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Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recovery, selectivity and effects on sample matrix

Steven B. Hawthorne*, Carol B. Grabanski, Esther Martin, David J. Miller

Energy and Environmental Research Center, University of North Dakota, P.O. Box 9018, Grand Forks, ND 58202-9018, USA

Abstract

Extractions of a polycyclic aromatic hydrocarbon (PAH)-contaminated soil from a former manufactured gas plant site were performed with a Soxhlet apparatus (18 h), by pressurized liquid extraction (PLE) (50 min at 100°C), supercritical fluid extraction (SFE) (1 h at 150°C with pure CO_2), and subcritical water (1 h at 250°C, or 30 min at 300°C). Although minor differences in recoveries for some PAHs resulted from the different methods, quantitative agreement between all of the methods was generally good. However, the extract quality differed greatly. The organic solvent extracts (Soxhlet and PLE) were much darker, while the extracts from subcritical water (collected in toluene) were orange, and the extracts from SFE (collected in CH_2Cl_2) were light yellow. The organic solvent extracts also yielded more artifact peaks in the gas chromatography (GC)–mass spectrometry and GC–flame ionization detection chromatograms, especially compared to supercritical CO_2. Based on elemental analysis (carbon and nitrogen) of the soil residues after each extraction, subcritical water, PLE, and Soxhlet extraction had poor selectivity for PAHs versus bulk soil organic matter (~1/4 to 1/3 of the bulk soil organic matter was extracted along with the PAHs), while SFE with pure CO_2 removed only 8% of the bulk organic matrix. Selectivities for different compound classes also vary with extraction method. Extraction of urban air particulate matter with organic solvents yields very high concentrations of *n*- and branched alkanes (~ C_{18} to C_{30}) from diesel exhaust as well as lower levels of PAHs, and no selectivity between the bulk alkanes and PAHs is obtained during organic solvent extraction. Some moderate selectivity with supercritical CO_2 can be achieved by first extracting the bulk alkanes at mild conditions, followed by stronger conditions to extract the remaining PAHs, i.e., the least polar organics are the easiest organics to extract with pure CO_2. In direct contrast, subcritical water prefers the more polar analytes, i.e., PAHs were efficiently extracted from urban air particulates at 250°C, with little or no extraction of the alkanes. Finally, recent work has demonstrated that many pollutant molecules become ''sequestered'' as they age for decades in the environment (i.e., more tightly bound to soil particles and less available to organisms or transport). Therefore, it may be more important for an extraction method to only recover pollutant molecules that are environmentally-relevant, rather than the conventional attempts to extract all pollutant molecules regardless of how tightly bound they are to the soil or sediment matrix. Initial work comparing SFE extraction behavior using mild to strong conditions with bioremediation behavior of PAHs shows great promise to develop extraction methodology to measure environmentally-relevant concentrations of pollutants in addition to their total concentrations.

*Corresponding author. Tel.: +1-701-777-5000; fax: +1-701-777-5181.

E-mail address: shawthorne@eerc.und.nodak.edu (S.B. Hawthorne).

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1. Introduction

Several new approaches for extracting organic analytes from environmental (and other) matrices have been developed including pressurized liquid extraction (PLE; also known under the trade name ASE, accelerated solvent extraction), supercritical fluid extraction (SFE), subcritical water extraction and others [1–9]. With proper understanding of the various methods' application, high recoveries of most organic pollutants can be achieved using any of these methods. However, comparisons of extraction methods usually discuss only relative recoveries of target analytes and the amount of organic solvent required, and tend to ignore other important chemical characteristics of the extraction method. Of special importance is the selectivity of an extraction method for target analytes in preference to bulk matrix organic compounds (e.g., humic matter from soil), since the presence of co-extracted matrix organics frequently requires post-extraction clean-up steps before chromatographic analysis.

In the present study, each of the above methods were used to extract polycyclic aromatic hydrocarbons (PAHs) from a soil contaminated several decades ago by a manufactured gas plant (MGP). Extractions were performed with a Soxhlet apparatus (18 h), by PLE (50 min at 100°C), SFE (1 h at 150°C with pure CO_2), and subcritical water (1 h at 250°C, or 30 min at 300°C). In addition to comparing the PAH recoveries of the different methods, the effect of the extraction on the sample matrix and on the presence of co-extracted (non-target) matrix material is reported. The potential for selectively extracting different classes of target organics is also investigated using alkanes and PAHs from urban air particulate matter.

Finally, the relevance of ''quantitative'' extraction methods to environmental processes is questioned, and initial results utilizing selective SFE to predict PAH behavior during bioremediation of a field site are presented.

2. Experimental

2.1. Samples

PAH-contaminated soil from an abandoned MGP site was collected, sieved to <6 mm, mixed and stored as received at 4°C until use. Air particulate matter [''urban dust'' SRM 1649 from the National Institute of Science and Technology (NIST), Gaithersburg, MD, USA] was used as received.

Since organic solvent extraction methods often use drying agents mixed with soils to retain water, some samples for Soxhlet and PLE were mixed 1:1 with sodium sulfate before extraction, and replicate samples were used as received (no sodium sulfate added) so that the C and N content could be determined on the soil residue after extraction. No differences in PAH recovery resulted with or without sodium sulfate for either Soxhlet or PLE.

2.2. Extractions

The conditions used for each extraction method are summarized in Table 1. SFE was performed using an Isco Model SFX-210 extractor (Isco, Lincoln, NE, USA) equipped with a heated co-axial restrictor (80°C) and a Model 260D pump filled with SFC-grade CO_2 with a helium headspace from Scott Specialty Gases (Plumsteadville, PA, USA). The 2-g samples were placed in a 10 ml cell at the bottom (outlet) end. The cell was placed in the extractor, immediately pressurized, and extraction performed in the dynamic mode at ~1 ml/min (measured as compressed CO_2 at the pump). Collection was performed by inserting the restrictor into a vial containing 15 ml of methylene chloride (CH_2Cl_2).

PLE was performed on a laboratory-assembled system using an Isco Model 260D pump for the solvent CH_2Cl_2–acetone (1:1), and a 3.5 ml (50 mm×9.4 mm I.D.) SFE cell from Keystone Scientific (Bellefonte, PA, USA). Both the extraction cell and a 1-m preheating coil of 1/16 in. (1.6 mm) stainless

Table 1
Conditions used to compare Soxhlet, PLE, SFE and subcritical water extractions

	Soxhlet[a]	PLE[b]	SFE[c]	Subcritical water
Sample size (g)	2	2	2	2
Extraction solvent	CH_2Cl_2–acetone	CH_2Cl_2–acetone	Pure CO_2	Water
Collection solvent	–	–	CH_2Cl_2	Toluene
Pressure (bar)	ambient	70	400	50
Temperature (°C)	b.p. of solvent	100	150	300, 250
Flow-rate	15 min/cycle	1 ml/min	1 ml/min	1 ml/min
Time	18 h	50 min	60 min	30, 60 min
Solvent volume (ml)	150	15	15 (60)[c]	10, 20 (30, 60)[d]

[a] Conditions similar to EPA method 3540C.
[b] Conditions similar to EPA method 3545. PLE was performed with 30 min of static extraction followed by 10 min of dynamic extraction. ~10 min additional time was required for filling the cell with solvent before the extraction, and flushing the cell with nitrogen after the extraction.
[c] SFE used 60 ml of CO_2 and 15 ml of CH_2Cl_2 for collection. Conditions were chosen based on Refs. [5,11].
[d] Subcritical water extractions used 30 ml water and 10 ml of toluene (for the 300°C extractions) or 60 ml water and 20 ml of toluene (for the 250°C extractions) [6,7].

steel tubing was placed in a GC oven (Hewlett-Packard 5890). A 1-m coil of 1/16 in. tubing at the end of the extraction cell exited the GC oven, and ran through a beaker of room temperature water to cool the solvent before collection. Outlet flow was controlled at 70 bar by a miniature back pressure regulator (Upchurch, Oak Harbor, WA, USA). Shut off valves (HIP Model 155-11AF1-316 from High Pressure Equipment, Erie, PA, USA) were placed outside the oven between the pump and the cell inlet, and between the cell outlet and the outlet back pressure regulator. For extraction, the 2-g soil sample was placed in the extraction cell, and the cell was placed in the GC oven. Room temperature solvent was pumped into the extraction cell until it was full (as evidenced by drops of solvent exiting the back pressure regulator). Both the inlet and outlet valves were shut off, and the oven was heated to 100°C for a 30-min static extraction. The outlet valve was then slowly opened to release the cell pressure (and a small amount of solvent was collected in a 22-ml collection vial). The pump flow was then begun and 10 ml of solvent was pumped through the cell and into the collection vial at 1 ml/min (during which the outlet pressure was controlled by the 70 bar back pressure regulator). Both shut off valves were then shut, a nitrogen tank was connected to the inlet line (set at ~3 bar), the 70 bar regulator was removed from the outlet, and the residual solvent in the cell was purged into the collection vial for 10 min.

Subcritical water extraction was performed as previously described [10]. In brief, an Isco Model 260D pump was used in the constant flow mode to pump water (HPLC-grade, Fisher Scientific, Pittsburgh, PA, USA) through a 1.5-m preheating coil of 1/16 in. stainless steel tubing connected to a 3.5-ml SFE cell (Keystone Scientific) placed in a GC oven (Hewlett-Packard 5890). ~One gram of clean sea sand was placed at the outlet of the extraction cell with the soil sample to prevent plugging of the cell frit. For collection of the extracted analytes, 0.3 ml/min of toluene (''Optima'' grade, Fisher Scientific) was introduced with a second pump (Isco Model micro-LC 500) into a stainless steel ''tee'' fitting placed in the oven at the outlet of the extraction cell. The water–analyte–toluene mixture was then cooled in the ice bath using a 1.5-m coil of stainless steel tubing, and transferred to the collection vial. This arrangement allowed the extracted analytes to partition into the toluene in the heated zone and prevented deposition of the analytes upon cooling [10]. Shut off valves (HIP Model 155-11AF1-316 from High Pressure Equipment) were placed in the connecting tubing between the pumps and oven, and at the end of the cooling coil just before the collection vial. All system components are rated for at least 350 bar, and both pumps are supplied with overpressure rupture discs to ensure safety. To perform an extraction, the toluene valve is closed while the outlet and the water supply valves

are opened. Water is pumped through the preheating coil at a low flow-rate (0.1 ml/min) to fill the cell from bottom to top. The system outlet valve is then closed until the pressure builds to ~100 bar and the oven is heated to 250 or 300°C. The outlet valve is then used to maintain the pressure at ~100 bar (still at 0.1 ml/min) until the GC oven reaches the set point temperature. At this point, the water pump is set to 1 ml/min, the toluene flow is started at 0.3 ml/min, and the outlet valve is used to control the system pressure at 100 bar. The cooled analyte–water–toluene stream is collected in 40-ml glass vials. After phase separation (a few min), the toluene layer is removed, the water is washed twice with additional aliquots of toluene, and the internal standard is added to the combined toluene aliquots before analysis.

2.3. Analyses

GC–flame ionization detection (FID) analyses were performed using a Hewlett-Packard Model 5890 (Series II) gas chromatograph equipped with a 60 m DB-5 column (J&W Scientific, Folsom, CA, USA, 0.25 mm I.D., 0.25 μm film thickness). Injections were performed in the splitless mode (300°C injection port) for 0.2 min. The oven temperature program was 80°C followed by a 6°C/min temperature ramp to 320°C followed by a 10-min hold. GC–mass spectrometry (MS) (HP Model 5973) analyses were performed in the same manner. Internal standards were *n*-undecane and 2-chloronaphthalene for GC–FID and GC–MS, respectively. Calibration curves were generated from gravimetrically-prepared solutions of PAHs. Quantitations of PAHs by GC–MS were based on the area of their molecular ion peak (versus the chloronaphthalene internal standard) as compared to the PAH standards. (When the PAH standard was not available, quantitations were based on the response of the standards having the same molecular mass as the target compound).

To reduce the possibility of bias in the GC analyses (e.g., change in detector performance from day to day), autosampler vials containing the extracts from all of the extraction methods were randomly mixed along with solvent blanks and calibration vials, and analyzed in one large batch each for GC–FID and GC–MS.

3. Results and discussion

3.1. PAH recoveries

A soil contaminated with PAHs was chosen for this comparison because (first), PAH-contaminated soils from coal processing (and other sources) are present in large numbers throughout the world, and (second) PAHs represent a group of pollutants which include a great range of vapor pressures and water solubilities (as well as solubilities in extraction solvents) as shown in Table 2. Thus, PAHs might be expected to show a wide range of extraction behavior.

The quantities of representative PAHs extracted by each method tested is shown in Table 3. With few exceptions, all of the extraction methods yield similar extraction efficiencies, and the total PAH content (based on total FID peak areas versus the *n*-undecane internal standard) determined by each extraction method are in good agreement. The slightly higher total PAH concentrations shown for the Soxhlet and PLE extractions are likely a result of co-extracted artifact peaks found in the FID chromatograms (discussed below). All extraction methods show good reproducibilities for the majority of PAHs, especially considering the complexity of the chromatograms (Fig. 1). In addition, the agreement between GC–FID quantitations (Table 3) and GC–MS quantitations (not shown) for individual PAHs was good, generally agreeing within a few percent.

The most notable differences in PAH recoveries were for the PAHs with molecular mass of 252 u and greater, especially for the SFE performed with pure CO_2, and to a lesser degree, for the 250°C water extraction. These results are consistent with earlier reports that either pure water or pure CO_2 gives lower recoveries for very high-molecular-mass PAHs [6,7,11], presumably since the solubilities of higher-molecular-mass PAHs in both supercritical CO_2 and in subcritical water are orders of magnitude lower than those of lower-molecular-mass PAHs at the same conditions [12,13]. Of course, adding an organic modifier to supercritical CO_2 would increase

Table 2
General characteristics of common PAHs

Compound	Molecular mass	Boiling point (°C)	Water solubility (mg/l)[a]
Naphthalene	128	218	32
1-Methylnaphthalene	142	245	29
2-Methylnaphthalene	142	241	25
Acenaphthylene	152	270	4
Fluorene	166	297	2
Phenanthrene	178	340	1.3
Anthracene	178	340	0.073
Fluoranthene	202	393	0.26
Pyrene	202	394	0.14
Benz[*a*]anthracene	228	438	0.014
Chrysene	228	436	0.002
Benzo[*a*]pyrene	252	496	0.0038
Benzo[*ghi*]perylene	276	500	0.00026

[a] Taken from Ref. [24].

the recoveries of the higher-molecular-mass PAHs [11], but at the expense of extracting more matrix components.

Subcritical water also yielded higher quantities of the lower-molecular-mass PAHs than the other methods. The reason for this is unclear. Several blanks (performed identical to a sample run, except with an empty cell) showed no detectable PAHs. Subcritical water extractions of residues from Soxhlet, PLE, and SFE did show a few mg/kg of the lower-molecular-mass PAHs, but not nearly enough to account for the higher values shown in Table 3. At present, we

Table 3
Mean concentrations (mg/kg) of PAHs extracted from an MGP soil by different methods

Peak	Soxhlet		PLE		SFE		250°C Water		300°C Water	
	Mean[a]	RSD (%)	Mean[a]	RSD (%)	Mean[a]	RSD (%)	Mean[a]	RSD (%)	Mean[a]	RSD (%)
(1) Naphthalene	ND[b]	15	53	6	59	4	95	4	90	9
(2) 2-Methylnaphthalene	147	12	132	6	137	4	192	3	171	9
(3) 1-Methylnaphthalene	151	11	143	6	146	3	195	4	169	9
(4) Acenaphthene	58	10	65	7	64	5	78	5	89	12
(5) Fluorene	134	10	149	6	149	3	168	5	141	9
(6) Dibenzothiophene	73	11	82	5	85	2	88	7	87	8
(7) Phenanthrene	429	11	489	5	502	2	546	5	493	8
(8) Anthracene	86	12	99	7	101	2	139	6	146	9
(9) Fluoranthene	156	14	166	4	165	3	163	5	150	9
(10) Pyrene	205	12	239	3	229	4	234	7	216	9
(11) Benz[*a*]anthracene	80	13	94	2	81	4	72	5	64	10
(12) Chrysene	89	11	106	1	89	6	77	7	74	10
(13) Benzo[*e*]pyrene	41	11	43	13	23	3	26	10	35	11
(14) Benzo[*a*]pyrene	58	12	61	14	25	7	36	10	50	11
(15) Indeno[1,2,3-*cd*]pyrene	20	15	21	10	6	12	11	20	18	12
(16) Dibenz[*a*,*h*]anthracene	5	12	5	18	2	6	3	14	5	11
(17) Benzo[*ghi*]perylene	31	10	31	18	7	7	17	12	28	11
Total PAHs	7025	10	7359	5	6407	4	6936	4	6902	9

[a] Based on the extraction of six replicate soil samples for Soxhlet, four replicates for PLE, and five replicates for the other methods.
[b] ND=Not determined because of a co-eluting interference.

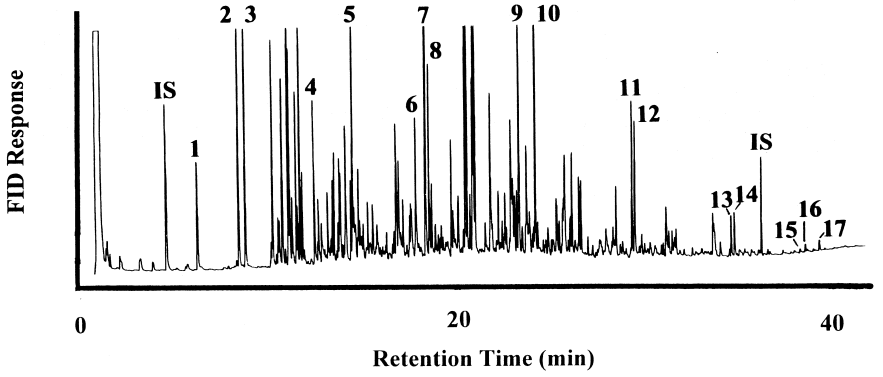

Fig. 1. GC–FID chromatogram of an MGP soil (PAH-contaminated) SFE extract. The numbers refer to PAHs identified in Table 3.

theorize that, since subcritical water causes clay particles to swell much more than other extraction methods, some of the PAH molecules which are highly sequestered in clay nanopores are available to subcritical water, but not to the other solvents which cause less clay swelling.

3.2. Selectivity: extraction of non-target matrix components

Although some differences in recoveries for higher-molecular-mass PAHs resulted from the different methods, quantitative agreement between all of the methods was generally good. However, the extract quality differed greatly among the methods tested. Differences in the extract characteristics, and the effect of the extraction method on the sample matrix are summarized in Table 4.

The organic solvent extracts (Soxhlet and PLE) were much darker and highly turbid (the color of black coffee), while the extracts from subcritical water (collected in toluene) were orange and somewhat turbid, and the extracts from SFE (collected in CH_2Cl_2) were light yellow and clear. The organic solvent extracts also yielded more artifact peaks in the GC–MS and GC–FID chromatograms, especially compared to supercritical CO_2, as shown in Fig. 2. While the PAH concentrations in this soil were high enough that class-fractionation was not necessary to remove matrix organics from the extracts prior to analysis, determination of PAHs at lower concentrations (e.g., a few ppm rather than the hundreds of ppm found for individual PAHs in this sample) would have required removal of the matrix organics extracted by the organic solvents (either Soxhlet or PLE) prior to GC analysis. It should also be noted that analysis of a large number of samples would require that both the Soxhlet and PLE extracts undergo class-fractionation because injection of a large number of such extracts will contaminate the

Table 4
Comparison of soil extract characteristics using SFE, PLE, Soxhlet and subcritical water extraction

	Extract color	Extract turbidity	Residue[a] (mg/g soil)	% Removed by extraction[b]	
				C	N
Soxhlet	Black/brown	Heavy	107±24	32	34
PLE	Brown	Moderate	15±1	22	<5
SFE (pure CO_2)	Light yellow	Clear	8±1	8	<5
Subcritical water:					
250°C	Orange	Moderate	8±1	34	79
300°C	Dark orange	Moderate	13±2	35	79

[a] Residue remaining after evaporation of the solvent.

[b] The original soil had 3.9% (w/w) carbon (after adjusting for the 0.7%, w/w, of total PAHs extracted) and 0.15% (w/w) nitrogen. All values are based on the average of two determinations.

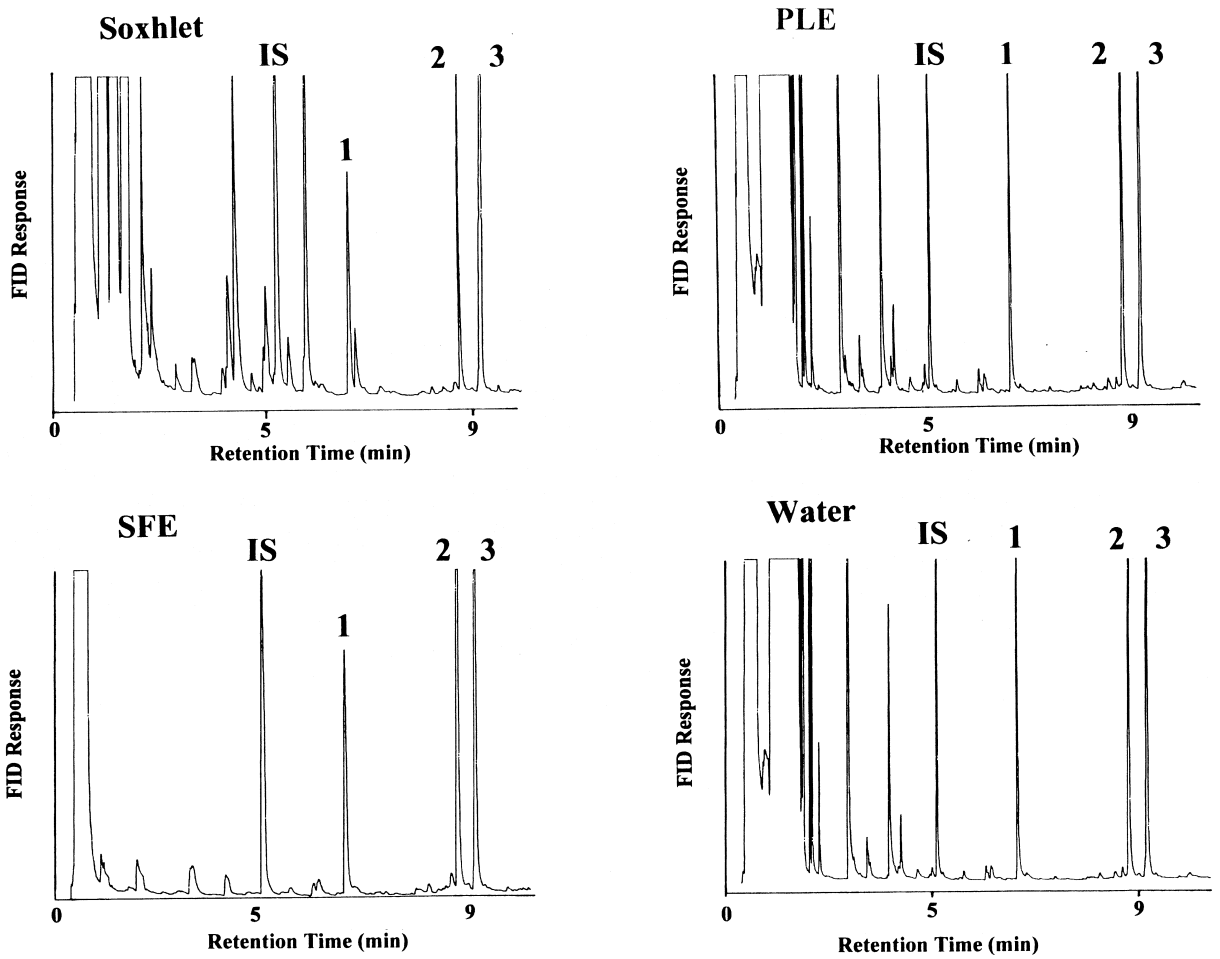

Fig. 2. GC–FID chromatograms of the early-eluting artifact and solvent peaks from Soxhlet, PLE, SFE and subcritical water extracts of an MGP (PAH-contaminated) soil. The numbers refer to PAHs identified in Table 3.

GC injection port. These extracts were analyzed using a split/splitless injection port, and the injection of unfractionated extracts from Soxhlet and PLE requires frequent replacement of the injection port liner (e.g., every 20 or so sample injections). Obviously, these extracts are unsuited for on-column injection.

In contrast, the SFE extract using pure CO_2 could be analyzed by GC–FID with no additional clean-up, simply because pure CO_2 does not extract matrix organics as easily as the organic solvents. More than a hundred splitless injections of SFE extracts from the same soil have been performed on several occasions with no noticeable discoloration of the injection port liner or degradation in chromatographic performance. As discussed below, subcritical water extracts large amounts of soil matrix organics, but the water extract is somewhat cleaned by the partitioning into the toluene collection solvent. Thus, the final PAH solution (in toluene) contains less matrix organics (at least as observed by GC–FID and GC–MS analysis) than Soxhlet or PLE extracts, but more than the pure CO_2 SFE extracts.

Quantitative differences in the extraction of non-target matrix organics were investigated by measuring the carbon and nitrogen content in the soil residues from each extraction method, (after allowing any residual solvent to evaporate overnight in a

laboratory hood) and comparing them to the carbon and nitrogen content of the original soil (adjusted for the carbon content of the PAHs which were extracted by each method). As shown in Table 4, subcritical water, PLE and Soxhlet extraction have poor selectivity for PAHs versus bulk soil organic matter, and removed ~1/4 to 1/3 of the bulk soil organic content from the soil during extraction. In contrast, SFE with pure CO_2 removed little or none of the bulk organic matrix. Both SFE and PLE removed little, if any, nitrogen, but Soxhlet and subcritical water extractions removed 34 and 80% of the soil nitrogen, respectively.

Further quantitative comparisons of the effect of each extraction method on the sample matrix and the quality of the resultant extract was performed by evaporating the organic solvents (i.e., the collection solvents for SFE and subcritical water extracts) and weighing the residue. As shown in Table 4, ~100 mg/g of the original soil mass is present in the Soxhlet extracts, while the remaining extracts had much less residue.

3.3. Selectivity: analyte compound class

Selectivities for different analyte compound classes also vary with extraction method. Organic solvent extractions generally show little or no compound class selectivity, and any required fractionation is performed after the extraction is complete. For supercritical CO_2, earlier reports have shown that some selectivity for non-polar organics over more polar organics can be achieved with CO_2 by sequentially increasing the pressure and/or temperature of the SFE step, but the degree of selectivity among semivolatile organics [e.g., alkanes, PAHs, polychlorinated biphenyls (PCBs)] is moderate at best. In essence, less polar analytes extract most easily in supercritical CO_2 because it is, of course, non-polar. In contrast, subcritical water starts as a very polar solvent (at lower temperatures) and becomes less polar as the temperature is increased, until its polarity (in terms of dielectric constant, ϵ) becomes similar to methanol or acetonitrile at ~200°C [14]. Thus, more polar analytes extract most readily in subcritical water (i.e., at lower temperatures), while less polar analytes (e.g., PAHs) require less polar water (i.e., at temperatures up to 250 or 300°C – note that pressure has little effect as long as the water remains liquid, [14]). Although it is difficult to compare solvent polarities (especially considering the chemical types of polarities possible), a feeling for the range of polarities available can be based on the dielectric constant. The dielectric constant of some common extraction solvents are hexane (ϵ=2), acetonitrile (ϵ=36), acetone (ϵ=21), methanol (ϵ=33), and methylene chloride (ϵ=9). Compared to all of these solvents except hexane, supercritical CO_2 is fairly non-polar with ϵ ranging from ~1 to 2 [15] (depending on the temperature and pressure of the CO_2). In contrast, the dielectric constant of water starts at about ϵ=80 at room temperature, and gradually lowers to ϵ=27 at 250°C [14]. Thus, subcritical water is always substantially more polar than CO_2 (and many common extraction solvents), regardless of the temperature used for extraction. (This is, of course, not true for supercritical water since the ϵ of supercritical water is ~1 at temperatures and pressures above the critical parameters, i.e., >374°C and >218 bar). Although little work on exploiting the selectivity of subcritical water has been reported, the potential seems to be greater than with supercritical CO_2 considering the wide range of polarities which can be generated using subcritical water (e.g., ϵ can be controlled anywhere from 80 to 20 by heating liquid water from ambient to 300°C), compared to supercritical CO_2 (ϵ=~1 to 2, [15]).

These selectivity concepts are shown in Fig. 3 by the Soxhlet, SFE, and subcritical water extracts of urban air particulates. Because of the large contribution of diesel exhaust particulates to this sample, the major organics extracted by all methods are *n*- and branched alkanes from about C_{18} to C_{30}, and PAHs with a similar range in vapor pressure (i.e., major PAHs range from those with molecular masses of 178 to 276 u). As expected, the Soxhlet extract shows a large amount of *n*-alkanes and a branched alkane ''hump'' in the chromatogram. Because of the large interferences from the alkanes, only a few of the PAHs can be observed in the total ion chromatogram (Fig. 3, upper left). SFE with pure CO_2 can provide some selectivity by first extracting alkanes at milder conditions followed by stronger extraction conditions for the remaining PAHs. For example, when the urban air particulate matter was first

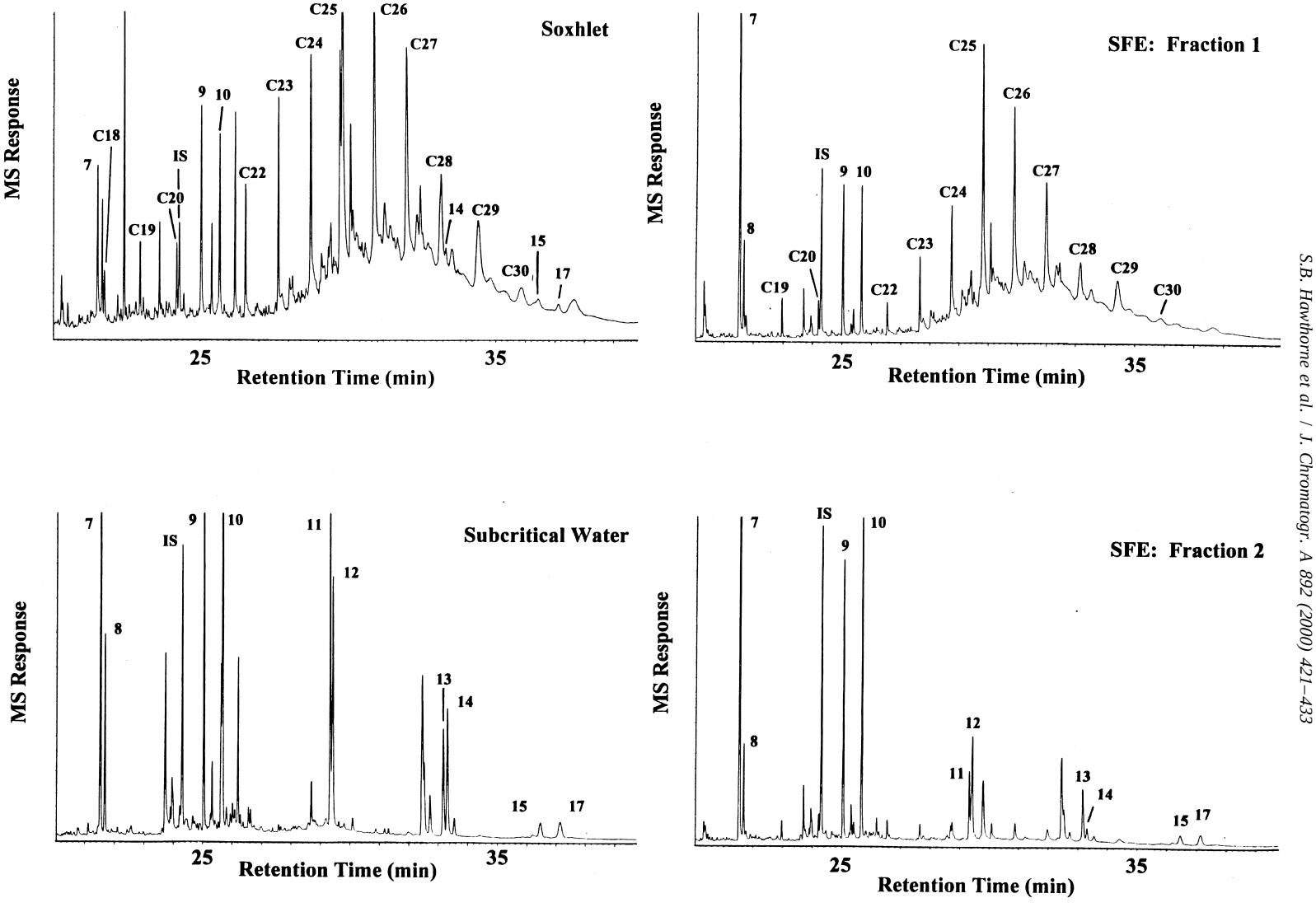

Fig. 3. Selectivity of SFE with pure CO_2 and subcritical water compared to Soxhlet extraction for alkanes and PAHs from urban air particulate matter. All chromatograms are composites of the GC–MS selected ions monitored for alkanes (m/z 57) and PAHs (the molecular ion for each PAH). I.S. denotes the internal standard. The numbers above the peaks denote the PAHs listed in Table 3. Major *n*-alkanes in the Soxhlet and first SFE extracts are designated by their chain length (e.g., C_{18} denotes *n*-octadecane).

extracted at 200 bar for 30 min (50°C), the extract contains much of the alkane content found in the Soxhlet extract, along with the lower-molecular-mass PAHs including phenanthrene, fluoranthene and pyrene (Fig. 3, upper right). When the extraction conditions are then increased to 400 bar and 150°C (for sequential extraction of the same sample), the second fraction contains an extract nearly free of alkanes, and PAHs are the major peaks in the chromatogram (Fig. 3, lower right). In fact, the 200 bar (50°C) fraction removed ~92% of the alkanes from the sample, and left only~8% of the alkane mass for extraction by the second SFE condition (400 bar, 150°C). While the ideal case for selectivity would be for none of the PAHs to be extracted in the first SFE fraction, unfortunately, PAHs are found in the first alkane fraction, especially the lower-molecular-mass PAHs. For example, nearly 60% of the phenanthrene was found in the first SFE fraction, and ~50% of the fluoranthene and pyrene. As molecular mass of the PAH increases, the selectivity of the two-step SFE procedure increases. For example, ~65% of the benz[*a*]anthracene and chrysene are found in the second ''PAH-rich'' SFE fraction (with 35% of these PAHs found in the first ''alkane-rich'' fraction), and ~80% of the higher-molecular-mass PAHs are found in the second ''PAH-rich'' fraction.

In contrast to CO_2, subcritical water extracts PAHs at the ''milder'' conditions, as might be expected since PAHs are more polar than alkanes. At least for this sample, the selectivity of water for PAHs vs. alkanes is much better than supercritical CO_2. When the urban air particulate sample is extracted with 250°C water, none of the *n*- and branched alkanes extracted by Soxhlet or found in the first ''alkane-rich'' SFE fraction are found in the subcritical water extract (Fig. 3, lower left), and the chromatogram shows a quite clean ''PAH-rich'' fraction. In fact, even if the temperature of the subcritical water is raised to 300°C, only ~13% of the alkane mass was found in the subcritical water extract as compared to the same size sample extracted by Soxhlet extraction.

The alkane vs. PAH selectivity shown in Fig. 3 are similar to those shown by Yang et al. for the extraction of a petroleum waste sludge [6]. From that sample, >90% of the phenols were extracted at 50°C, >90% of the PAHs at 100 to 250°C, and alkanes larger than ~C_{12} were largely unextracted unless the water was allowed to flash to steam [6]. Other reports on using subcritical water for analytical extractions of soils suggest that the potential selectivity of subcritical water may best be used for more polar analytes such as acid herbicides, and either proceeding or following the water extraction with supercritical CO_2 for the less polar analytes. For example, Field et al. successfully separated different ester and acid forms of dicamba by simply extracting the soil with supercritical CO_2 (for the esters) followed by subcritical water (for the acid forms) [9].

3.4. Relevance of analytical extractions to the environment

Finally, the relevance of extraction methods to environmental analyses is questioned.

Historically, analytical extractions have focussed on ''100% recoveries'' and workers have generally assumed that the method that gave the highest concentrations of a particular analyte was the ''best'' method. However, much recent work has demonstrated that many pollutant molecules become ''sequestered'' as they age for decades in the environment (i.e., more tightly bound to soil particles, and less available to organisms or transport processes) [16,17]. Therefore, it may be more important for an extraction method to only recover pollutant molecules that are environmentally-relevant, rather than the conventional attempts to extract all pollutant molecules regardless of how tightly bound they are to the soil or sediment matrix. Of the extraction methods discussed above, subcritical water and SFE have the most ability to change solvent conditions by controlling simple parameters (temperature and pressure for SFE, and primarily temperature for subcritical water), and may have the most potential for selectively extracting ''mobile'' versus ''bound'' pollutant molecules from soils and sediments. No work has been reported with subcritical water, but initial studies with supercritical CO_2 have been promising [18–22]. Weber Jr. and Young have followed sorption of phenanthrene and other PAHs to soils over time using SFE [21,22], and we have recently studied sorption and desorption of PCBs from sediments using sequentially-stronger SFE conditions [18–20]. Indeed, it is very interesting to note

that kinetic models describing the extraction of recalcitrant organics with SFE are very similar to recent models describing the transport of pollutants in the environment [16,17,23].

Recently, we have begun using selective SFE conditions to determine if the relative extraction rates of PAHs can be linked to environmental processes in a semi-quantitative manner. Subsamples of a field plot at a former MGP site undergoing bioremediation were collected over one year of treatment. The samples collected at different treatment times are being extracted for 30 min with each of four sequentially stronger SFE conditions (all pure CO_2), i.e., 120 bar at 50°C, 400 bar at 50°C, 400 bar at 100°C, and 400 bar at 150°C. Our hope is that the milder SFE conditions will extract the PAH molecules which are most susceptible to bioremediation, while the molecules which remain untreated in the field site will only extract at the more severe SFE conditions.

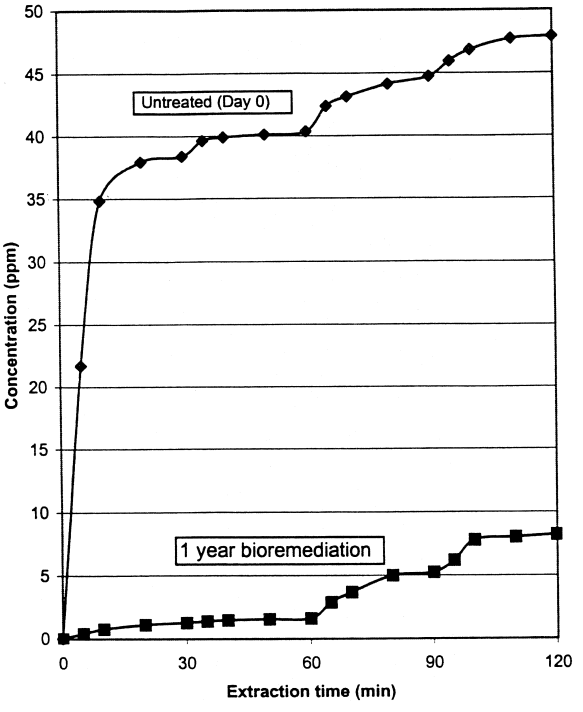

Fig. 4. Selective SFE extraction of naphthalene from a PAH-contaminated soil undergoing bioremediation for one year. Each sequentially-stronger SFE condition was used for 30 min. Thus, the most ''loosely'' bound naphthalene molecules extracted from 0 to 30 min, and the most ''tightly'' bound molecules at the strongest SFE condition from 90 to 120 min. SFE conditions are given in the text.

Initial results from these studies are shown in Fig. 4. For example, the extraction of the untreated (day 0) soil shows most of the naphthalene extracted in the ''loose'' fraction, i.e., at the mildest SFE conditions (from 0 to 30 min). Most of the remaining naphthalene molecules in this sample require the strongest two extraction conditions (from 60 to 120 min) to be removed from the soil. In contrast, the same soil (after one year of bioremediation) shows almost no naphthalene in the ''loose'' 0 to 30 min fraction, but nearly the same amounts (and extraction rates) of the naphthalene molecules located in the ''tight'' 60 to 90 and 90 to 120 min fractions. In fact, there is an almost perfect correspondence between the naphthalene molecules extracted by the mildest SFE conditions and the amount of naphthalene which was removed after one year of bioremediation. Of the 48 ppm of naphthalene present in the untreated soil, 8 mg/kg remained after one year of bioremediation (essentially the same quantity of naphthalene which was extracted only at the strongest two SFE conditions). Although this may be a fortuitous result, it is interesting to note that all PAHs which were not removed by bioremediation after 1 year were only extracted under the stronger SFE conditions. For example, higher-molecular-mass (and mutagenic) PAHs such as benzo[*a*]pyrene and indeno[1,2,3-*cd*]pyrene showed virtually no molecules extracted in the ''loose'' (0 to 30 min) SFE fraction (Fig. 5), and also showed no measurable removal after one year of bioremediation. Although these results are initial, they and the results from other initial studies clearly demonstrate the potential for developing analytical extraction methods which have more relevance to environmental fate and exposure issues.

4. Conclusions

With proper understanding of the techniques, many approaches can be used to achieve high recoveries of hydrophobic organic pollutants from soils and sediments. However, different methods have varying degrees of ability to provide extracts free from large amounts of co-extracted matrix material, and to obtain selective fractions of target

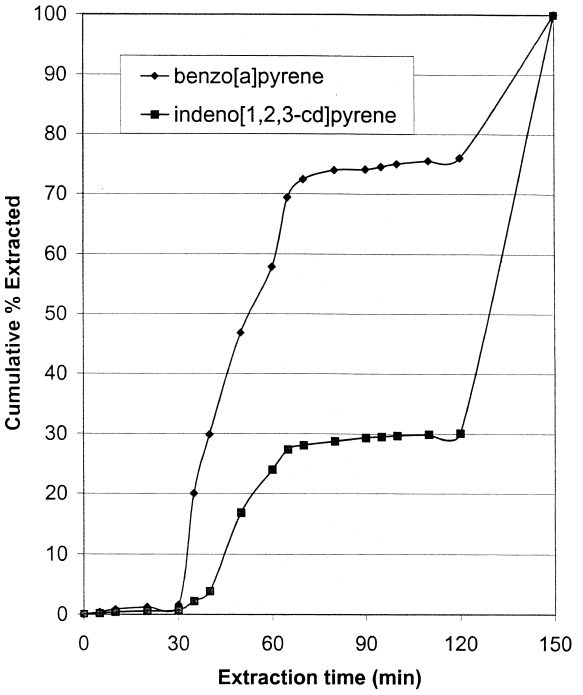

Fig. 5. Selective SFE extraction of benzo[*a*]pyrene and indeno[1,2,3-*cd*]pyrene from untreated MGP soil. Each 30-min SFE extraction was performed using stronger conditions (given in the text). The data after 120 min corresponds to the PAHs extracted by Soxhlet extraction of the soil residue after the four-step SFE sequential extraction.

analytes. At the present stage of development, the extraction methods which are most easy to understand and implement (those based on organic solvents), also yield the dirtiest extracts and have the least potential for selectivity. In contrast, the methods with the greatest potential for selectivity (either against the sample matrix, or for different analyte classes) also require the largest amount of expertise to use (e.g., SFE and subcritical water extraction). Perhaps most important, the goal of analytical extractions needs to be re-evaluated for environmental samples. The traditional goal of ''quantitative'' extraction may not be highly relevant as the weight of evidence increases that only a fraction of a certain pollutant's molecules on a soil or sediment may be available to environmental processes such as transport and biological uptake.

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