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Optimization and Comparison of Microwave-Assisted Extraction, Supercritical Fluid Extraction, and Eucalyptus Oil-Assisted Extraction of Polycyclic Aromatic Hydrocarbons from Soil and Sediment

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Abstract: Polycyclic aromatic hydrocarbons (PAHs) are persistent organic compounds of major concern that mainly accumulate in soils and sediments, and their extraction from environmental matrices remains a crucial step when determining the extent of contamination in soils and sediments. The objective of the present study was to compare the extraction of PAHs (phenanthrene, pyrene, chrysene, and benzo[a]pyrene) from spiked soil and sediment using supercritical fluid extraction (SFE) with ethanol as the modifier, microwave-assisted extraction (MAE), and eucalyptus oil-assisted extraction (EuAE). Recoveries of PAHs were comparable between the three methods, and >80% of applied pyrene, chrysene and benzo[a]pyrene were recovered. The most efficient method of extracting PAHs from naturally incurred soils with different levels of contamination was SFE. A longer extraction time was required for the EuAE method compared with SFE and MAE under optimized conditions. However, EuAE required lower extraction temperatures (15–20 °C) compared with SFE (80 °C) and MAE (110–120 °C), and consumed less solvent than SFE and MAE. Compared with hexane/acetone used in MAE, the use of ethanol in SFE and eucalyptus oil in EuAE can be considered as more sustainable approaches to efficiently extract PAHs from spiked/naturally contaminated soils and sediments. And, although less efficient for matrices containing higher carbon content, EuAE offered a cheap, low-tech approach to extracting PAHs. *Environ Toxicol Chem* 2023;42:982–994. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Eucalyptus oil-assisted extraction; Microwave-assisted extraction; Polycyclic aromatic hydrocarbon; Soil; Sediment; Supercritical fluid extraction

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants, which show teratogenic, mutagenic, and in some cases carcinogenic effects in humans and other organisms (Parales et al., 2002; Yu et al., 2013). These compounds can accumulate in soils, sediments, air, marine organisms, and plants, with 90% of the global environmental PAH burden present in soils and sediments (Wild & Jones, 1995).

Polycyclic aromatic hydrocarbons are released into the environment via biological processes and during incomplete combustion of organic matter from natural or anthropogenic sources (Abdel-Shafy & Mansour, 2016), mainly during pyrolysis when organic compounds are combusted at high temperatures under depleted oxygen conditions. This commonly occurs during the production of coke and coal tar, incomplete combustion of fossil fuels in vehicles, forest fires, and when using fuel oil heating systems (Abdel-Shafy & Mansour, 2016). Polycyclic aromatic hydrocarbons released into the air can enter humans and other organisms through inhalation, while PAHs in soil and water bodies account for the highest impact on human and animal health through bioaccumulation in food chains (Zhang et al., 2015). Therefore, an accurate assessment of these environmental contaminants is vital to guide potential remediation.

Soxhlet, ultrasonic, liquid–liquid, and solid-phase extractions are some conventional techniques used to extract

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PAHs from various matrices (Imam et al., 2019; King et al., 2003; Temerdashev et al., 2021). However, these techniques tend to require large volumes of industrial organic solvents and can be time-consuming, and many involve multistep processes that increase the risk of the loss of some analyte. Microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and plant oil-assisted extraction of PAHs show advantages over conventional solvent extraction owing to the potential reduction in extraction time, solvent consumption, and waste generation (Camel, 2001; Kariyawasam, Doran, et al., 2022; Lau et al., 2012), which reduces risks to human operators. These greener extraction techniques will be the focus of the present study.

Microwave-assisted extraction allows rapid extraction of analytes from the sample matrix into the solvent by heating the solvent with microwave energy (Sparr Eskilsson & Björklund, 2000) and minimizes energy consumption and waste generation via extraction of sample batches (Ibáñez & Cifuentes, 2017; Sparr Eskilsson & Björklund, 2000). The hexane/acetone solvent system has a greater reported efficiency for PAH extraction from soils and sediments compared with cyclohexane/acetone, cyclohexane/water, and hexane/water solvent systems (Flotron et al., 2003; Sánchez-Uría & Castillo-Busto, 2018; Shu & Lai, 2001). The ionic conduction and the dipole rotation occur simultaneously both in the solvent and in the analyte to convert microwave energy to heat energy (Moret et al., 2019). Therefore, incorporating polar solvents such as water or acetone allows a better ability to absorb microwave energy and produce bulk heating, facilitating the extraction process. Microwave-assisted extraction can be affected by the nature of the solvent, solvent volume, microwave power, temperature, sample size, and the moisture level of the sample (Ibáñez & Cifuentes, 2017). However, the effect of the environmental matrix on PAH extraction has not been extensively studied.

Supercritical fluid extraction uses a gas, such as CO₂, which has been heated and compressed above its critical temperature and pressure, resulting in a fluid with properties of both a gas and a liquid. Supercritical carbon dioxide (SC-CO₂) is the most commonly used solvent in SFE because it is effective, nontoxic, nonflammable, and chemically inert and has a moderate critical point of 31 °C and 7.4 MPa, which is relatively easy to reach (Saldana et al., 2005). The nonpolar nature of CO₂ makes it an ideal solvent for extracting nonpolar organic compounds, including *n*-alkanes, *n*-alkyl-phthalates, and PAHs (Librando et al., 2004). Although extraction temperature and pressure are the main factors that govern SFE, CO₂ flow rate, static and dynamic extraction times, and cosolvent composition also play a vital role. Enhanced extraction efficiency of PAHs has been achieved through adjusting the polarity of the solvent by incorporating an organic modifier because SC-CO₂ has a different polarity when compared with an SC-CO₂ modifier/cosolvent system (Librando et al., 2004). As reported by Hawthorne and Grabanski (2000), Meskar et al. (2018), Saldana et al. (2005), and Wicker et al. (2018), organic modifiers such as pentane, acetone, acetonitrile, dichloromethane, and methanol can facilitate PAH extraction from soils. However, as far as the authors are aware, no studies

have used ethanol as a modifier, which is less hazardous than many other industrial organic solvents.

The potential to use vegetable oil as a nontoxic, biodegradable, and cost-effective alternative solvent to extract PAHs from contaminated soils has been explored in recent studies (Arca-Ramos et al., 2015; Gong et al., 2006; Lau et al., 2012; Pannu et al., 2004), which indicated that the oils have the ability to desorb and dissolve PAHs bound to environmental matrices, while potentially also acting as nutrients to support plant growth in remediated soils (Villemur et al., 2000). Another type of plant-derived oils are the essential oils, and Kariyawasam, Prenzler, et al. (2022) explored the application of eucalyptus oil (Eu-oil) to extract PAHs from soils and sediments, which allowed direct analysis of the extract without the use of hazardous industrial organic solvents and sophisticated technology. The major compound in Eu-oil is eucalyptol (84.39%), with 19 other compounds, including limonene and α -terpineol occurring at <6% (Campos & Berteina-Raboin, 2022). Eucalyptol has a polarity comparable to toluene and hexane, with a log octanol–water partition coefficient (K_{OW}) of 2.74 (PubChem, 2022b), which is analogous to toluene (log K_{OW} = 2.73; PubChem, 2022a) and slightly lower than that of hexane (log K_{OW} = 3.90; PubChem, 2022c). Although Eu-oil-assisted extraction (EuAE) was efficient at PAH extraction (80%–100%) from spiked soil and sediment, its application to naturally contaminated aged soil/sediment has not been investigated. Sequestration of PAHs in the soil/sediment matrix with aging is the main process which governs the desorption of PAHs into extractant solvents.

Previous studies on MAE and SFE have reported recoveries of PAHs using a common set of extraction conditions which have been optimized for a PAH mixture and not specifically for individual PAHs or for specific groups of PAHs (Amezcuca-Allieri et al., 2012; Ávila-Chávez & Trejo, 2010; Portet-Koltalo et al., 2008; Yang et al., 2008). Consequently, the present study aims to optimize MAE and SFE conditions for phenanthrene (PHE), pyrene (PY), chrysene (CHY), and benzo[a]pyrene (BAPY) spiked into soil and sediment by employing a Box-Behnken experimental design (BBD) combined with response surface methodology, which has not been reported previously, and to contrast the efficiencies of the two optimized extraction processes with EuAE. Four PAHs with varying ring numbers were chosen to represent the most commonly found 21 PAHs, as well as for the ability to be readily separated and quantified by gas chromatography–mass spectrometry (GC-MS). The efficiencies of these three extraction methods will be further compared by applying optimal conditions to naturally contaminated and aged samples.

MATERIALS AND METHODS

Chemicals

The PAH standards PHE, PY, CHY, and BAPY and their deuterated analogues (phenanthrene-d₁₀, pyrene-d₁₀, chrysene-d₁₀, and benzo[a]pyrene-d₁₂) were purchased from Sigma-Aldrich. High-performance liquid chromatography–grade acetonitrile and acetone were purchased from Chem-Supply

(RCI Labscan), and pharmaceutical Eu-oil was purchased from a local supermarket (Bosisto's).

Soil and sediment for PAH spiking studies

An agricultural soil was collected from Yanco, New South Wales (NSW), Australia, and was characterized (Isbell, 2016) as a brown chromosol (34°36'0"S, 146°24'0"E). Sediment was collected from Bullenbung Creek, Galore, NSW, Australia (35°18'2"S, 146°9'44"E). The pH (Rayment, 2011), conductivity (Rayment, 2011), texture (Black & Evans, 1965), and total organic carbon (Rayment, 2011) of soil and sediment are shown in Supporting Information, Table S1. Oven-dried and sieved (mesh size 2 mm) soil and sediment with PAH levels below the limit of quantification (LOQ) were used for spiking and recovery experiments to optimize extraction parameters.

Incurred soil samples for method comparison

Two soils were collected from sites in NSW, Australia, affected by bushfires (wildfires) in 2019 and were assumed to contain PAHs. Incurred Soil 1 was collected from Burra (35°51'01"S, 148°08'14"E), which is a semielevated (77 m) farming region. Incurred Soil 2 was collected from Kosciuszko (36°31'27"S, 148°15'36"E) in a heavily forested alpine region (1593 m) near the highest point in Australia, Mt. Kosciuszko (2228 m). A third soil contaminated by petrochemicals was collected near a diesel pump (35°03'09.4"S, 147°21'02.7"E) at Wagga Wagga, NSW (Incurred Soil 3). These three soils were used to compare the three optimized extraction methods.

Spiking of soil and sediment

Spiking of the soil and sediment was carried out as described by Kariyawasam, Prenzler, et al. (2022) for MAE and SFE parameter optimization using the four PAH standards. Two grams of blank soil or sediment were weighed into glass screw-cap scintillation vials (20 ml) in triplicate and covered with acetone (1800 µl). The solutions were spiked with PAHs (800 µl, 5 µg/ml), and the acetone was evaporated under a gentle stream of nitrogen, resulting in a PAH concentration in soil and sediment of 2 mg/kg.

For the method validation, 400 µl were spiked from either 2.5, 5, 10, 20, or 50 µg/ml PAH solutions (PHE, PY, CHY, and BAPY) onto soil/sediment to obtain 0.5, 1, 2, 4, and 10 mg/kg, respectively, as the final PAH concentration. All spiking and evaporation processes were conducted as mentioned previously.

Extraction of PAHs

Experimental design. A second-order multivariate BBD was employed to identify optimal extraction conditions, through response surface modeling. Three extraction parameters, temperature (X_1), solvent volume (X_2), and time (X_3), were selected as independent variables for MAE; and four extraction

TABLE 1: Selected factors and levels for microwave-assisted extraction according to the Box-Behnken experimental design

Factor	Code	Level		
		−1	0	+1
Temperature (°C)	X_1	100	110	120
Solvent volume (ml)	X_2	10	15	20
Time (min)	X_3	10	15	20

parameters, temperature (X_1), pressure (X_2), CO₂ flow rate (X_3), and dynamic duration (X_4), were selected as independent variables for SFE. Extraction efficiency was the response for the combination of the independent variables. The three levels of each variable were designated as −1, 0, and +1; represent low, medium, and high values; and are listed in Tables 1 and 2 for MAE and SFE, respectively. Minimum and maximum values for SFE parameters were chosen based on the minimum and maximum values of temperature, pressure, and CO₂ flow rates that can be used with the machine in our laboratory, to have an uninterrupted run, whereas in MAE minimum and maximum values were determined based on the values used in literature. Minimum and maximum values for EuAE parameters were obtained based on the preliminary studies conducted. Based on a factorial design, a total of 15 experiments for MAE and 27 for SFE were performed, including three center points, as described in Supporting Information, Tables S2 and S3. Optimization of EuAE was carried out as described in Kariyawasam, Prenzler, et al. (2022).

The mathematical relationship of the response to the independent variables can be approximated by the second-order polynomial equation

$$Y = \beta_0 + \sum_{i=1}^K \beta_i X_i + \sum_{i=1}^K \beta_{ii} X_i^2 + \sum_{i=1}^{K-1} \beta_{ij} X_i X_j \quad (1)$$

where Y represents the response variable; β_0 , β_i , β_{ii} , and β_{ij} are intercept, linear, and quadratic coefficients, respectively (Dahaghin et al., 2017); and X_i and X_j are the independent variables. The analyses of the experimental design and optimization were performed using Minitab 17 and SigmaPlot 13 software. The optimal extraction conditions were established through regression analysis and response surface plots.

MAE. A CEM MES-1000 microwave extraction system (Matthews) equipped with a solvent detector was used to carry out MAE. Soil samples spiked with PAH (2 g) were weighed into

TABLE 2: Selected factors and levels for supercritical fluid extraction according to the Box-Behnken experimental design

Factor	Code	Level		
		−1	0	+1
Temperature (°C)	X_1	40	60	80
Pressure (MPa)	X_2	10	20	30
CO ₂ flow rate (ml/min)	X_3	2	6	10
Dynamic duration (min)	X_4	10	20	30

Teflon-lined extraction vessels together with 10–20 ml of hexane:acetone (1:1). The slurry temperature was increased from room temperature to 100–120 °C over 15 min, held for a further 10–20 min, then cooled to room temperature. After MAE, the samples were centrifuged (3220g, 5 min), and the supernatants were spiked with the internal standards (50 µl, 10 µg/ml) to account for the losses during the filtration and evaporation steps and minor variations in resuspension volume. The solvent was evaporated and the residue reconstituted with 2 ml of hexane:acetone (1:1), and the filtered (0.22 µm polytetrafluoroethylene) extract was analyzed by GC-MS.

SFE. Supercritical fluid extraction was performed using an MV-10 ASFE (Waters), which was equipped with pumps for carbon dioxide delivery, cosolvent and make-up solvent, a column oven, and a back pressure regulator. The system was controlled by ChromScope Ver 1.20 software (Waters). Spiked soil/sediment samples (2 g) were placed in 50-ml stainless steel extraction cells, and the remaining volume was filled with glass beads. Ethanol was used as the cosolvent and the make-up solvent at flow rates of 1 and 0.5 ml/min, respectively. Extraction was carried out at an SC-CO₂ flow rate of 2–10 ml/min, oven temperature of 40–80 °C, pressure of 10–30 MPa, and dynamic duration of 10–30 min. Static duration was kept constant throughout the experiment (5 min). After SFE, samples were spiked with the internal standards (50 µl from 10-µg/ml solution) and evaporated under a gentle stream of nitrogen. Samples were then reconstituted with 2 ml of hexane:acetone (1:1), and the filtered extract was analyzed by GC-MS.

EuAE. Eucalyptus oil-assisted extraction was conducted under the conditions optimized in Kariyawasam, Prenzler, et al. (2022). In brief, Eu-oil (5 ml) was added to PAH-spiked soil samples (2 g), and the soil slurries were mixed on a rotary shaker (23 rpm) for 5 days at 20 °C. The extracts were centrifuged (3220g, 5 min), and the recovered supernatants were spiked with 50 µl of the internal standards (10 µg/ml). The filtered extracts were analyzed by GC-MS.

GC-MS analysis of PAHs

The PAHs were analyzed using GC with a mass-selective detector (Agilent 8890 A GC coupled to a 5977B MSD, 7693 A) based on the method of Guo and Lee (2013) with modifications. The PAHs were separated using a 30-m high-resolution glass capillary column DB5-MS (inner diameter 0.25 mm, 0.25 µm film; J&W Scientific, Agilent Technologies). The oven temperature was held initially at 90 °C for 1 min and then ramped to 200 °C at 20 °C/min. The temperature then increased to 210 °C at 3 °C/min and to 290 °C at 5 °C/min, followed by a 10-min hold. The helium column flow rate was 1.7 ml/min with a source temperature of 300 °C.

Validation of extraction methods

Under the most favorable MAE and SFE conditions, the limits of detection (LODs), LOQs, linearity, repeatability,

reproducibility, and recovery efficiencies (in the range of 0.5–10 mg/kg) were used to validate the proposed methods. The LODs were taken as the lowest concentration of a PAH that could be detected through visual evaluation, and a relative standard deviation (RSD) <10% was considered as the LOQ. Repeatability and reproducibility were theoretically determined by the prediction of the RSD (percentage) using the Horwitz function (Zuas et al., 2018). Statistical analysis was conducted using one-way analysis of variance and Tukey's post hoc test (Wawra et al., 2018). A two-tailed $p < 0.05$ was considered statistically significant.

Application of MAE, SFE, and EuAE to aged and naturally incurred samples

The soil and sediment used for aging were sterilized by gamma irradiation (Steritec) and spiked with PAHs (2 mg/kg) in triplicate before incubation for 30 days at room temperature. Air-dried naturally incurred soils and aged spiked soils ($n = 3$) were extracted using the optimized extraction methods previously identified and analyzed by GC-MS. To study the effect on extractability of PAHs due to matrix inhibition, PAH standards were spiked into a separate set of naturally incurred soils; the extractions were performed and analyzed by GC-MS (Shu & Lai, 2001). Ionization suppression of PAHs in the extracts after conducting MAE, SFE, and EuAE for nonspiked naturally incurred soils was investigated by spiking PAH standards to the extracts before the GC-MS analysis.

RESULTS AND DISCUSSION

The inherent properties of PAHs are associated with the number of aromatic rings and the ring arrangement (linear, angular, or cluster) in molecules (Abdel-Shafy & Mansour, 2016). Depending on the number of aromatic rings present in the compound, PAHs are classified as low-molecular weight PAHs (LMW-PAHs; having two or three rings) and high-molecular weight PAHs (HMW-PAHs; having more than three rings). The HMW-PAHs are mainly associated with soils and sediments in the environment, while the LMW-PAHs are commonly found with atmospheric particulates (Abdel-Shafy & Mansour, 2016). Increase in the size and angularity of PAH molecules results in enhanced hydrophobicity and electrochemical stability (Sahoo et al., 2020), thus increasing resistance to desorption from complex matrices such as soils and sediments. In the present study, four PAHs were chosen, covering a range of molecular weights (PHE, three rings; PY and CHY, four rings; BAPY, five rings) and shapes to more fully explore the relationship between the different extraction methods and the recoveries of PAHs with concomitantly different properties.

Optimization of MAE of PAHs from spiked soil and sediment

The influence of microwave extraction parameters (temperature, time, and solvent volume) on the extraction of a

mix of four PAHs at one concentration was investigated in a soil and a sediment. Response surface models were used to identify optimal values for the parameters tested. The pairwise effects of extraction parameters on extraction efficiencies are presented in the form of contour and three-dimensional plots (Supporting Information, Figure S1–S4). A nonsignificant lack of fit ($p < 0.05$), R^2 values of >0.78 , and closeness (<0.2) of R^2 and R^2 -adjusted obtained for the models indicated model adequacy for predicting responses for all of the PAHs in sediment and soil. Predicted recoveries based on the model and experimental values are presented in Table 3.

Optimum extraction conditions for MAE of all of the PAHs (Table 3) show that optimum temperature and volume were relatively consistent for the four PAHs in soil and sediment, while the optimum extraction time ranged between 10 and 20 min. Over 90% of the PAHs spiked into sediment were recovered under these extraction conditions, whereas 78%–100% of PAHs were recovered from soil. The different results show that matrix would appear to have some impact on MAE of PAHs. Based on the optimized conditions for individual PAHs (Table 3), a set of extraction conditions which consisted of a compromise for the four PAHs was also obtained (temperature, 120 °C; volume, 15 ml; and time, 15 min), resulting in $>90\%$ recoveries for all PAHs in sediments. In soil, recoveries were 100% for PY and BAPY and $>75\%$ for PHE and CHY.

Sánchez-Uría and Castillo-Busto (2018) optimized MAE for PAHs in soil and found that a higher temperature (120 °C) and a longer extraction time (20 min) improved extraction of two- to four-ring PAHs compared to those with five to seven rings, which required 100 °C temperature and 15 min extraction time when using hexane:acetone (1:1) as the solvent. When compromise conditions for all two- to four-ring and five- to seven-ring PAHs were applied, 48%–116% recoveries were obtained from a soil reference material. Portet-Koltalo et al. (2008) obtained recoveries of 2%–57% for four- to five-ring PAHs, under the common set of extraction conditions optimized for a PAH and alkane mixture. Similar to the present study, these authors used a chemometric approach to optimize MAE conditions for PAHs in diesel soot, but the methylene chloride and chloroform used in that study are more hazardous than the hexane:acetone solvent system. Nevertheless, the authors obtained a common set of extraction conditions that were optimal for PAHs, nitro-PAHs, and *n*-alkane and not specifically

for PAHs. Chee et al. (1996) optimized the MAE conditions for a PAH mixture in marine sediment reference materials using hexane:acetone (1:1) and reported 74%–105% recoveries for PHE, PY, CHY, and BAPY using 30 ml of solvent, a temperature of 115 °C, and a 5-min extraction time. Recovery of CHY was among the lowest of the four PAHs extracted. A longer extraction time may have improved CHY recovery, but this may have been at the expense of other PAHs in the mixture. Despite optimization of MAE having been done for one type of matrix, variations in the optimum conditions due to different matrices such as soil and sediment have not been considered before.

Recoveries of the four PAHs with MAE were comparable to those with EuAE (Supporting Information, Table S4) when using optimized conditions. Nonetheless, when extracting LMW-PAHs, more consistent and higher recoveries were achieved with EuAE than MAE, while the opposite was true for HMW-PAHs. This may be due to the higher affinity of more polar LMW-PAHs toward the relatively polar solvent Eu-oil compared with the hexane/acetone solvent system. Under the optimized conditions, EuAE offers the use of significantly lower temperature and solvent volume compared with MAE but requires a longer extraction time. Notably, EuAE is advantageous for performing bulk extraction because many extractions can be performed simultaneously with limited operator input. In terms of the hazardous nature of the solvents used, Eu-oil is a safer solvent than the hexane:acetone solvent system used in MAE.

Optimization of SFE of PAHs from spiked soil and sediment

The SFE parameters, including temperature, pressure, CO₂ flow rate, and dynamic duration, were optimized for the extraction of a mix of four PAHs at a single concentration in a soil and a sediment. Model adequacy based on the nonsignificant lack of fit ($p < 0.05$), R^2 (>0.76), and closeness (<0.2) of R^2 and R^2 -adjusted for all of the PAHs in sediment and soil denotes that the model can be used as a predictor of the responses. The optimized conditions are listed in Table 4, which shows that BAPY, PY, and CHY share optimized extraction conditions within a given matrix but require slightly different dynamic durations between matrices. The results indicate that the highest temperature (80 °C) and time (~30 min) selected for the optimization

TABLE 3: Experimental and predicted values of responses under optimum microwave-assisted extraction conditions

Matrix	PAH	MAE conditions			Extraction efficiency (%)	
		Temperature (°C)	Volume (ml)	Time (min)	Experimental	Predicted
Sediment	PHE	120	15	15	90.7 ± 3.6	93.5
	PY	110	15	15	92.3 ± 3.1	90.3
	CHY	120	20	10	93.8 ± 4.7	120.9
	BAPY	120	15	15	104.3 ± 4.5	120.4
Soil	PHE	120	10	15	78.2 ± 4.4	72.8
	PY	120	10	10	99.7 ± 3.8	101.3
	CHY	120	10	20	82.6 ± 4.3	72.4
	BAPY	120	10	10	99.8 ± 3.9	109.6

PHE = phenanthrene; PY = pyrene; CHY = chrysene; BAPY = benzo[a]pyrene; MAE = microwave-assisted extraction; PAH = polycyclic aromatic hydrocarbon.

TABLE 4: Experimental and predicted values of responses under optimum supercritical fluid extraction conditions

Matrix	PAH	SFE conditions				Extraction efficiency (%)	
		Temperature (°C)	Pressure (MPa)	CO ₂ flow rate (ml/min)	Dynamic duration (min)	Experimental	Predicted
Sediment	PHE	80	30	10	30	32.6 ± 4.5	12.8
	PY	80	20	6	25	107.7 ± 3.2	94.8
	CHY	80	20	6	25	87.4 ± 4.1	100.3
	BAPY	80	20	6	30	116.7 ± 3.3	125.7
Soil	PHE	80	30	10	30	44.9 ± 4.7	7.3
	PY	80	20	6	30	98.7 ± 3.1	82.2
	CHY	80	20	6	30	87.4 ± 3.9	65.9
	BAPY	80	20	6	25	90.5 ± 3.7	73.0

PHE = phenanthrene; PY = pyrene; CHY = chrysene; BAPY = benzo[a]pyrene; PAH = polycyclic aromatic hydrocarbon; SFE = supercritical fluid extraction.

experiment were the best conditions for all of the PAHs. Recoveries of 87%–116% were reported for PY, CHY, and BAPY, whereas only 30%–45% of PHE could be removed using optimal extraction conditions. Rezaee et al. (2010), however, observed a reduction in PAH recovery with increasing temperature and time, due to the loss of PAHs by volatilization because their collection vessel was not an enclosed container. Further, considering the optimized conditions for each PAH (Table 4), a common set of optimized conditions was determined. Extraction temperature of 80 °C, 20 MPa pressure, CO₂ flow rate of 6 ml/min, and 30 min dynamic duration were chosen; and >90% recoveries for PY, CHY, and BAPY and >35% for PHE in soil and sediment were observed. Unlike MAE and EuAE, SFE can effectively extract a wide spectrum of PAHs ranging from PY to BAPY in different matrices, using the same set of extraction conditions.

Previous studies reported the application of SFE at pressures ranging from 7.5 to 65.9 MPa and temperatures ranging from 40 to 200 °C to extract PAHs from contaminated soils (Kariyawasam, Doran, et al., 2022). As revealed by Notar and Leskovšek (1997), optimization of SFE conditions for PAHs grouped based on the number of rings or polarity is important when extracting from a sample with a wide spectrum of PAHs. Underestimation of PAH concentrations as a result of lower recoveries is possible when a single set of extraction conditions is used to extract a mixture of PAHs (Amezcuá-Allieri et al., 2012; Ávila-Chávez & Trejo, 2010; Hawthorne et al., 2000; Yang et al., 2008).

Compared with the recent study conducted by Al-Marzouqi et al. (2019), which used a lower CO₂ flow rate (1 ml/min) with heptane as the modifier, the present study indicated 6–10 ml/min as the most favorable flow rate when ethanol was used as the modifier. When a cosolvent with a higher polarity is used, a higher CO₂ flow rate is favored for PAH extraction because the percentage of the cosolvent decreases. While methanol (0.5%–10%) has been used as the cosolvent in previous studies (Librando et al., 2004; Notar & Leskovšek, 1997; Rezaee et al., 2010; Szolar et al., 2004) and showed recoveries of 30%–100%, ethanol has not been investigated and offers the advantage of being less hazardous than methanol. However, compared with the percentage of methanol used in the previous studies, a slightly higher (9%–14%) percentage of ethanol was used in the present study.

The model in the present study suggests that full recovery of PHE will not be achieved using the selected optimal conditions

and would actually require more extreme temperature, pressure, and CO₂ flow rate; but these conditions are unattainable using most laboratory-grade SFE units and beyond the capacity of MV-10 ASFE. Such low PHE recoveries (<50%) were also reported by Bielská et al. (2012) at 12 MPa, 50 °C, and 30 min in spiked soil when using pure CO₂. However, increasing the extraction temperature and pressure from 50 °C to 150 °C and from 12 to 40 MPa, respectively, resulted in >92% recovery of PHE. These increased temperature and pressure conditions may be able to break stronger PAH–soil interactions due to the alterations in diffusivity and solvation power of the SC-CO₂ (Anitescu & Tavlarides, 2006). Yamini and Bahramifar (2000) also reported increased solubility of LMW-PAHs in SC-CO₂ when increasing temperature from 35 °C to 75 °C and the pressure from 10 to 40 MPa. Increasing temperature mainly involves decreasing the SC-CO₂ density, allowing better penetration of the solvent into the matrix (Rezaee et al., 2010).

Supercritical fluid extraction had a longer extraction time than MAE but shorter than EuAE, while the extraction temperatures of the three methods followed the order of MAE > SFE > EuAE; SFE used the highest solvent volume compared with MAE and EuAE, but the solvents used for SFE were SC-CO₂ and ethanol, which are less hazardous than the hexane and acetone employed in MAE. The solvent used in EuAE is the least hazardous of those used in the three extraction methods, can be handled with the least risk to the user, and has potential as a cosolvent in SFE under mild extraction conditions. Recovery efficiencies of all three methods are comparable and exceed 78% for all PAHs, except for PHE by SFE. Both MAE and EuAE are more efficient at extracting PHE from spiked soils and sediments compared with SFE, but it should be noted that this may not be true in more complicated matrices for incurred samples.

When compared with the previous studies on conventional PAH extraction techniques such as Soxhlet, ultrasonic, and sonication (Camel, 2001; Khodadoust et al., 2000; Oluseyi et al., 2011; Silalahi et al., 2021), MAE, SFE, and EuAE conducted in the present study resulted in higher recoveries of PY, CHY, and BAPY. Oluseyi et al. (2011) reported very low recoveries of 10%–30% for PY, CHY, and BAPY after Soxhlet extraction and conventional shaking. Similarly, Dupeyron et al. (1999) observed relatively low recoveries for CHY and BAPY in soil when Soxhlet extraction or sonication was used, compared with MAE and SFE. In contrast, Silalahi et al. (2021) reported

TABLE 5: Limits of detection and quantification for the polycyclic aromatic hydrocarbons extracted using microwave-assisted extraction, supercritical fluid extraction, and eucalyptus oil–assisted extraction

PAH	Type of matrix	Extraction method					
		MAE		SFE		EuAE ^a	
		LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
PHE	Sediment	15.6	31.3	31.3	125	15.6	62.5
	Soil	31.3	62.5	31.3	125	15.6	62.5
PY	Sediment	7.8	62.5	3.9	31.3	31.3	62.5
	Soil	7.8	62.5	3.9	31.3	15.6	62.5
CHY	Sediment	15.6	62.5	3.9	62.5	62.5	125
	Soil	15.6	62.5	3.9	62.5	31.3	125
BAPY	Sediment	15.6	62.5	3.9	62.5	62.5	125
	Soil	15.6	62.5	3.9	62.5	15.6	62.5

^aKariyawasam, Prenzler, et al. (2022).

PHE = phenanthrene; PY = pyrene; CHY = chrysene; BAPY = benzo[a]pyrene; MAE = microwave-assisted extraction; SFE = supercritical fluid extraction; EuAE = eucalyptus oil–assisted extraction; LOD = limits of detection; LOQ = limits of quantification; PAH = polycyclic aromatic hydrocarbon.

>80% extraction of BAPY with Soxhlet, sonication, and shaking. However, MAE, SFE, and EuAE under the studied extraction conditions were more efficient at extracting (>80%) CHY and BAPY, with the use of lower volumes of greener solvents. Optimization of the extraction conditions, choice of solvent, and the complexity of the matrix have played significant roles in variations in the PAH recoveries with different techniques.

Validation of MAE and SFE using spiked samples

Validation of MAE and SFE was undertaken by spiking four PAHs into soil and sediment at five concentrations. The linearity, LODs, LOQs, repeatability, reproducibility, and recovery efficiencies were determined using a single set of optimized conditions for each extraction technique. Good linearity of the calibration plots based on analyte peak areas was obtained between 0.5 and 10 mg/kg with satisfactory regression coefficients (r^2) >0.99 for all analytes. While several studies report optimized conditions for PAH extractions in soils/sediments (Bangkedphol et al., 2006; Chee et al., 1996; Portet-Koltalo et al., 2008), none reported the LOD and LOQ values for direct comparison.

Supercritical fluid extraction showed the best LOD and LOQ values (Table 5) for PY, CHY, and BAPY, while both MAE and EuAE were better for PHE under the conditions studied. Because the polarity of SC-CO₂ is similar to nonpolar hydrocarbon and halocarbon solvents (Hyatt, 1984), the relatively higher polarity of hexane:acetone (1:1) and Eu-oil may contribute to the higher efficiency of PHE extraction compared with SFE. Minimal interaction of SC-CO₂ with the matrix compared with MAE explains the better performance of SFE compared with MAE (Oukebdane et al., 2010). Oukebdane et al. (2010) also observed slightly better LOD and LOQ values for SFE of PAHs in soot compared with MAE. Compared with SFE and MAE, EuAE demonstrated the poorest LOD and quantitation. Because Eu-oil is a more chemically complex solvent than SC-CO₂ and hexane, there is a potential of coelution of some oil components with analytes on the GC-MS, resulting in potential ionization suppression of the analyte.

Repeatability or reproducibility of a method is considered acceptable if the %RSD is <0.67 of the coefficient of variation (CV)–Horwitz (0.67 CV–Horwitz; Zuas et al., 2018). The calculated CV–Horwitz (CV–Horwitz [%] = $2^{1-0.5 \log c}$, where c is the concentration of the PAH stated in a decimal fraction) based on the PAH concentration (2 mg/kg) was 14.41 and the corresponding 0.67CV–Horwitz value was 9.7. It can be seen from Table 6 that all PAHs have calculated %RSDs <0.67 CV–Horwitz (9.7) for MAE and EuAE, except for PHE in SFE. These results imply that the MAE and EuAE methods are repeatable and reproducible for all of the studied PAHs, while PHE extraction using SFE needs further optimization to improve the repeatability and reproducibility.

Recoveries of all four PAHs were generally good for SFE (Figure 1) and EuAE across the 0.5–10 mg/kg range tested, but the low results for MAE for the 0.5 mg/kg spike might be due to the presence of more matrix-bound PAHs, which are less extractable with this technique. When the concentration of the PAH is low, matrix has more unoccupied binding sites, which can accommodate a higher percentage of PAHs. These PAHs are less prone to extraction because they are strongly trapped inside the matrix (Wang et al., 2021).

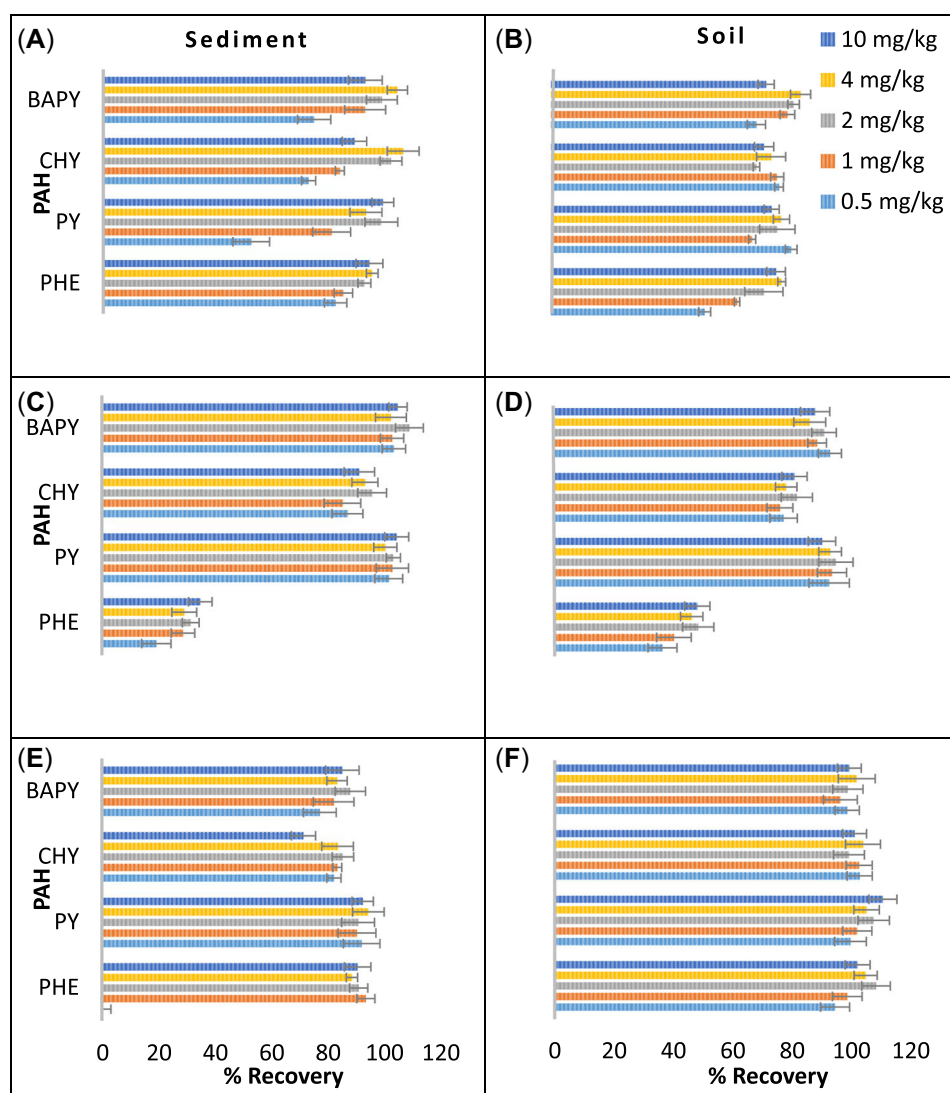
Application of MAE, SFE, and EuAE to aged PAH-contaminated soil and sediment

The efficiencies of the three extraction methods for recovery of PAHs from aged soil and sediment were investigated using gamma irradiation prior to spiking, to minimize microbial degradation of PAHs. In sediment (Figure 2), slightly reduced recoveries of PHE were observed after 30 days of aging with both MAE and EuAE, compared with reductions for PHE, PY, and BAPY in aged sediment when using SFE. Aging did not significantly impact the recovery of CHY in sediment using SFE. According to Luo et al. (2012), PAHs bound to the soft organic carbon fraction can be more easily solubilized using polar extractants; and because SC-CO₂ is less polar (Hyatt, 1984) compared with the solvents used in MAE and

TABLE 6: Repeatability and reproducibility data of the microwave-assisted extraction, supercritical fluid extraction, and eucalyptus oil–assisted extraction methods

PAH	Type of matrix	Extraction method					
		MAE		SFE		EuAE	
		%RSD ^r	%RSD ^R	%RSD ^r	%RSD ^R	%RSD ^r	%RSD ^R
PHE	Sediment	4.0	4.6	13.8	15.3	3.8	6.3
	Soil	5.6	6.4	10.5	11.5	6.0	8.2
PY	Sediment	3.4	3.6	3.0	4.2	3.8	4.9
	Soil	3.8	3.9	3.1	3.9	4.4	6.0
CHY	Sediment	5.0	5.9	4.7	5.8	4.3	6.0
	Soil	5.2	7.0	4.5	5.8	7.2	9.4
BAPY	Sediment	4.3	4.8	2.9	3.7	5.9	6.7
	Soil	3.9	4.4	4.1	5.8	8.0	9.6

PHE = phenanthrene; PY = pyrene; BAPY = benzo[a]pyrene; CHY = chrysene; EuAE = eucalyptus oil-assisted extraction; MAE = microwave-assisted extraction; SFE = supercritical fluid extraction; PAH = polycyclic aromatic hydrocarbon; r = repeatability (n = 3); R = reproducibility (n = 3); %RSD = relative standard deviation.

**FIGURE 1:** Recovery efficiencies of polycyclic aromatic hydrocarbons extracted using microwave-assisted extraction: (A,B) supercritical fluid extraction, (C,D) andeucalyptus oil–assisted extraction (E,F). PAH = polycyclic aromatic hydrocarbon; PHE = phenanthrene; PY = pyrene; CHY = chrysene; BAPY = benzo[a]pyrene.

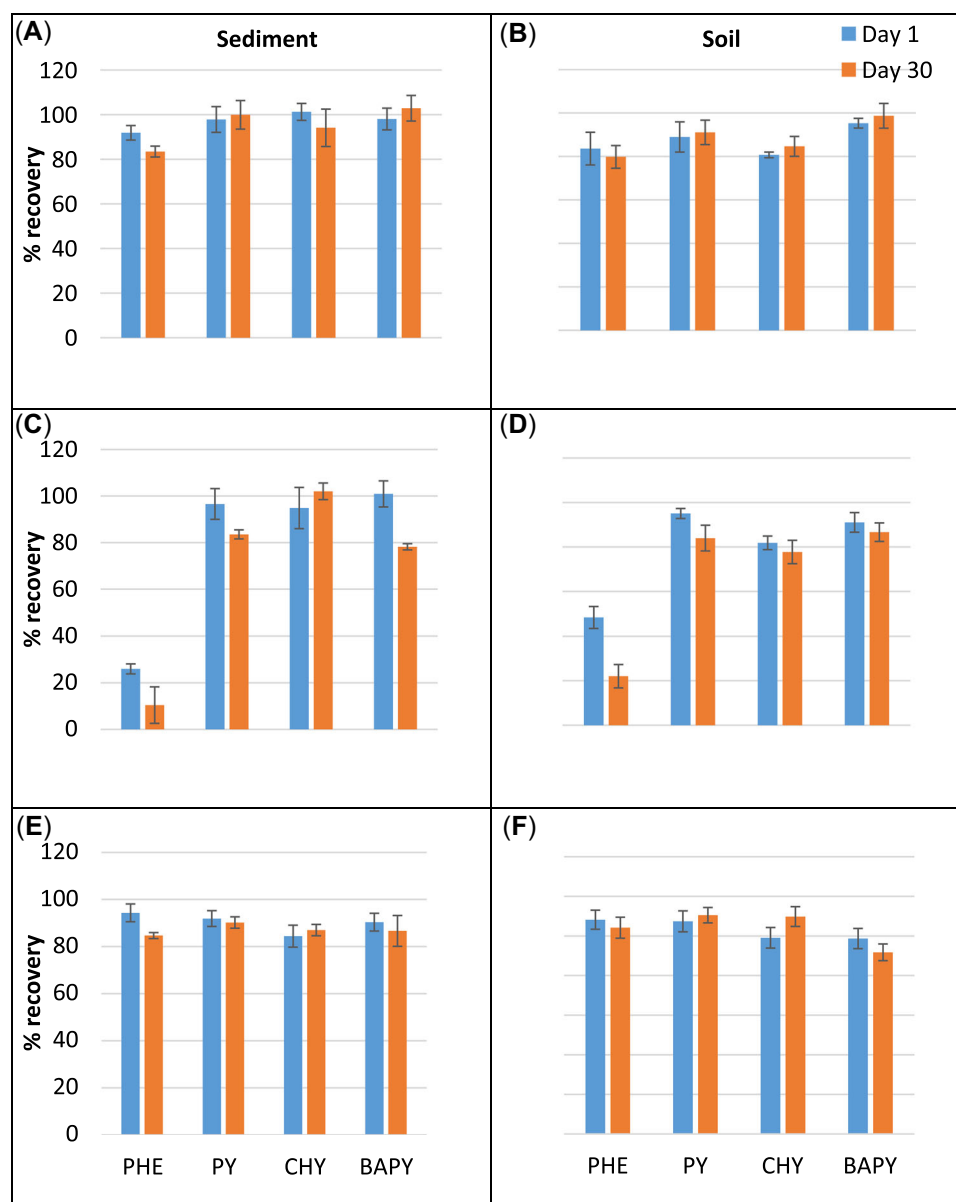


FIGURE 2: Effect of aging on the recovery of polycyclic aromatic hydrocarbons (2 mg/kg) in soil and sediment. (A,B) microwave-assisted extraction, (C,D) supercritical fluid extraction, and (E,F) eucalyptus oil-assisted extraction. PHE = phenanthrene; PY = pyrene; CHY = chrysene; BAPY = benzo[a]pyrene.

EuAE, increased partitioning of PAHs in soft carbon with aging may have resulted in reduced SC-CO₂ extractability of PAHs in aged soil. Moreover, 30 days of aging did not markedly influence the extractability of the selected PAHs in soil when extracted using MAE, SFE, and EuAE, except for the SFE of PHE. In soil, PHE showed a similar behavior to that in sediment, resulting in a reduced recovery by SFE after 30 days of aging. A nonlabile fraction of PHE is mainly retained in the narrow meso- and micropores because of the relatively lower molecular size of PHE (Luo et al., 2012). The presence of narrower meso- and micropores relative to the molecular size of PAHs can also limit the diffusion of PAHs into nonpolar solvents like SC-CO₂. The aging process of PAHs in soils and sediments is closely associated with sorption and sequestration (Luo et al., 2012), which are mainly governed by the

structure and composition of organic carbon and pore distribution in soil and sediment. Soil organic carbon is mainly composed of humic substances, black carbon, kerogen, and bitumen (Ukalska-Jaruga & Smreczak, 2020), which, combined with the different rigidities and distributions of meso- and micropores in soil, results in complex PAH–matrix interactions (Luo et al., 2012; Tan et al., 2020). With time, PAHs enter the glassy rigid structures of organic matter in soil, reducing their dissolution in extractants.

Tan et al. (2020) reported that PAHs tend to accumulate with particulate humic acid, microaggregate occluded humic acid during the early period of aging, and occur with non-aggregated silt and clay later. However, molecular size, polarity, redox active functional groups, complexing sites, and polyelectrolytic characteristics of humic acid are the key factors

that control PAH binding affinity (Tan et al., 2020; Ukalska-Jaruga & Smreczak, 2020). Many factors influence the diffusivity of PAHs, and because of the heterogeneous nature of soil/sediment matrices, the effect of a single factor could be impacted by the other factors. Hence, the changes in the extractability of PAHs due to aging cannot be reasonably explained by only considering one or two factors. Consequently, the analyst must give due consideration to all of these factors when choosing a suitable extraction method. Our results suggest that the MAE, SFE, and EuAE methods can be successfully applied to extract PAHs from aged soil and sediment, and MAE and EuAE are more efficient when extracting more polar LMW-PAHs such as PHE.

Comparison of MAE, SFE, and EuAE using naturally incurred samples

The three extraction methods were applied to soils naturally contaminated with PAHs by bushfires (Incurred Soils 1 and 2) and petrochemicals (Incurred Soil 3). Incurred Soil 3 showed a higher level of PAH contamination (1000–3500 µg/kg) compared with the soils contaminated by bushfires (4–200 µg/kg), and this is mainly due to fuel spillage resulting from daily use of the fuel nozzle over more than three decades. Figure 3 shows higher recoveries for SFE than MAE and EuAE in soils with low PAH concentrations (Incurred Soils 1 and 2) compared with soil with high PAH concentrations (Incurred Soil 3), while MAE performed similarly to SFE when soil PAH concentrations were high (Incurred Soil 3). The superiority of SFE at low PAH concentration was more evident for Incurred Soil 2 (Figure 3) and may be due to a higher soil organic carbon concentration (seven times greater than Incurred Soil 1) or the differences in particle size distribution because of the presence of different sand, silt, and clay contents (Supporting Information, Table S1). Differences in the types of organic matter present in Incurred Soil 2, such as a greater char content, may also contribute to the different method performances; however, these soil characterizations were beyond the scope of the present study.

Extractions were repeated using increased moisture content of Incurred Soil 1 to match Incurred Soil 2 to determine whether moisture content impeded the extraction of PAHs by MAE and EuAE. Higher moisture content reduced the extractability of PAHs when extracted by EuAE (Supporting Information, Figure S9), suggesting that EuAE may be influenced by soil moisture content and that soils should be air-dried prior to extraction. Reduced MAE efficiency was not observed when Incurred Soil 1 moisture content was increased. This explains that the reduced extractability of PAHs in Incurred Soil 2 with MAE compared with SFE was not associated with the moisture contents.

Furthermore, Incurred Soils 1 and 2 were extracted, and then the extracts were spiked with PAHs (1.25 µg/ml) immediately prior to GC-MS analysis to look at the influence of the matrix on the MS ionization efficiency. Supporting Information, Figure S10, shows that suppression of PAHs in GC-MS was not significant for any of the extracts, but recoveries from spiked

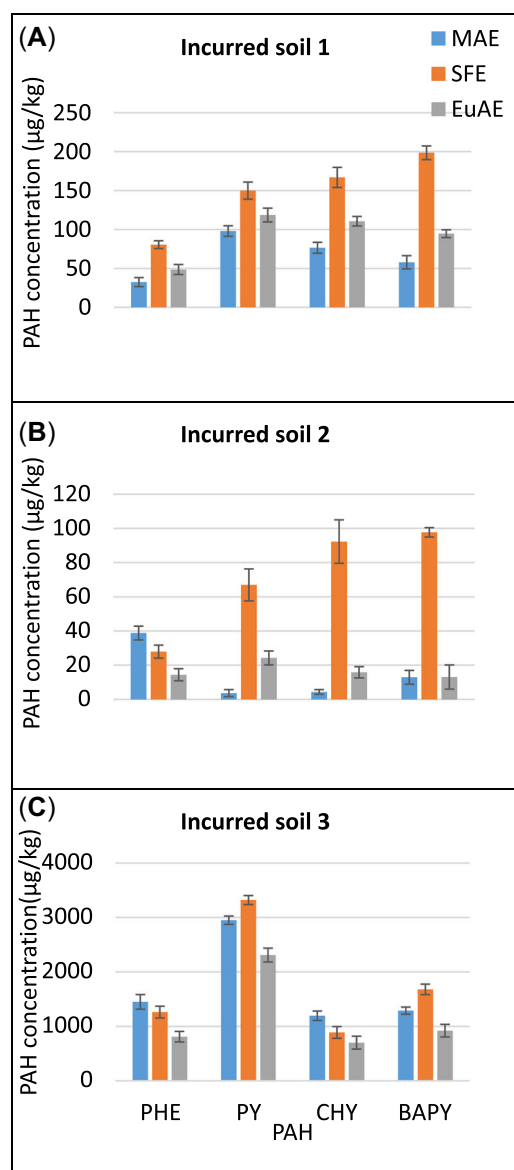


FIGURE 3: Concentration of polycyclic aromatic hydrocarbons in soils contaminated by bushfires (A,B) and petrochemicals (C). PAH = polycyclic aromatic hydrocarbon; MAE = microwave-assisted extraction; SFE = supercritical fluid extraction; EuAE = eucalyptus oil-assisted extraction; PHE = phenanthrene; PY = pyrene; CHY = chrysene; BAPY = benzo[a]pyrene.

SFE extracts were >100%, which may be due to the enhanced ionization by matrix components dissolved in the extracts.

In addition, Incurred Soils 1 and 2 were spiked with PAHs (1.25 mg/kg) and then extracted to look at matrix influence on the entire extraction and analysis process. The matrix effect associated with the strong binding of PAHs with matrix constituents was distinct for all of the spiked PAHs extracted using the three methods. Notably, a higher matrix suppression was observed for Incurred Soil 1 (unextractable PAHs 17%–41% for MAE, 5%–13% for SFE, and 31%–53% for EuAE) compared with Incurred Soil 2 (unextractable PAHs 8%–20% for MAE, 2%–11% for SFE, and 15%–43% for EuAE). Moreover, SFE showed the best recovery of spiked PAHs in spiked-incurred soils, showing

the least impact on extraction by the matrix, followed by MAE and EuAE.

In particular, spiked PAHs do not mimic the PAHs in long-term field-contaminated soils because the extractability of PAHs declines, mainly because of aging for incurred soils (Wei et al., 2014, 2017). Pannu et al. (2004) reported a significantly lower extractability of HMW-PAHs in naturally contaminated soil compared with spiked soil when extracted using peanut oil. The PAH extraction efficiency is governed mainly by the strength of PAH interactions formed with the soil organic matter and clay fractions (Wei et al., 2014). Clay and silt-associated soil organic matter is highly stable; therefore, PAHs with clay-/silt-bound organic matter are less extractable than those in the sand fraction. Furthermore, in industrial soils, anthropogenic organic matter is predominant; therefore, natural recent carbon is limited. Organic matter in industrial soils greatly varies from natural soils such as in forests; thus, PAHs behave differently in the two soil types (Boulangé et al., 2019).

Thus, the results suggest that SFE is the most efficient at extracting PAHs from a greater variety of sample matrices contaminated by various sources, which is due to the potential impact of moisture and soil organic matter content on extraction efficiency.

CONCLUSION

The findings of the present study indicate that SFE is the more robust method for extracting PAHs over the 0.5–10 mg/kg range from matrices of varying compositions, compared with MAE and EuAE. Moreover, unlike MAE and EuAE, SFE, using ethanol as the cosolvent, can be successfully applied to quantify PAHs even in aged soils/sediments, when the PAH concentration is <0.5 mg/kg. The SFE method was revealed to be highly efficient for different levels of PAH contamination in different matrices compared with MAE and EuAE. In terms of analysis time, SFE and MAE were faster than EuAE. Despite the longer extraction time and the poorest LOD and LOQs, EuAE is low on user time and input, does not need continual attendance, requires very low technology, and is therefore low-cost. Hence, it might be useful for low-budget screening of lots of samples when time is not a concern. Notably, with regard to the solvents used, SFE and EuAE are more sustainable extraction techniques because of the requirement of the cleaner SC-CO₂-ethanol solvent system and Eu-oil. However, when extracting LMW-PAHs such as PHE, incorporation of a higher proportion of a polar modifier and/or application of a higher temperature and pressure may enhance recoveries in SFE. Further, the effect of increasing the number of SFE cycles needs to be considered. Future research should focus on the application of these extraction methods to highly contaminated soils and sediments and the optimization of SFE, while also considering the ethanol flow rate as a variable. Moreover, further studies should concentrate on greener cleanup processes suitable for the three extraction methods and optimization to enhance the efficiency further.

Supporting Information—The Supporting Information is available on the Wiley Online Library at <https://doi.org/10.1002/etc.5593>.

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Data Availability Statement—Data will be provided to readers on request (gdoran@csu.edu.au). Data directly linked to the article are available in the Supporting Information.

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