipitation and dry-ice formation originating from carbon dioxide cooling. However, valve systems based on regulation, as opposed to restriction, significantly reduce the possibility of blockage of the valve flow path. Fluid flow is regulated by means of a rapidly oscillating needle valve with an electronic feedback to the SF pump. A pressure transducer in the valve assembly ensures a constant pressure is maintained by the system. Should particulates enter the valve, the aperture defined by the oscillating needle is increased, thereby contributing to a minimisation in blockages.

An efficient on-site extraction method requires an appropriate field-based method of analysis. Field-based chromatographic or spectroscopic systems exist, but complexity, high apparatus cost and time-consuming analysis make them less suited to rapid cost-effective use [2]. However, immunoassays are being increasingly developed for screening of polluted sites [2,11–13]. Methods based on enzyme-linked immunosorbent assays (ELISAs) have greater throughputs and are more cost effective and less solvent intensive than conventional methods, being readily deployable on site, with a simple equipment requirement [13]. In addition, the EPA has accepted the use of immunoassays for soil screening application under SW-846 methods #4030 and #4035 for petroleum hydrocarbons and PAHs respectively. This study therefore further aimed to investigate the direct performance of immunoassays on methanolic SFE extracts for the PAH screening of soils. The SFE instrumentation utilised in this study was either commercially available or was simply adapted from readily available hardware to widen the applicability and uptake of the proposed approach.

EXPERIMENTAL

Samples and Standards

The PAH-contaminated certified reference material (CRM) was obtained from LGC (Middlesex, UK). Field-based tests were performed on a former gas works in London, UK, where gas manufacturing, principally through the carbonisation of coal and coke, had been continuously practised for 125 years. The borehole depth of the samples varied from surface level to 16 m. Sample materials were collected and transferred to 1-L capacity sealable plastic containers and stored under ambient conditions. Soil samples were homogenized by manual mixing after removal of large stones, sticks and other significant debris to provide a finer consistency. No other treatment, including drying, was performed. Quantities of 2–3 g of soil were weighed and mixed with 1 g of hydromatrix (Varian, Surrey, UK), for removal of excess moisture, and 1 g of copper powder (45 μm, 99% pure, ACROS Organics, Loughborough, UK) to retain sulfur compounds. Table I lists the key physico-chemical properties of the soil samples examined in this study.

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Solvents and Standards

Methylene chloride 99.8% and MeOH 99.8% (both HiperSolv) were from Fisher Scientific (Leicester, UK). GC-MS and immunoassay calibration was performed using a stock solution containing the EPA-defined 16 priority PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthrene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*] and benzo[*k*] fluoranthrene, benzo[*a*]-pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a*,*h*]anthracene and benzo[*ghi*]perylene), all $10\,mg\,L^{-1}$ in acetonitrile (QMX, Thaxted, UK). Diluted standards were prepared in MeOH.

SFE Instrumentation

The field-transportable SFE system incorporated a CO_2 reciprocating pump [PU-1580-CO_2; 22.5(W) × 43(D) × 31.5(H) cm; Jasco, Great Dunmow, UK], back-pressure regulator [BPR; 5(W) × 34(H) × 31(D) cm; BP-1580-81, Jasco] and a heating unit adapted

TABLE I Organic matter content, water content and physical aspect of contaminated soil samples

Sample ID	*Organic matter* (% w/w)	*Water* (% w/w)	*Physical aspect*
VS698	5.3	15	Black, ashy
VS1214	3.1	17	Wet clay
VS1234	4.6	17	Powdery soil
VS1237	8.3	18	Very compact clay
VS657	7.5	19	Dispersed clay
VS1233	6.8	21	Compact soil
VS1235	9.1	23	Oily tar
VS1202	1.57	24	Dispersed clay
VS691	11.0	32	Grey, oily, dispersed clay

from a digital block heater BT5D [14(H) × 20.5 (W) × 40(D) cm; Grant, Cambridge, UK].

The pump (maximum pressure 50 MPa) was selected for its amenability to field-based usage, being more compact than the widely used syringe-pump alternatives. The pump was equipped with an integrated electronic (Peltier) cooling unit to control the heat generated during CO_2 compression, negating the need for an external cooling water supply. The pump inlet was connected to a 20-kg SFE-grade CO_2 cylinder pre-mixed with 10% v/v MeOH (BOC Speciality Gases, Guildford, UK). The pump outlet fed pressurised CO_2/MeOH to a 10-mL Thar Design extraction vessel (70 MPa rating, Presearch, Hitchin, UK), housed within the heater unit. The BT5D heater contained a machined aluminium block for housing test tubes, which was disconnected and replaced with a heating block cut from aluminium [75(H) × 14 (W) × 19(D) cm] and machined on site to house two extraction vessels plus tubing, such that one extraction vessel could be pre-heated off-line during system operation if required. All fittings were fashioned from stainless steel, pressure rated to 70 MPa, with a tubing i.d. of 1.59 mm.

Downstream of the extraction vessel, the extract passed through the BPR, which regulated fluid flow/pressure across the system by means of a solenoid-driven oscillating needle valve. This design effectively eliminated the blockage problems encountered in the more widely used restrictor-type systems [2]. A pressure transducer and feedback system precisely controls the needle aperture allowing it to open/close to a greater or lesser extent to maintain the pre-set fluid flow rate and pressure. The feedback loop was implemented through electronic linkage of the valve to the pump.

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Carriage and Set-up

The field-based SFE device [72(W) × 57(D) × 50(H) cm; 40 kg total weight, excluding cylinder] was mounted on a robust 2.5-cm thick wooden board, surrounded by a cuboid metallic frame that anchored the components and allowed secure carriage (Fig. 1). The assembled device was transported in a vehicle licensed and insured to carry pressurised cylinders. On site, the SFE device was linked to the power supply using outdoor electrical cables. The device was earthed and operated in the vehicle,

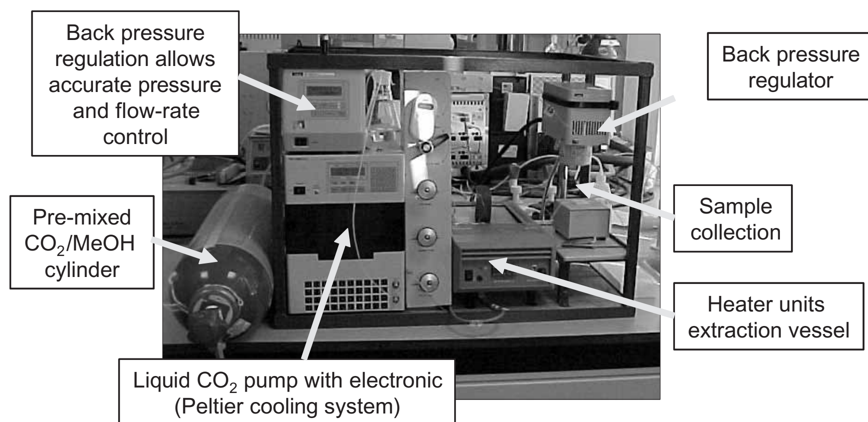

FIGURE 1 Field-based SFE instrument with associated pressurised CO_2/MeOH supply.

which was well ventilated and earthed. In the absence of on-site electricity, an electric generator would provide a suitable alternative supply. Set-up of the system took 30 min on day 1 and 5 min on subsequent days. During on-site testing, the SFE device remained in the vehicle, the system being isolated from the electrical supply during non-operation. Set-up of the device, extractions and analysis were all performed by a single operator with no technical problems being encountered during on-site testing.

SFE Extraction

Samples were loaded into the extraction vessel and packed with hydromatrix to minimise vessel void volume. The vessel was placed in-line within the heater unit and equilibrated at 60°C. Extractions were performed as follows: (1) SF entered the vessel to a pressure of 13.8 MPa and static-flow (i.e., zero flow rate) conditions maintained for 5 min at 60°C; (2) 10 min dynamic flow at 0.8 $mL\,min^{-1}$; 13.8 MPa, 60°C; (3) Pressure ramped to 32.5 MPa and temperature to 120°C, 5 min static flow; (4) 30 min dynamic flow, 0.8 $mL\,min^{-1}$, 32.5 MPa, 60°C; (5) Extracts were collected in 7 mL MeOH, this volume increasing during extraction owing to entrapment of MeOH co-solvent; (6) Final extracts were made up to 10 mL with MeOH, then appropriately diluted in 0.1 M phosphate buffer, pH 7, for analysis by immunoassay or GC-MS, with no further sample manipulation. After each extraction, the system was cleaned with SF (20 MPa, 60–80°C, 0.8 $mL\,min^{-1}$, 5–30 min) using a clean extraction vessel filled with 2–3 mL MeOH-spiked hydromatrix.

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Soxhlet Extraction

The SFE method was validated against EPA Soxhlet method #3540C. Soil (5–10 g, weighed) was mixed with an equal amount of anhydrous sodium sulfate (Merck, Gillingham, UK) and thoroughly ground by mortar and pestle before transfer to a 33×80 mm cellulose extraction thimble (Fisher Scientific). Samples were extracted for 18 h in 350 mL methylene chloride, the resultant extract being concentrated to 10 mL by rotary evaporation then appropriately diluted for analysis.

Solvent-shake Extraction

Soil samples (3 g) were extracted by the Strategic Diagnostics Incorporated (SDI) soil solvent shake extraction (SSE) kit method (Strategic Diagnostic Inc., Newark, NJ US). MeOH (10 mL) was decanted into 50 mL plastic screw-top bottles containing metal ball bearings and manually shaken for 1 min. Samples were then left to settle (~15 min) until some separation of the MeOH layer from the settling soil matrix was observed. This upper layer (~7 mL) was carefully removed by pipette and filtered (5 μm) to remove larger soil particles, then appropriately diluted for analysis.

Field-based Immunoassay Analysis

The total-PAH RaPID Assay kit (SDI) was chosen for its broad antibody cross-reactivity towards a wide range of PAH compounds, thus providing a total PAH measurement. The assay principle was that of indirect competitive enzyme-linked immunosorbent assay (ELISA), with measurement in the low microgram per kilogram

range. Soil screening for PAHs by immunoassay has been approved by the EPA (method #4035) for semi-quantitative determinations.

Assay Details

250 μL of diluted extract, 250 μL of PAH-horseradish peroxidase (HRP) enzyme conjugate and 500 μL of anti-PAH antibody, bound to paramagnetic beads, were mixed in a disposable polypropylene tube. Following incubation for 30 min under field conditions, during which analyte PAH and PAH-HRP conjugate competed for antibody binding sites, the tubes were placed in a magnetic rack, to separate the paramagnetic beads from unbound reagents. The beads were washed twice with the wash solution provided. Residual enzyme activity which, by virtue of the competitive nature of the assay, is inversely proportional to analyte PAH concentration, was determined by addition of 500 μL "colour solution" (hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine chromogen). Following 20-min incubation, the reaction was halted and stabilised with 500 μL of 2 M sulfuric acid and the absorbance recorded at 450 nm (OD_{450}) using a Model S2000 photometer (WPA, Cambridge, UK). The assay was calibrated each time, using a 16 priority PAH mixture in 10% v/v MeOH (2.0, 10.0 and 50.0 $\mu L\,L^{-1}$; $n = 2$). Full assay details are provided with the test kit. Data was normalised as $\%B/B_0$ where B and B_0 were the OD_{450} of the sample and blank control (i.e., zero analyte) respectively.

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GC-MS Analysis

GC-MS analyses were performed using a Hewlett-Packard Model 5890 series II instrument with an RTX-5MS column (30 m × 0.25 mm i.d., 0.25 μm film; Restek Corp., Windsor, UK) and coupled to a Hewlett-Packard 5971 mass-selective detector. The mass spectrometer was operated in Selective Ion Monitoring (SIM) mode for the 16 priority PAHs. A 4-min column delay prevented detector saturation by sample solvent. The injector was maintained at 290°C and the detector at 300°C. The column temperature was programmed as follows: (i) 55°C, 2 min; (ii) 55–180°C at 30°C min^{-1}; (iii) 180–300°C at 5°C min^{-1}; (iv) 300°C, 5 min.

RESULTS AND DISCUSSION

Modifier Addition

Modifiers have long been used in SFE to improve the solubility of polar analytes in non-polar CO_2 and to aid recovery of analytes having significant polar interactions with the sample matrix [1,10,14,15]. MeOH is the most frequently used modifier for PAH extraction [10,15–18] and was selected here for its beneficial solvation properties and miscibility with aqueous solutions, essential for subsequent extract analysis by immunoassay. Most SFE systems utilise a second pump to deliver co-solvent, an approach not considered here because of field-based operational constraints. Two alternative methods of modifier addition were examined: direct addition to sample within the extraction vessel and addition as a pre-mixed CO_2/MeOH (10% v/v) mixture.

The extraction efficiencies of selected PAHs from the CRM are given in Table II. The use of pre-mixed CO_2/MeOH led to an increase in recoveries for all native PAHs, with a significant impact on the recovery of the higher-molecular-weight PAHs (HMW-PAHs). Direct addition of MeOH to the sample gave no benefit, as it was stripped during extraction. Use of the pre-mixed fluid ensured continuous sample contact with 10% v/v MeOH, thus promoting analyte desorption and solubility throughout the extraction process. The solubility of CO_2 and MeOH vary with pressure, so as the cylinder reservoir is depleted, a slight but progressive change in the CO_2 : MeOH ratio will occur.

Effect of Moisture Content on SFE Extraction

A prerequisite of any field-based analytical method is that the process, including sample preparation, be as simple as possible. Therefore the effect of sample moisture on SFE extraction efficiency was examined. Seven ''natural'' (i.e., non-spiked PAH-contaminated) samples were either extracted as collected, or air dried under ambient conditions then extracted by both SFE and Soxhlet. Total recoveries for the 16 priority PAHs are given in Fig. 2 and show that five of the seven samples yield similar or superior recoveries when extracted wet (17–32% moisture). In common with other studies, it is believed that the water acted as a polar modifier, but also as a deactivating and swelling agent in soil, favouring PAH extraction [3,19–21]. However, water effects vary according to soil properties, which may explain the reduced recoveries of samples VS689 and VS1237 [3,20,21]. Overall, the results were encouraging, given that natural samples typically contain 0–40% w/w water.

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Effect of MeOH on Immunoassay Performance

Antibodies, as part of the vertebrate immune response, favour predominantly aqueous environments. However, reports suggest that antibody preparations can retain a degree

TABLE II Comparison of direct addition of MeOH modifier to the soil matrix and use of premixed CO_2/MeOH. PAH recoveries from the CRM for each method of addition are calculated against recoveries obtained by Soxhlet extraction (100%)

	Soxhlet (%)	*Direct spike* (%)	*Premixed* $CO_2/MeOH$ (%)
Naphthalene	100 ± 5	52 ± 7	82 ± 1
Acenaphthylene	100 ± 6	26 ± 1	56 ± 10
Fluorene	100 ± 9	67 ± 7	85 ± 5
Phenanthrene	100 ± 16	82 ± 4	84 ± 11
Anthracene	100 ± 13	51 ± 9	82 ± 14
Fluoranthene	100 ± 7	86 ± 7	91 ± 13
Pyrene	100 ± 7	88 ± 13	92 ± 14
Benzo[*a*]anthracene	100 ± 8	77 ± 10	85 ± 17
Chrysene	100 ± 8	83 ± 11	89 ± 15
Benzo[*b*]fluoranthene	100 ± 5	59 ± 23	87 ± 15
Benzo[*k*]fluoranthene	100 ± 8	63 ± 8	91 ± 7
Benzo[*a*]pyrene	100 ± 5	45 ± 9	78 ± 19
Indenopyrene	100 ± 14	25 ± 1	68 ± 11
Benzoperylene	100 ± 0	16 ± 3	47 ± 10

Values shown ± Relative Standard Deviation, $n = 3$.

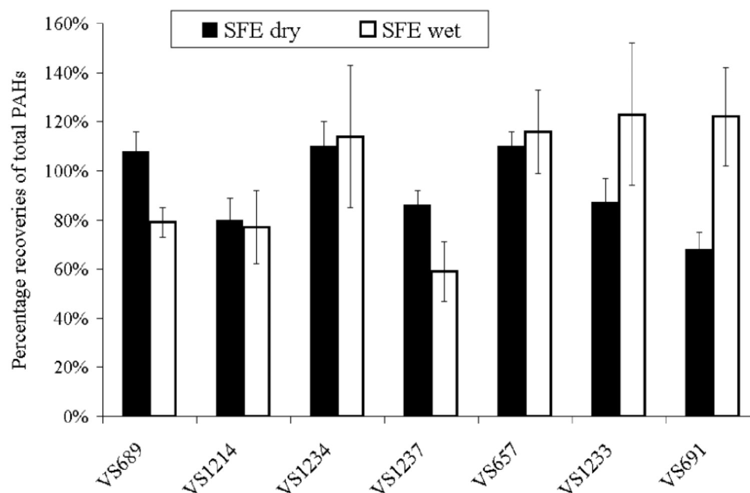

FIGURE 2 Percentage recoveries of 16 priority PAHs extracted by SFE from non-dried and air-dried laboratory samples, analysed by GC-MS. Percentage values are relative to those obtained by Soxhlet extraction of the same samples (100%). Error bars = SD, $n = 3$.

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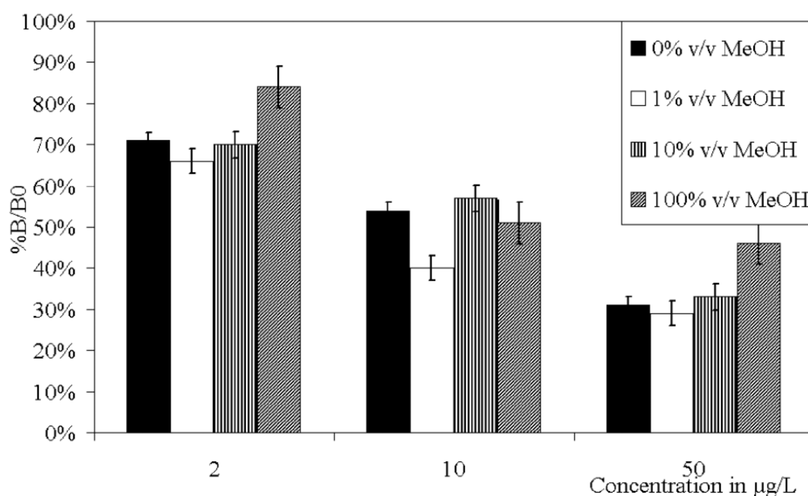

FIGURE 3 Effect of MeOH on immunoassay response. Comparison of phenanthrene standards (2, 10, $50\,\mu g\,L^{-1}$) in 0, 1, 10 and 100% v/v MeOH.

of binding activity in the presence of organic solvents such as MeOH and ethanol (to 90%v/v) and MeOH/acetone, diethyl ether and benzene (all to 50% v/v) [23,24]. Since MeOH was used as both collection solvent and modifier during the SFE process, the effect of this solvent on PAH immunoassay performance was investigated.

Figure 3 shows the PAH ELISA response to phenanthrene standards, prepared in 0–100% v/v MeOH with 0.1 M phosphate buffer (pH 7). Standards containing 10% v/v MeOH gave responses comparable to those at 0% v/v. The reduction in signal evident at the higher MeOH level was attributed to solvent-induced disruption of the

antibody binding site due to distortion, water displacement, altered binding interactions and denaturation of the HRP enzyme label [25]. These results indicate that the PAH test kit is able to function in the presence of 10% v/v MeOH. However, the assay calibration obtained in pure MeOH (non-dried) were significantly different from those obtained in 0% v/v MeOH, owing to solvent-induced alterations in the antibody–analyte binding interactions, and thus was in agreement with the findings of Matsuura *et al*. [26].

Assay Cross-reactivity

A linear logit–log calibration was obtained on assaying the 16 priority PAH mixture in 10% v/v MeOH (Fig. 4(a)). Comparison of the curve with that obtained using phenanthrene indicates that the 16 PAH mixture concentration is over-calculated by a factor of 2.5 across the assay dynamic range (Table III). This difference was observed consistently throughout the study and was a function of the combined cross-reactivity of the PAH mixture. Immunoanalytical methods, although target selective owing to high-specificity biorecognition, rely on the particular structural and physico-chemical properties of the target analyte species. PAH-specific antibodies, as noted by Fähnrich *et al*. [27], recognise many PAH congeners. Thus antibodies raised against a specific PAH will exhibit cross-reactivity towards other members of the PAH

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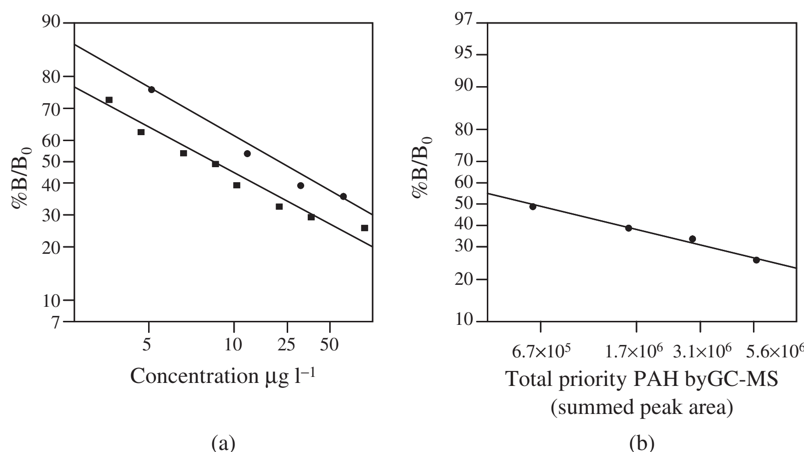

FIGURE 4 (a) Kit calibration using phenanthrene standards in 0% v/v methanol (•) and priority PAH standards in 10% v/v MeOH (■); (b) Validation of immunoassay technique against GC-MS for priority PAHs mixture (overall concentrations: 4, 8, 16, 32 $\mu g\,L^{-1}$).

TABLE III Measurement of 16 priority PAH mixture by total PAH immunoassay

Concentration of standards ($\mu g\,L^{-1}$)[a]	0.9	1.6	3.2	5.6	8.0	16	32
Concentration by immunoassay using phenanthrene calibration curve ($\mu g\,L^{-1}$)[b]	2.2	6.0	11	14	29	49	80
Over-calculation factor[c]	2.5	3.8	3.4	2.5	3.6	3.0	2.5

[a]Row 1: Actual concentration of 16 PAH standard dilution series; [b]Row 2: PAH concentration obtained by immunoassay using calibration curve constructed using phenanthrene standards; [c]Row 3: 'Over-calculation factor' = Row 2/Row 1.

family. However, for measuring total PAHs, cross-reactivity is advantageous, since the use of a single antibody species permits recognition of the majority of the 16 priority PAHs, in addition to other PAH species present.

The commercial immunoassay used in this study utilised phenanthrene standards. The concentration of phenanthrene required to inhibit 50% of the colour produced by the negative control (IC50 value) was 21.9 $\mu g\,L^{-1}$. Fluoranthene and benzo[*b*]fluoranthene have IC50 values of 6.25 and 72.1 $\mu g\,L^{-1}$ respectively and therefore exhibit antibody binding affinities approx. three times greater and three times lower than phenanthrene. Thus the particular composition of the 16 PAH mixture is responsible for the observed change in the calibration profile.

Comparison of the immunoassay response ($\%B/B_0$) against the summed concentrations of each of the individual 16 PAHs determined by GC-MS yielded a linear correlation:

$$y = -0.5x + 6.72, \qquad r^2 = 0.9940$$

(logit–log plot, Fig. 4(b)). This relationship supports the use of the immunoassay as a semi-quantitative tool for determining PAH mixtures in methanolic extracts.

In certain situations, immunoassay data may be considered complimentary to that obtained by GC-MS. Given that several hundred PAH congeners have been identified in the environment, it is impractical to determine each species in routine analysis. The immunoassay method, whilst only semi-quantitative owing to the cross-reactivity issue, will respond to the majority of these species via the broad binding specificity of the antibody. It may be argued that the immunoassay approach provides a truer evaluation of the ‘‘risk’’ posed by a PAH-contaminated sample as opposed to the absolute quantification of selected PAH species offered by chromatography. Since tests were performed on real samples with widely varying PAH compositions, the PAH mixture was used for assay calibration purposes.

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Field-based SFE of Soils

Total PAHs recovered by SFE varied from 59–137%, in comparison to Soxhlet, whilst recoveries by SSE varied from 22 to 76% (Table IV). The SFE recoveries, whilst demonstrating the field-based potential of the method, appeared dependent upon the physical nature of the samples. For example, SFE extracts of VS1237 (very compact clay), VS1214 (wet clay) and VS1235 (oily tar) gave lower total PAH recoveries than Soxhlet. For the latter two samples, incomplete PAH extraction was primarily attributed to high contaminant concentrations, or to the compactness of the sample matrices, preventing maximum PAH uptake by the modifier/CO_2 over the selected extraction time. Longer extraction times would be expected to increase analyte recoveries, but at the expense of field-based assay throughput.

In contrast, Soxhlet is a laboratory-based, solvent-intensive and time-consuming process (18 h), requiring subsequent extract concentration, and in which the sample requires drying and thorough grinding prior to extraction. Employing the SFE device as a rapid, field-amenable screening tool, the values obtained were acceptable for grading contaminated samples with an extraction time of 75 min (Table V and Fig. 5). The SSE method, although very simple and rapid, gave poorer recoveries for the 16 priority

TABLE IV Comparison of priority PAHs levels by Soxhlet, SFE and SSE sample extracts with GC-MS analysis of the 16 priority PAHs. Concentration values are shown for Soxhlet and relative recoveries (%) for the SFE and SSE extracts

Sample	*Soxhlet* ($mg\,kg^{-1}$)	*SFE* (%)[a]	*SSE* (%)[a]
VS1214	5889	77	69
VS1235	23 481	70	56
VS1234	640	114	76
VS1233	4916	123	22
VS1202	121	137	22
VS1237	24	59	28
VS689	2456	81	32

[a]Percentage recovery versus Soxhlet (Column 2).

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TABLE V Concentrations of priority PAHs (GC-MS analysis) and total PAHs (immunoassay) for Soxhlet, SFE and SSE extracts, with SFE, SSE and immunoassay procedures being performed in the field

	Soxhlet extract (GC-MS)		*SFE extract (GC-MS)*		*SFE extract (immunoassay)*		*SSE extract (GC-MS)*		*SSE extract (immunoassay)*	
VS1237	32	**L**	14	**L**	77	**L**	7	**L**	31	**L**
VS1202	149	**M**	166	**M**	389	**M**	27	**L**	57	**L**
VS1234	340	**M**	731	$\mathbf{M^+}$	792	$\mathbf{M^+}$	486	**M**	903	$\mathbf{M^+}$
VS689	1795	**H**	1978	**H**	4342	**H**	775	$\mathbf{M^+}$	940	$\mathbf{M^+}$
VS1233	4916	**H**	6055	$\mathbf{H^+}$	4320	**H**	1062	**H**	3640	**H**
VS1214	5940	$\mathbf{H^+}$	4544	**H**	3560	**H**	4077	**H**	8795	$\mathbf{H^+}$
VS1235	21 820	$\mathbf{H^{++}}$	16 546	$\mathbf{H^{++}}$	5102	$\mathbf{H^+}$	13 090	$\mathbf{H^{++}}$	11 572	$\mathbf{H^{++}}$

Characters L, M, H denote broader sample PAH concentrations to illustrate similarities/differences between the methods by consideration of data in purely semi-quantitative terms. $\mathbf{L} = 0$–$100\,mg\,kg^{-1}$; $\mathbf{M} = 100$–$500\,mg\,kg^{-1}$; $\mathbf{M^+} = 500$–$1000\,mg\,kg^{-1}$; $\mathbf{H} = 1000$–$5000\,mg\,kg^{-1}$; $\mathbf{H^+} = 5000$–$10\,000\,mg\,kg^{-1}$; $\mathbf{H^{++}} = > 10\,000\,mg\,kg^{-1}$ priority PAH contamination.

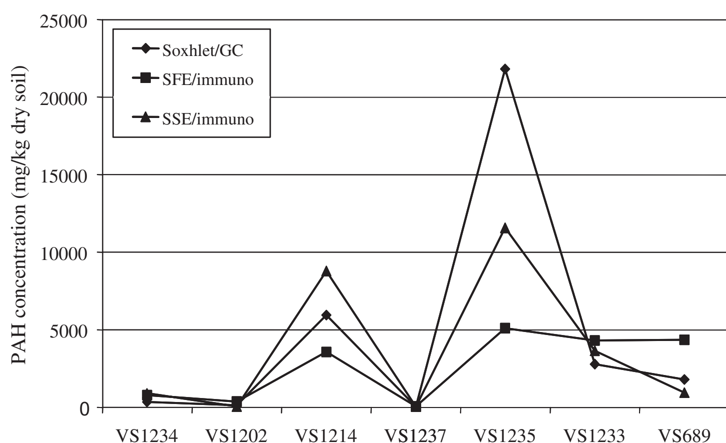

FIGURE 5 Comparison of total PAH loadings by field-based SFE/immunoassay, solvent-shake/immunoassay and laboratory-based Soxhlet/GC-MS. GC-MS method measured sum concentration of the 16 priority PAHs.

PAHs for most of the samples and failed to identify the moderately and highly contaminated samples VS1202 and VS689 respectively (Tables IV and V).

On examining the recoveries of individual PAH species (Table VI), no obvious trends were observed. Acceptable recoveries were obtained for all the HMW-PAHs, but some samples (VS1214 and VS1235) yielded lower recoveries of LMW-PAHs, especially naphthalene and acenaphthylene. However, naphthalene and acenaphthylene extraction by both Soxhlet and SFE are vulnerable to the volatile nature of the analyte, which may be lost during SFE solvent entrapment or repeat higher-temperature Soxhlet refluxing. Naphthalene and acenaphthylene are generally easier to extract from solid environmental matrices owing to their low molecular weight and weaker matrix binding and thus should be more amenable to recovery using simple solvent-extraction procedures. Many studies on PAH contamination are aimed at the HMW-PAHs as these have been shown to be the most potent carcinogens, although naphthalene is listed as a priority pollutant by the EPA.

Field-based Immunoassay

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All SFE extracts were analysed by immunoassay at the end of the SFE field trial (Fig. 5). Dilutions and analysis of the 10 samples, including standards and subsequent data analysis, took 4 h. Discrepancies between the SFE extracts analysed by immunoassay and GC-MS were attributed to co-extracted interferents and cross-reactivity effects. Such discrepancies arise owing to the relative amounts of PAH congeners in the samples. For example, the underestimated PAH concentrations of samples VS1214, VS1235, VS1233 by immunoassay may be attributed to very high levels of naphthalene in the extract (IC50 > 1330 $\mu g\,L^{-1}$), compared to substantially lower amounts of the higher affinity PAHs such as benzo[*a*]pyrene, pyrene and anthracene (IC50s of

TABLE VI Priority PAH composition of samples VS1214, VS1235 and VS1233, as extracted by Soxhlet and SFE. Recoveries by SFE are expressed as a percentage of Soxhlet values

	VS1214		*VS1235*		*VS1233*	
	Soxhlet ($mg\,kg^{-1}$)	*SFE recovery* (%)	*Soxhlet* $mg\,kg^{-1}$	*SFE recovery* (%)	*Soxhlet* $mg\,kg^{-1}$	*SFE recovery* (%)
Naphthalene	2217	78	10 864	78	1538	133
Acenaphthylene	159	50	1265	59	166	93
Acenaphthene	96	78	416	67	61	130
Fluorene	229	79	1190	71	214	146
Phenanthrene	1000	80	3825	72	1118	134
Anthracene	223	69	860	63	184	139
Fluoranthene	470	91	952	61	337	88
Pyrene	372	90	1540	59	577	91
Benzo[*a*]anthracene	210	77	688	53	182	101
Chrysene	188	79	625	54	201	111
Benzo[*b*]fluoranthene	128	73	204	55	46	152
Benzo[*k*]fluoranthene	149	73	283	59	72	143
Benzo[*a*]pyrene	159	65	388	60	98	118
Indeno[1,2,3]pyrene	119	53	144	57	41	171
Dibenzanthracene	53	45	69	68	27	167
Benzo[*g,h,l*]perylene	117	49	168	56	54	156
Total PAHs	5889	77	23 481	70	4916	123

9.18, 10.24 and 14.6 $\mu g\,L^{-1}$ respectively). Sample VS1235 contained 10 864 $mg\,kg^{-1}$ naphthalene, compared to 388, 1540 and 860 $mg\,kg^{-1}$ benzo[*a*]pyrene, pyrene and anthracene respectively. As a result, the PAH contamination was classified as high level by SFE/immunoassay, being ~70% of the summed 16 PAH value obtained by Soxhlet/GC-MS. The total PAH concentration by immunoassay was over-estimated in comparison to GC-MS for all other samples, but followed the same trend as the GC-MS data (Fig. 5). Again, over-estimation may be attributed to cross-reactivity factors, co-extracted interferents and the presence of other (non-priority) PAHs.

Comparison of Combined Extraction/Analysis Methods

The extraction efficiencies of field-based SFE/immunoassay and SSE/immunoassay were compared to laboratory Soxhlet/GC-MS (EPA #3540C and #4035). PAH concentrations reported by SFE/immunoassay were in broad agreement with Soxhlet/GC-MS (Fig. 5, Table VI), being suitable for identifying high and low contamination levels. In contrast, the SSE/immunoassay data under-estimated total PAH concentrations for four of the seven samples, yielding recoveries of < 32% (VS689, VS1202, VS1233, VS1237). Recoveries of 56–76% were recorded for the other samples.

These under-calculated values were attributed to the poor extraction efficiency of the SSE method, as evidenced by comparison of SSE extracts by immunoassay and GC-MS, the former method over-estimating the total PAH loading for all seven samples. The SSE/immunoassay approach offers rapidity, low cost and simplicity, all desirable characteristics of a field-based analytical tool, but with questionable reliability for all but the most cursory of on-site investigations. Ultimately the site-analyst is required to make a judgement as to the type and quality of data required, considering the simplicity but imprecision of SSE/immunoassay against the more quantitative field-based SFE/immunoassay approach and highly quantitative centralised Soxhlet/GC-MS method, with associated cost and time penalties. The key features of each method, regarding the information obtained and the quality and cost inherent in each procedure are listed in Table VII for comparative purposes.

CONCLUSIONS

Evaluation of the developed field-portable SFE device, linked to commercially available immunoassay, shows promise as a rapid screening tool for on-site monitoring of PAH-contaminated sites. The SFE method proved a highly efficient extraction tool, yielding recoveries similar to those of the "gold-standard" Soxhlet method, but within a shorter time (75 min vs. 16–24 h) and directly in the field. Both SFE and immunoassay have EPA approval for environmental PAH analysis. Furthermore, SFE reduced solvent consumption 35-fold in comparison to Soxhlet, lowering the environmental burden of the analysis. Furthermore, SFE also proved more efficient, accurate and reliable than the solvent-shake extraction (SSE) method, the only alternative commercially available field-amenable extraction method. SSE proved highly dependent on the physical characteristics of the sample.

GC-MS analysis of SFE and Soxhlet extracts yielded similar quantitative data for the 16 priority PAHs. The differences observed between SFE/immunoassay and Soxhlet/GC-MS were therefore primarily due to the performance characteristics of

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TABLE VII Characteristics of laboratory-based Soxhlet-GC/MS, field-based solvent extraction/immunoassay and SFE/immunoassay for extraction and analysis of organic pollutants from soil matrices. Comparison of the information obtained, and the quality and cost provided by each method

	Soxhlet-GC/MS	*Field-based SDI extraction kit/immunoassay*	*Field-based SFE/immunoassay*
Information obtained	Accurate concentration of individual/and or total 16 priority PAHs	Screening of total PAHs Evaluation of degrees of contamination	Screening of total PAHs Evaluation of degrees of contamination
Extraction time	16 to 24 h	15 min	30–75 min
Extraction efficiency for PAHs[a]	++	±	++
Selectivity	No	No	Yes
Solvent consumption	350 mL	10 mL	10–20 mL
Complexity of the process	Complex procedure	Easy procedure but not automated and not very reproducible	Easy procedure automated and programmable
Time of analysis	40 min/sample (6–7 h for 10 samples)	2–4 h from 1 to 10 samples	2–4 h from 1 to 10 samples
Efficiency of method of analysis	Qualitative and quantitative	Qualitative and semi-quantitative	Qualitative and semi-quantitative
Total time of extraction and analysis for one sample	2 days	Half day	Half day
Cost of equipment for extractions[b]	45	1000 (test kit for 10 extractions)	31 000
Cost of equipment for analysis[b]	67 000	1400 (test kit for 100 analysis)	1400 (test kit for 100 analysis)
Cost per analysis[b]	225–300	150	100–150

[a] + + high efficiency; ± low efficiency.
[b] Approximate cost in (US)$.

the EPA-approved immunoassay procedure. GC-MS can be used to provide a more quantitative PAH evaluation of a sample, but not on a routine practical basis owing to the presence of co-extracted interferents and the vast number of PAH congeners present in a typical environmental sample. Because of this, it is usual to express PAH content in terms of the 16 priority PAHs. Thus, the data provided by Soxhlet/GC-MS is only one way of defining PAH content. In contrast, the immunoassay method, which is based on structural recognition, is responsive to the wider PAH population of a sample, but again is ultimately limited in quantitative terms because of interference and cross-reactivity effects. Thus, neither method can be considered absolutely quantitative regarding the total PAH content of a sample, but both provide a valuable measure of the threat posed by a PAH-contaminated soil, as recognised by the EPA.

The instrumentation procured and developed in this study was more compact, easier to transport and more user-friendly than that proposed by Bowadt *et al*. [2]. The device did not exhibit technical problems during field-analysis, whilst Bowadt *et al*. encountered serial episodes of restrictor plugging.

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