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ABSTRACT

SUPERCRITICAL FLUID EXTRACTION (SFE) AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBON (PAH) CONTAMINATED SOIL

by
Marie Renee Gargas

Methods for the extraction and analysis of polycyclic aromatic hydrocarbons (PAHs) from contaminated soil were evaluated for use in a treatability study. Candidate procedures were selected from EPA SW-846 (Third Edition) methods. Soxhlet extraction (3540B) was selected to determine initial PAH concentrations. Supercritical fluid extraction with carbon dioxide (SFE: 3561) and high performance liquid chromatography (HPLC: 8310) were evaluated for use as the extraction and analysis methods. Experimental soil was obtained from a petroleum product refinery site; a Certified Reference Material (CRM) was also studied.

Experiments focused on determining concentrations of anthracene, chrysene, fluoranthene, phenanthrene, and pyrene in untreated experimental soil; recoveries from SFE and Soxhlet extraction were compared. SFE method 3561 uses a 5%, 1/4 (v/v) methanol/water modifier; a 5% methanol modifier was also evaluated. Soxhlet extractions yielded the highest recoveries of the PAHs with the exception of fluoranthene, recovered only after SFE with the pure methanol modifier.

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APPROVAL PAGE

SUPERCRITICAL FLUID EXTRACTION (SFE) AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBON (PAH) CONTAMINATED SOIL

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CHAPTER 1

INTRODUCTION

1.1 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are molecules consisting of various numbers of rings, comprised entirely of carbon and hydrogen; some PAHs are illustrated in Figure 1. Characteristics of a particular PAH, such as its ability to adsorb to a surface or its retention in a chromatographic column, are often related to the degree of planarity of the molecules. The strength of the π-orbitals of their rings contributes to their high boiling points, ability to tightly sorb to various sample matrices, and ability to resist microbial degradation.[1,2,3]

1.2 PAHs in the Environment

PAHs are formed by both natural and anthropogenic sources; natural sources include volcanic activity and forest fires. Anthropogenic sources are generally classified as either stationary or mobile sources, such as the burning of coal at industrial facilities or automobile exhaust emissions.[4] They commonly occur in coal and petroleum derived products, but are generally formed by the incomplete combustion of fossil fuels, or through carbonization processes.[2,3,4]

PAHs can be released into the air directly, or by evaporation of surface waters or soil. Industrial and wastewater treatment plants may release them into water through waste discharges; since most PAHs are only sparingly soluble in water, they tend to settle to lake and river bottoms and can accumulate to high concentrations in sediment. PAHs in soil may be the result of atmospheric deposition or fossil fuel spills; some may be able to be

transported to ground water supplies. Microorganisms present in soil have been shown to degrade the less recalcitrant PAHs, but uptake by different type of plants, consumption of those plants by animals, and potential exposure in general is a concern.[1] Whether the PAHs were transported to a particular site, or if the site was contaminated due to an activity or spill that occurred on that site, PAH contaminated soils have been a focus of remediation efforts for some time.

1.3 Concerns with Exposure to PAHs

Their presence in soil, water, and atmospheric environments is of concern due to the known or suspected carcinogenic, teratogenic, or mutagenic potentials of many of these chemicals.[4,5] Known carcinogens or mutagens have been placed on the United States Environmental Protection Agency (USEPA) priority pollutant list, such as fluoranthene and benzo[a]pyrene.[6]

1.4 General Remediation Techniques

Depending on the level of contamination and amount of contaminated soil, different in situ or ex situ techniques can be used to remediate soil containing PAHs. Ex situ techniques, such as excavation and incineration or land disposal, may be impractical due to the location of the contaminated material. Surface material is easy to access, but subsurface layers require a knowledge of the site hydro-geology and may require sophisticated modeling techniques to predict the range and extent of contamination. Excavating and removing material may result in the spread of contamination during transport. In general, ex situ methods can also be quite expensive.

In situ techniques treat contaminated soil or groundwater on site, without removing any material. A surfactant or solvent may be pumped into the soil to solubilize the contaminants; the liquid is then treated as waste. Vapor extraction or steam stripping has been successfully used for lower molecular weight contaminants.[7]

1.5 Bacterial Bioremediation

Pure cultures of bacteria have been reported to degrade PAHs through three main mechanisms: complete mineralization, cometabolic degradation, and unspecific radical oxidation. All involve oxidation of the ring structure of the PAH molecule, either by some type of enzyme, or radicals derived from hydrogen peroxide. With complete mineralization, the PAH is used as the sole source of carbon and energy, and the end products are carbon dioxide and water. PAHs are not used for growth under cometabolic degradation; these other two processes generally result in a variety of intermediate byproducts.[5]

In several field studies, adding PAH-degrading bacteria to contaminated soil was shown to significantly enhance the rate and degree of degradation or mineralization.[8] Different species of bacteria have been shown to degrade fluoranthene, anthracene, phenanthrene, chrysene, and pyrene, among other 2-4 ring PAHs; one particular species, *Mycobacteria,* have been extensively studied for their PAH degrading abilities.[6,8,9,10]

1.6 Treatability Study - The Project at Hand

The overall aim of the project is to investigate the role of *Mycobacteria sp.* in the enhanced bioremediation of PAH contaminated soil, with a focus on five particular PAHs: anthracene, chrysene, fluoranthene, phenanthrene, and pyrene. Experimental soil for the project was obtained from a local petroleum product refinery. Amendments chosen for addition to the soil include peat moss, pine bark, and cedar mulch; it is anticipated that their benefits will include aeration of the soil, contribution to a favorable pH for bacterial growth, and surfactant-like oils which may bring the PAHs and bacteria in closer contact.

Initial concentrations of each PAH in the experimental soil needed to be determined prior to beginning the studies, since exact concentrations were not known; however, the suppliers of the soil reported it contained low levels of contamination. It is anticipated that bacteria native to the soil obtained for the study will biodegrade PAHs, especially with the addition of the various amendments. Thus, the concentrations of the PAHs will need to be monitored as the study progresses; reliable methods for extraction and analysis needed to be established.

CHAPTER 2

OBJECTIVE

The objective of this study was to evaluate methods for the extraction and analysis of anthracene, chrysene, fluoranthene, phenanthrene, and pyrene in a contaminated soil being used for a treatability study. A Certified Reference Material (CRM) would be used to compare recoveries from that material with its certified values; the concentrations of the PAHs in the experimental soil were unknown.

Different extraction techniques would be selected and evaluated based on the recoveries of the PAHs, as well as the amenability of the method to the requirements of the study. A large number of samples was anticipated to be generated, so a rapid yet reliable method was desired. Reproducibility was another important consideration; since decreased recoveries could indicate microbial degradation of the PAHs, potential instrument fluctuations or opportunities for analyst error needed to be minimized.

CHAPTER 3

LITERATURE SURVEY

3.1 Solvent Extraction Methods

A variety of methods for the extraction and analysis of PAHs from different matrices exist for both qualitative and quantitative determinations; the most common extraction methods are solvent based, such as the Soxhlet extraction and sonication. Soxhlet extraction involves the cycling of solvent through the sample matrix, whereas sonication employs the continuous mixing of solvent with the sample. Conventional solvent extraction techniques may be impractical when large numbers of samples need to be processed for a particular study; these extractions may run a few hours or as much as a day, and often involve subsequent concentration or cleanup steps which may result in the loss of analytes. A variety of solvents or mixtures can be used, depending on the matrix, analytes, and subsequent method of analysis; acetone and methylene chloride have been commonly used to extract PAHs from soil. The use of relatively volatile extraction solvents in methods using reflux, as well as lower concentration temperatures, can minimize extraction time and potential losses when thermal degradation and sublimation of the analytes are a concern. Oxidation during concentration steps can be minimized by keeping the sample under a gentle stream of nitrogen. Potential sources of contamination include the solvents, the extraction thimbles, glass wool, the glassware, and drying agents.[1]

3.2 Supercritical Fluid Extraction (SFE)

3.2.1 Defining Supercritical Fluids

A supercritical fluid is a substance above its critical temperature and pressure; it will not boil at a temperature above its critical temperature, or compress when the pressure is above its critical pressure. Hence, it is truly neither a liquid or gas, and is termed a supercritical fluid above the point at which the critical pressure and critical temperature meet, the critical point.[11] The usefulness of fluids in this region is that they have the mobility of a gas, but the solvating properties of a liquid; the density of a supercritical fluid is typically 100-1000 times that of the gas.[12]

3.2.2 Comparison to Liquid Solvent Extraction Methods

SFE offers several advantages to conventional solvent extractions in that it uses significantly less quantities of solvent, may take as little as an hour or less to perform, and usually involves fewer concentration or cleanup steps, if any at all.[2,3,13,14] Recoveries and data reproducibility have been reported as comparable or better to Soxhlet extractions or sonication in some instances, and not as well in others.[2,12] In either case, recoveries are strongly influenced by the sample matrix, analyte properties, and extraction parameters.

3.2.3 Extraction Systems and Modes of Operation

Supercritical fluid extraction systems have been designed and used with success in many laboratories, but are also commercially available. Samples must be placed in extraction vessels, such as stainless steel tubes with specially designed end caps, which are able to

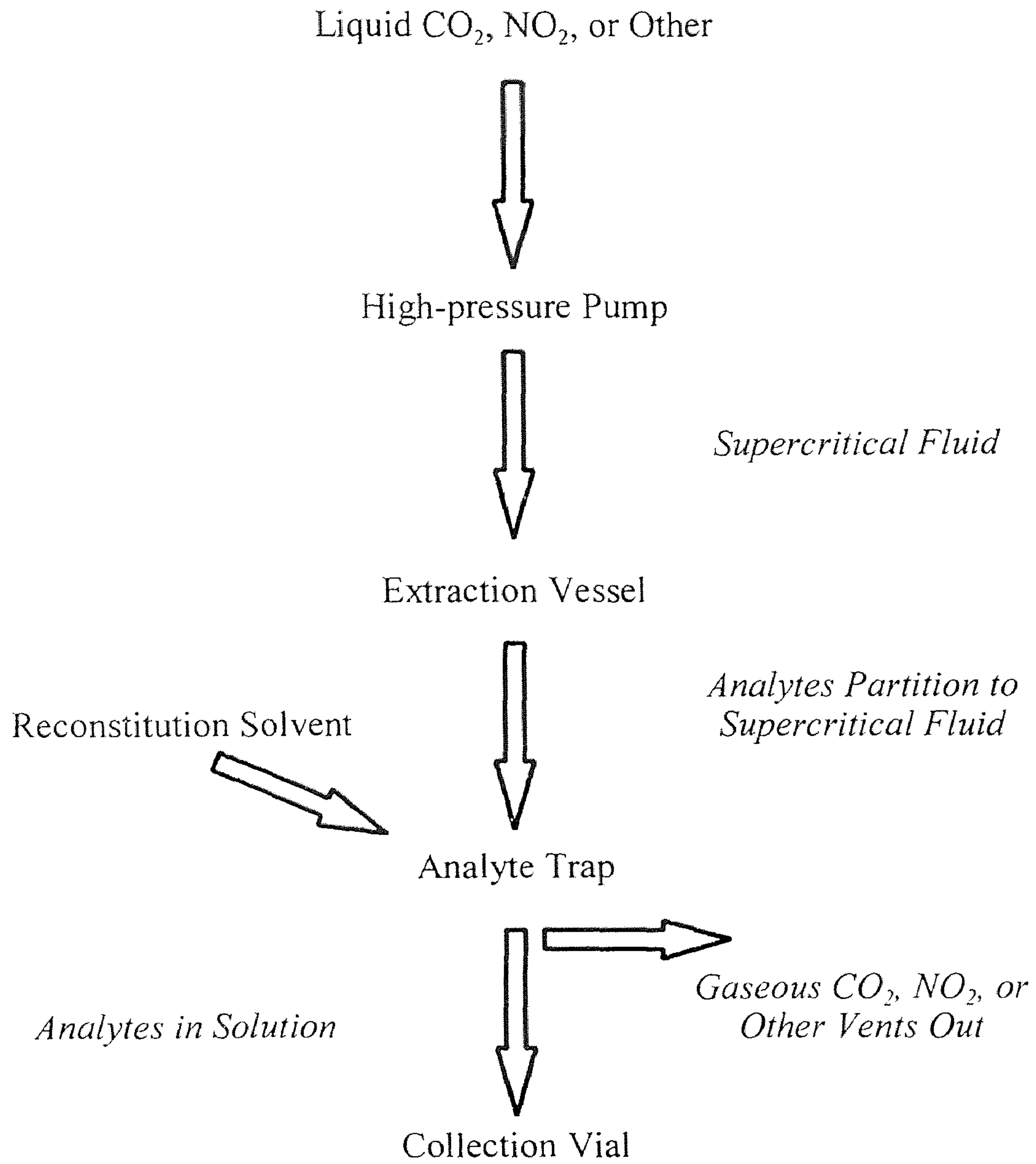


Figure 2 Supercritical Fluid Extraction Process

withstand the high pressures involved in SFE. The general SFE process is shown in Figure 2. The supercritical fluid may begin as a gas, or the liquid form of the gas aspirated from a cylinder equipped with a full length eductor tube. A vacuum pump and temperature control system are used to increase the pressure and temperature, forming the supercritical fluid.

A dynamic, or continuous, mode of extraction constantly pumps the supercritical fluid through a restrictor into the vessel in a single step; a static/equilibrium step may first be used to allow the supercritical fluid greater contact time with the sample matrix before being pumped through. The supercritical fluid is depressurized after the desired extraction time, and the gas is vented.

The method of collection of the analytes depends on whether on-line or off-line analysis of the extracts will be used. On-line methods for direct analysis require appropriate interfaces with the chosen separation method, such as gas chromatography. In off-line methods, analytes are commonly collected by depressurizing the supercritical fluid into a vial of liquid solvent. Otherwise, they may first be deposited on a trap of adsorbent material, such as octadecylated (C-18) silica; reconstitution solvent is introduced to flush the analytes out of the trap into vials. Multiple extraction steps or different reconstitution solvents can often be used to fractionate the samples. Changing the pressures and temperatures of the various system components, density and flow rate of the supercritical fluid, and the type reconstitution solvent can have significant impacts on the results of the extraction.[11,12]

3.2.4 The Extraction Process

The basic process of supercritical fluid extraction involves partitioning of the analyte from the matrix to the fluid, sweeping it out of the extraction cell, and collecting it in a form compatible with the chosen method of on-line or off-line analysis.[11] The first step has been termed by Hawthorne et al. as the "desorption/kinetic" step, which occurs when the analyte initially leaves the site to which it was bound and enters the supercritical fluid. The second step has been termed the "solubility/elution" step, and involves reversible partitioning of the analyte between the matrix and the fluid. This partially depends on the solubility of the analyte in the fluid, as well as the retention of the analytes on the active sites of the matrix. Highly contaminated soils are generally restricted by solubility/elution factors; much of the analyte is often held by weaker sites when the analyte is present in high concentrations, so these molecules are more readily desorbed. Lesser contaminated matrices generally have the analytes bound to the stronger sites, and desorption/kinetic factors are often more difficult to overcome.[15]

3.2.5 Types of Supercritical Fluids Used for Extractions of PAHs

Experiments have been conducted using ethane, nitrous oxide (N_2O), Freon-22 ($CHClF_2$), and carbon dioxide (CO_2) as supercritical fluids to extract PAHs from a variety of matrices.[3,14,16] The critical temperature, T_c, and critical pressure, P_c, of a candidate supercritical fluid must first be easily and safely achieved to be practical. The critical temperatures of CO_2 (31.1 °C), ethane (32.3 °C), and N_2O (36.5 °C), are not extremely high temperatures, which is desirable to avoid overheating certain analytes; Freon-22 has a T_c of 96 °C. The critical pressures of

CO_2 (72.8 atm), ethane (48.2 atm), N_2O (71.7 atm), and Freon-22 (49 atm) are also reasonable.[14,17] Of these, a study by Hawthorne and Miller showed ethane gave the poorest recoveries when compared to CO_2 and N_2O.[3] Freon-22 is reported to be relatively inexpensive, non-toxic, and nonflammable, yielding higher recoveries than CO_2. This has been attributed to its greater polarity; it has a dipole moment of 1.4 debye (D), whereas CO_2 has no dipole moment.[14] Despite this, concerns with its environmental impact and cost have made it unpopular. Nitrous oxide, with a dipole moment of 0.2 D, has achieved better recoveries compared to CO_2, but it presents an explosion hazard in the presence of large amounts of easily oxidizable material in the sample matrix. Because of its low critical parameters, low cost, and non-toxicity, CO_2 has been the most commonly used supercritical fluid in the extraction of many nonpolar to moderately polar analytes from a variety of sample matrices.[3,14,16]

3.2.6 Modified Supercritical Fluids

Various modifiers in different concentrations have been experimented with to increase the polarizability of both CO_2 and N_2O, aimed at increasing analyte solubility and partitioning from the matrix.[3,12,14,15] Lower alcohols, such as methanol (MeOH), are common; higher recoveries have also been reported using toluene.[11,13,14] The modifier can be introduced by spiking it directly on the sample, through the use of a pre-mixed gas, or through a dual pump system which introduces the gas and modifier separately before mixing. This method is preferred, as premixed gases may contain impurities, or separate during storage. Modifiers are commonly used for PAHs since desorption can be problematic; modifier addition usually

requires an increase in the extraction temperature to achieve comparable recoveries.[2,11] A variety of explanations for the success of modifiers have been postulated, such as the swelling of the matrix by the polar modifier or matrix-modifier interactions.[13] For example, the hydrogen in methanol preferentially bonds to active sites in some matrices, such as silanol groups, thus aiding the desorption process.[2]

The success of the modifier depends in part on the percent of the modifier added, as well as the type of sample. Recoveries of fluoranthene in urban dust, as well as recoveries of phenanthrene and pyrene in fly ash and river sediment, were all improved with additions of 5% MeOH to both CO_2 and N_2O compared to the respective pure fluids; the modified N_2O gave the best overall recoveries.[3] At this percentage, the polarity of the fluid is not affected much, and the reactions at the active sites of the matrix are more significant. Reindl and Höfler observed that the extraction fluid became too polar at 10% MeOH, typically resulting in lower PAH recoveries than at 5% MeOH for a loamy soil with a high number of active sites; 8% MeOH generally yielded the best recoveries.[2] Detection and overall chromatographic performance during analysis may also be impaired by too high a concentration of any modifier.[16]

3.2.7 Extraction Fluid Volumes and Flow Rates

The amount of extraction fluid used and the rate at which it is introduced are also important considerations. The absolute minimum volume of extraction fluid required, under conditions of unlimited solubility and extremely rapid desorption, is the void volume of the cell. For quantitative extractions, a good sweep is considered to be five times the volume of the cell.[2]

Typical flow rates range from 0.5 to 1.0 mL/min.[2,14,16,17,18,19] In many cases, regardless of analyte concentration, solubility/elution considerations tend to control the extraction at flows up to approximately 1.2 mL/min. Above this flow rate, there is sufficient supercritical fluid being swept through the extraction cell such that the rate of extraction depends more on the initial desorption of the analytes.[15]

The amounts recovered, however, have been found to be somewhat dependent on the molecular weights and concentrations of the analytes. Matrices with lower analyte concentrations are more challenged by the initial desorption step; Hawthorne et. al. found increasing the extraction flow rate from 1.3 to 1.9 mL/min (400 atm, 80 °C, CO_2) had little or no effect in these cases. However, more heavily contaminated soils, which require more focus on the solubility/elution factor, showed a slightly greater dependence on flow rate for higher molecular weight PAHs (MW = 252 or greater). Increasing the flow rate from 1.2 to 1.9 mL/min slightly improved recoveries in these cases (400 atm, 60 °C, CO_2). PAHs with moderate molecular weights (phenanthrene, anthracene; MW = 178) experienced marginal improvement, and lower molecular weight PAHs extract rather quickly with rates as low as 0.2 mL/min. Flow rates from 0.2 - 1.9 mL/min showed comparable recoveries after 30 minutes at each rate for fluoranthene and other PAHs in highly contaminated soil, approaching 100% when compared to total concentrations after multiple SFE and sonication extractions.[15]

3.2.8 Duration of Extractions

Optimum extraction times have been determined experimentally by collecting fractions of extractions and plotting the percent of the total recovery of a PAH as a function of time.[13,14,15,18] Fluoranthene recoveries from a railroad bed soil reached nearly 75% after 60 minutes of extraction (CO_2, 400 atm, 60 °C) at flow rates from 0.3 - 1.9 L/min. However, regardless of flow rate, more than 50% had been recovered in less than 10 minutes of extraction. The initial rate of extraction was rapid, but decreased after the first 10-30 minutes; this is an example of a common situation in which increasing the total extraction time is not particularly effective in enhancing recoveries of less concentrated contaminants.[15]

The dependence of the required extraction time on the specific PAH and sample matrix was observed for phenanthrene, pyrene, and chrysene (constant CO_2 density of 0.67 g/mL, 200 °C) in both a railroad bed soil and marine sediment. Phenanthrene recoveries from the marine sediment were 60% or greater after the first few minutes, but required 45 minutes to approach 100% of the total fraction extracted; recoveries from the railroad bed soil approached 100% after the first 5 minutes. In contrast, high recoveries of chrysene were more rapidly achieved in the marine sediment. The total amount of pyrene recovered was rapidly extracted from both matrices, greater than 90% after approximately 5 minutes of extraction from either sample type.[18]

3.2.9 Extraction Pressures and Temperatures

Pressure and temperature have received considerable attention in evaluating their effect on analyte recovery. Increasing the pressure just above the critical pressure results in a rapid rise in the density of the supercritical fluid, increasing its solvating power. As the temperature increases, the solvating power tends to decrease for a period until the volatility of the solute rises enough to overcome this effect; the end result is again increased solvating ability.[11] Thus, the solvating strength of the supercritical fluid is directly related to its density, which is easily manipulated by changing the extraction temperature and pressure.[3] The restrictor temperature should ideally be low enough to minimize the loss of more volatile analytes, but high enough to avoid plugging of the restrictor.[2]

Optimum temperature and pressure combinations have been reported for PAH recovery from a variety of matrices with low to high levels of contamination. Typical extractions occur at mild temperatures, around 50 °C, and high pressures of about 400 atm.[16] One study determined that regardless of pressure (between approximately 198 to 590 atm), an extraction temperature of 80 °C yielded higher PAH recoveries as compared to 100 °C or 150 °C (CO_2 at approximately 0.56 mL/min). At this "ideal" temperature, the highest recoveries for PAHs ranging from 2-6 rings were achieved at pressures between 290-390 atm. Within this pressure range, 4-ring PAHs were optimally extracted at the low end (approximately 290 atm) and 3-ring PAHs in the middle (approximately 340 atm).[2]

For the same samples of marine sediment and railroad bed soil previously discussed with respect to extraction time (Section 3.3.8; previous), higher recoveries of PAHs were achieved for both matrices at temperatures of 200 °C, compared to 40 and 120 °C.

Recoveries of phenanthrene, pyrene, and chrysene in each were at or near 100% of the total fraction recovered within 60 minutes at this higher temperature. At 120 °C, only phenanthrene showed decreased recovery in the marine sediment; all of the PAHs showed decreased recoveries in the railroad bed soil. The results were generally poorer at 40 °C.[18]

Research has also focused on determining the ideal temperatures and pressures for maximizing the solubilities of various PAHs in supercritical CO_2.[20,21] Consistent increases in mole fraction solubilities of chrysene and pyrene in one study were observed when the temperature was raised incrementally at constant pressure, and the pressure was raised incrementally at constant temperature. Solubilities were determined by a flame ionization detector (FID) method previously established by the researchers. In each case, the solubility maxima were achieved at the highest pressures (450 bar) and temperatures (473 or 523 K) examined for each PAH.[20] A similar study used HPLC analysis to determine solubilities, reported to be capable of detecting responses at lower pressures than the FID method. The solubility of chrysene was found to increase with increasing pressure to the highest condition used (251 bar) at a temperature of 308.15 K. This trend was also observed for fluoranthene at a temperature of 308.15 K, but maximum solubility was achieved at 247 bar. The greatest solubility values for fluoranthene, however, were obtained for the highest temperature and pressure combination studied, 328.15 K and 209 bar. Experimental values, in agreement with other data previously reported, predicted maximum solubilities for phenanthrene at pressures of 175 bar at 318.15 K, and 145 bar at 328.15 K.[21] Of these three PAHs, chrysene was determined to be the least soluble; phenanthrene was predicted to be the most soluble. This seems to be consistent with the theory of decreased solubility with increasing molecular

weight or size; phenanthrene is a three-ring PAH, whereas chrysene is composed of four rings. While the solubilities of the different PAHs were considered low in both studies, varying the temperature, rather than the pressure, was found to have a more profound effect in each.[20,21]

3.2.10 Single and Combined Step Extraction Methods

The experimental data presented to this point was performed in the dynamic (continuous) extraction mode; research has been conducted which compares the recoveries of various PAHs from purely dynamic extractions and static/dynamic extraction combinations. One method may not necessarily be better than the other, as factors such as sample matrix and extraction temperature will still exhibit different effects. The recoveries of phenanthrene and fluoranthene from both marine sediment and diesel soot were compared using 15/15 static/dynamic and 30 minute dynamic extraction methods. The combined static/dynamic extraction method yielded higher recoveries than the dynamic extractions at both 80 and 200 °C for the sediment samples, but the recoveries from diesel soot were higher at using the dynamic extraction method at either temperature. While a higher extraction temperature generally yielded the higher recoveries for all PAHs, it can be seen that the sample matrix clearly has an effect on what type of extraction procedure should be used to obtain the highest overall recoveries.[19]

3.2.11 General Sample Considerations - Additives and Size

Additional sample preparation, other than the use of spikes, is usually not required unless the sample has a high content of moisture or elemental sulfur. Samples are commonly dried to about 1-5% moisture; drying agents such as magnesium chloride, magnesium sulfate, or sodium sulfate may be added. Clean copper granules may be added to remove organosulfur compounds, which can clog the instrument or interfere with chromatographic analysis.[1,2] Elemental sulfur can be quantified using capillary GC with atomic emission detection.[14] Soils are mixed well for homogeneity and generally sieved to particles sizes around 2 mm in diameter or smaller.[2,13,18] Void volumes may be filled with clean sand if the cells are not filled to capacity.[2,13,15] If a lab-designed setup rather than commercial instrument is to be used, void volume effects can also be minimized by positioning the extraction vessel vertically, with the flow of the extraction fluid moving from top to bottom.[15]

The sample amount can also have an effect on the overall recoveries. A study which extracted pyrene from 0.5 and 4 gram soil samples reported higher recoveries for the smaller sample size, regardless of extraction time, in a highly contaminated sample; recoveries approached 100% for the 0.5 g sample after 30 minutes, and 60 minutes for the 4.0 g sample. A less contaminated soil in the same study showed comparable recoveries throughout the duration of the extraction.[15] While these are not atypical sample sizes, the use of commercially designed systems may limit the sample size by the volumes of the extraction vessels available.

3.2.12 Method Evaluation - Extraction Recoveries

Spiking samples with native PAHs has not been considered a reliable method of evaluating extraction efficiencies or overall recoveries.[1] Chemical and physical adsorption processes are not truly accounted for, since unlike spikes, native analytes have to overcome stronger initial kinetic barriers to desorb. Spikes generally do not interact in the same manner with the sample matrix, even if the sample has been "aged" for a period of time before analysis.[2,11,13] One study recovered 78-99% of spiked PAHs using pure CO_2, and 90-100% of spiked PAHs using methanol-modified CO_2, after aging periods of 14 hours on petroleum waste sludge.[22]

However, spikes have been used to check the sweeping of the extraction cell and collection of the analytes. A clean material that the analytes would not be expected to react with, such as sand, is spiked and extracted. Low recoveries could indicate a leak in the extraction system, or inadequate extraction volumes, temperatures, or pressures. Another useful experiment analyzed fractions of extracts collected at specific time intervals; this information can be used to create a profile showing the fraction of total analyte extracted over a period of time.[11]

Comparisons with liquid solvent extractions and sequential extractions have been more useful in determining recoveries than spikes. In the first, SFE recoveries are compared to recoveries from a separate extraction method, such as Soxhlet extraction or sonication. The SFE recovery is reported as a percentage based on the solvent extraction recoveries obtained. The second approach begins with a supercritical fluid extraction; the residual sample is then subjected to liquid extraction, and the SFE recovery is based on the sum recovery from the two extractions. If subsequent extractions and analysis reveal measurable

quantities of PAHs, the SFE method may require some adjustment, depending on whether qualitative or quantitative information is desired.[11,15]

3.2.13 Analyzing the Supercritical Fluid Extract

GC and HPLC are common techniques for analyzing the extracts; these chromatographic methods employ a variety of detectors, including ultraviolet (UV), fluorescence, and mass spectrometers (as in GC-MS). SFE is the only extraction technique which can be used on-line with these methods of analysis. Complex matrices can be difficult to analyze with GC-MS, as the similar structures of the PAHs result in fragment ions of the same molecular weight; selected ion monitoring (SIM) mode is often necessary to use in these cases. Using SFE on-line with HPLC can be problematic with the possibility of gas formation in the interface; gas bubbles in the mobile phase tend to produce irregular baselines and contribute to poor detector responses.[11] If HPLC analysis is desired, an off-line technique is the better choice.

3.3 Reverse Phase High Performance Liquid Chromatography (HPLC)

3.3.1 Chromatographic Column

In using reverse-phase HPLC separation of PAHs, octadecylated silica (C-18) columns are among the most popular for their ability to separate several pairs or clusters of closely eluting analytes. Elution is related to the size and molecular weight of the molecule; higher molecular weight and slightly nonplanar PAHs tend to be retained longer on the chromatographic column.[4] Temperature controlling the column can help stabilize mobile phase temperatures and viscosities, resulting in more consistent retention times.

3.3.2 Mobile Phase Selection

The mobile phase for the reverse-phase HPLC analysis of PAHs is generally a combination of water and an organic solvent, such as methanol or acetonitrile. Increasing the proportion of the organic solvent decreases the retention of the analyte in the column, and generally produces narrower and better-separated peaks. The strength of the mobile phase can also be increased with the addition of a more polar solvent or surfactant as a modifier. Increasing the temperature at which the separation is carried out also decreases the retention of the more compact PAHs; the less compact PAHs experience the same effect to a lesser extent.[1]

3.3.3 Detectors

Detection of PAHs analyzed by HPLC is commonly achieved using an ultraviolet (UV) detector at 254 nanometers (nm), often coupled with a fluorescence detector for greater sensitivity and selectivity.[1,2] Several PAHs fluoresce, while potentially interfering substances may not; the excitation and emission wavelengths chosen can also be selected to minimize potential interference from closely eluting PAHs which also fluoresce. Deoxygenation of the mobile phase is especially important if this type of detection is to be used, in order to avoid fluorescence quenching.[1] In either case, the spectra of a pure component dissolved in solvent can be obtained for comparison with the spectra obtained for sample peaks to aid in peak identification.

CHAPTER 4

MATERIALS AND EXPERIMENTAL METHODS

4.1 Analytical Reagents and Instruments

4.1.1 Reagents

Carbon dioxide (bone dry and SFC grades), nitrogen, and helium were obtained from Matheson Gases (East Rutherford, NJ). The following were obtained from Fisher Scientific (West Chester, PA): HPLC grade acetonitrile and tetrahydrofuran (THF); pesticide grade acetone, cyclohexane, n-hexane, methanol, methylene chloride, pentane, and 2-propanol; anhydrous sodium sulfate (12-60 mesh); and silica gel (200 mesh). Neat compounds of all PAHs were obtained from ChemService, Inc. (West Chester, PA). All water used was filtered through a MilliQ system utilizing a 0.22 μm filter. Standards were stored in a refrigerator at 4 °C in amber EPA vials with Teflon-lined rubber septa caps (Fisher Scientific).

The reagents used directly with the SFE and HPLC were vacuum-filtered with 0.45 μm filters (Millipore; type HA for water and type HV for organics). Single thickness cellulose thimbles were used for Soxhlet extractions (Whatman Scientific, England; 33 mm external diameter x 94 mm external length).

Glassware used for the Soxhlet extraction and silica gel cleanup included the following: 40 mm ID Soxhlet extractors, 500 mL round bottom flasks, 20 mm ID and 10 mm ID chromatographic columns, 10 mL and 25 mL concentrator tubes, 500 mL Kuderna-Danish evaporation flasks, and three-ball Snyder columns. All glassware was manufactured by Kontes and Pyrex.

4.1.2 Analytical Instruments and Materials

The SFE system was manufactured by Hewlett Packard (Model 7680T), as was the octadecylated silica (ODS; C-18) trap and sample thimbles (7 mL stainless steel). Vials (2 mL volume) and Teflon-silicone-Teflon septa screw caps were also obtained from Hewlett Packard. Filters for both ends of the thimbles were cored from glass fiber filter paper, 0.45 µm (Whatman Scientific, 934-AH grade). HP Chemstation software was used to program the analytical methods, sequences, and sample fraction outputs.

The HPLC system used was manufactured by Waters (Milford, MA), and consisted of the following: autosampler (Model 717+), multisolvent delivery system (System Controller Model 660E), and photodiode array detector (PDA: Model 996). Mobile phase reservoirs were sparged with helium to degas the solvents. The column (Supelco) had the following specifications: LC_PAH column, 250 mm length, 3.0 mm inner diameter, C-18 packing with a particle size of 5 µm. In addition, µBondapak™ C-18 Guardpak™ HPLC precolumn inserts were used (Waters). A water bath connected with tubing to a column jacket was used to maintain the column temperature at 17 °C. Millennium Chromatography software (v. 2.15) was used to control all aspects of the HPLC analysis with the exception of the water bath temperature.

HPLC vials consisted of an outer vial and springs (Waters), 0.25 mL conical inserts (Kimble Glass, Inc.), screw caps, and self-sealing rubber-Teflon septa (Alltech). Soxhlet extracts were filtered through 0.45 µm certified non-sterile syringe filters (13 mm; Alltech).

4.1.3 Certified Reference Material - PAH Soil/Sediment

A USEPA Certified PAH soil/sediment, CRM 104-100 (lot CR912) was used; it was determined to have a moisture content of 3.5% (Resource Technology Corp.; Laramie, WY). The reference values provided with it were reported as determined by Soxhlet extraction and GC/MS analysis (Methods 3540A and 8270A, respectively; USEPA SW846, Third Edition,).

4.1.4 Experimental Soil for Study

Experimental soil was provided by a petroleum product refining facility in New Jersey. The exact location from which our soil was obtained from the site is maintained by the company, should the ongoing study require additional quantities. The soil was stored in airtight, 20-gallon buckets, and refrigerated at 4 °C.

A brass sieve (1.19 mm mesh) and pan was used to remove rocks, glass, and other debris, as well as ensure a small particle size consistent with efficient extractions. Samples taken at different times from this soil had moisture contents of 12.5 and 10.9%.

4.2 Standards

4.2.1 Surrogate Standard

To monitor the HPLC response, 2-fluorobiphenyl spikes were added to samples prior to extraction; 1.0 mL for Soxhlet, and 150 µL for SFE. For Soxhlet extractions, 2.63 mg of 2-fluorobiphenyl was dissolved in 25 mL of acetonitrile; the final concentration was 105.2 µg/mL. For samples processed by SFE, 10.57 mg of 2-fluorobiphenyl was dissolved in 10 mL of acetonitrile; the final concentration was 1.057 mg/mL.

Table 1 Calibration Standards - Preparation and Concentrations

Component	Stock Solution mg/mL[a]	Stock in Working Solution (mL)[b]	Working Solution mg/mL[c]	Level 1 mg/L[d]	Level 2 mg/L[e]	Level 3 mg/L[f]	Level 4 mg/L[g]	Level 5 mg/L[h]	Level 6 mg/L[i]	Level 7 mg/L[j]
naphthalene	2.035	2.0	0.081	1.628	4.070	6.512	16.280	32.560	40.700	65.120
acenaphthylene	2.007	2.0	0.080	1.605	4.013	6.421	16.052	32.104	40.130	64.208
2-fluorobiphenyl	2.038	4.0	0.163	3.261	8.152	13.043	32.608	65.216	81.520	130.432
acenaphthene	1.965	2.0	0.079	1.572	3.930	6.288	15.720	31.440	39.300	62.880
fluorene	2.008	4.0	0.161	3.213	8.032	12.851	32.128	64.256	80.320	128.512
phenanthrene	1.997	3.0	0.120	2.396	5.991	9.586	23.964	47.928	59.910	95.856
anthracene	2.006	1.0	0.040	0.802	2.006	3.210	8.024	16.048	20.060	32.096
fluoranthene	2.008	1.0	0.040	0.803	2.008	3.213	8.032	16.064	20.080	32.128
pyrene	2.018	1.0	0.040	0.807	2.018	3.229	8.072	16.144	20.180	32.288
benz(a)anthracene	1.999	1.0	0.040	0.800	1.999	3.198	7.996	15.992	19.990	31.984
chrysene	0.2029	4.0	0.016	0.325	0.812	1.299	3.246	6.493	8.116	12.986
benzo(b)fluoranthene	2.001	1.0	0.040	0.800	2.001	3.202	8.004	16.008	20.010	32.016
benzo(k)fluoranthene	0.2004	5.0	0.020	0.401	1.002	1.603	4.008	8.016	10.020	16.032
benzo(a)pyrene	0.2036	3.0	0.012	0.244	0.611	0.977	2.443	4.886	6.108	9.773
dibenz(a,h)anthracene	0.1984	5.0	0.020	0.397	0.992	1.587	3.968	7.936	9.920	15.872
benzo(g,h,i)perylene	0.1998	6.0	0.024	0.480	1.199	1.918	4.795	9.590	11.988	19.181
indeno(1,2,3-cd)pyrene	0.2021	4.0	0.016	0.323	0.808	1.293	3.234	6.467	8.084	12.934

[a]Prepared by dissolving solid compounds in 100% acetonitrile (ACN); all PAHs are certified 99.9% purity or higher. [b]Combined and adjusted to a final volume of 50 mL with ACN. [c]Concentrations of each PAH combined working solution. Calibration standards prepared by diluting the following volumes of working solution in the following volumes of ACN (v/v, each in mL): [d]2/100, [e]50/100, [f]4/50, [g]10/50 [h]10/25, [i]5/10, [j]8/10.

4.2.2 Calibration Standards

Stock solutions of 16 EPA priority PAHs were prepared from neat compounds dissolved in acetonitrile: acenaphthene, acenaphthylene, anthracene, benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene, and pyrene. A working solution was made from the stock solutions, combining all 16 PAHs, and then diluted to make seven different calibration standards, Level 1 through Level 7. These concentrations were selected to demonstrate the large working range of the instrument, and are given in Table 1.

4.3 Soxhlet Extraction of PAHs in Soil

4.3.1 Soxhlet Extraction Method

Soxhlet extractions performed were based on SW-846 (Third Edition) Method 3540. For each soil extraction, approximately 10 grams of soil and 10 grams of anhydrous sodium sulfate were combined in a cellulose extraction thimble; a glass wool plug was placed on top of the sample inside each thimble. Blank extractions were performed on 10 grams of anhydrous sodium sulfate. Thimbles were carefully placed inside individual Soxhlet extractors; surrogate standards were then applied to the soil in the thimbles (1.0 mL each).

A 1:1 (v/v) acetone/hexane extraction solvent was prepared by adding equal volumes of each solvent to a flask, then swirling it gently until it was thoroughly mixed. Three boiling chips and 300 mL portions extraction solvent were added to round bottom flasks. Each Soxhlet extractor was attached to a round bottom flask, and the apparatus was placed on a

heating mantle. Water-cooled condensers were added to each Soxhlet extractor. Samples were extracted for 24 hours, with 10-15 minute cycles (4-6 cycles per hour), then allowed to cool to room temperature.

Boiling chips (2-3) were added to 10 mL concentrator tubes and attached to 500 mL Kuderna-Danish evaporation flasks. Drying columns (20 mm ID) were prepared by plugging the bottom with glass wool, adding 50 mL of freshly prepared extraction solvent, and then adding 50 grams of anhydrous sodium sulfate while tapping the column to aid settling. The solvent in each drying column was eluted into a waste beaker so that the level of the extraction solvent was just above the level of the sodium sulfate. Kuderna-Danish flasks and concentrator tubes were placed under the columns. Extracts were passed through the drying columns at a rate of approximately 2 mL/min and collected in the Kuderna-Danish apparatus. Three 50-mL portions of extraction solvent were used to rinse each round bottom flask, then added to the appropriate columns to be dried and combined with the extracts. Once the extracts and rinses were collected, a three ball Snyder column was attached to each flask; the Snyder columns were then prewet with 1-2 mL of extraction solvent.

Hot water baths were prepared by filling Erlenmeyer flasks with water and placing them on a hot plate. Each Kuderna-Danish apparatus was placed in bath with the concentrator tube nearly immersed; the temperature was adjusted so that the Snyder columns "chattered" gently, without sputtering. Samples were concentrated to a final volume of approximately 1-2 mL, then removed from the bath to cool. Snyder columns were removed first; the Kuderna-Danish flasks and lower joints were then rinsed into the concentrator tubes with adequate volumes of cyclohexane.

Extracts were concentrated under a gentle stream of nitrogen and partial immersion in a warm water bath. A solvent exchange to cyclohexane was performed by reducing the extract volume to 2 mL, then adding 2 mL of cyclohexane and evaporating the total volume to 2 mL again a total of six times. After the last addition, the final volume was brought down to 1 mL. If the silica gel cleanup was not being performed immediately, samples were stoppered and stored in a refrigerator at 4 °C.

4.3.2 Silica Gel Cleanup

A silica gel cleanup was performed on all extracts based on SW-846 Method 3630C. Silica gel (100/200 mesh) was first activated by heating it in a glass tray, covered loosely with a sheet of foil, in a 130 °C oven for a minimum of 16 hours. Sodium sulfate was precleaned by washing it with methylene chloride. A 2:3 v/v solution of methylene chloride/pentane was prepared by combining appropriate portions of the solvents in a flask.

Columns (10 mm ID) were prepared by first plugging the bottom with glass wool, then adding slurries of 10 grams of activated silica gel and methylene chloride to each; columns were tapped to aid settling. The methylene chloride was eluted into a waste beaker; all elutions were at a rate of approximately 2 mL/min. Washed anhydrous sodium sulfate (3 grams) was added to the top of each column. Each column was pre-eluted with 40 mL of pentane until the pentane level was just above the level of the sodium sulfate; the eluate was discarded as waste.

Sample extracts (in cyclohexane) were transferred to columns; 2 mL of cyclohexane were used to rinse each tube and transfer any remaining extracts to the appropriate column. Columns were eluted until the solvent level approached that of the sodium sulfate. A 25 mL portion of pentane was added to each column, eluted as before, and discarded. Concentration tubes (25 mL) were placed under each column; columns were eluted with 25 mL each of the 2:3 v/v methylene chloride/pentane solution.

Final concentration of the extracts was accomplished using a warm water bath and nitrogen, with a final solvent exchange to acetonitrile. Extracts were concentrated to approximately 2 mL, and acetonitrile was added to 4 mL; this was repeated six times, with a final volume of 1 mL for HPLC analysis. Extract were filtered through 0.45 μm cartridges into conical 0.25 mL glass inserts in the HPLC autosampler vials.

4.4 Supercritical Fluid Extraction of PAHs of Soil

4.4.1 Modifier A - 1:4 (v/v) Methanol/Water

Filters for the stainless steel extraction thimbles were prepared by coring them from filter paper; a filter was placed at one end of each thimble before capping it. Approximately 5 grams of soil were used for each extraction; samples were weighed into the open ends of the thimbles, and spiked with 150 μL portions of the surrogate standard. A filter was placed on top of the sample, and the thimbles were capped.

Bone dry carbon dioxide was used to cryogenically cool the ODS (octadecylated silica) trap; SFC grade carbon dioxide was used for the extractions. A modifier of 1:4 v/v methanol/water and reconstitution solvent of 1:1 v/v THF/acetonitrile were prepared.

The procedure used as a starting point for establishing an SFE method was SW-846 (Third Edition) Method 3561; extraction conditions were programmed into the HP Chemstation software. This method is comprised of three extraction steps; the first is aimed at extracting the more volatile analytes, the second is aimed at extracting the less volatile analytes, and the third performs a final sweep of the thimble and trap. These three steps are further subdivided into three substeps: extraction of the sample, collection of the analytes on the trap, and reconstitution of the analytes by rinsing the trap with extraction solvent. Only the first and third extraction steps are followed by trap rinses, which are deposited into vials for collection and later analysis. The extraction conditions for each step were programmed as follows:

- Step 1:

Extraction: The extraction chamber pressure was raised to and maintained at a pressure of 120 bar (1750 psi) at 80 °C. The extraction fluid composition was 100% CO_2 at a density of 0.30 g/mL and flow rate of 2.0 mL/min. Static equilibration and dynamic extraction times were set at 10 minutes each.

Collection: The trap temperature was maintained by cryogenic cooling at -5 °C to prevent the loss of volatile analytes. The temperature of the nozzle before the trap was set at 80 °C to vent off the extraction fluid to a waste exhaust.

Reconstitution: The trap temperature was raised to 60 °C, and the nozzle temperature was lowered to 45 °C. A portion of 1:1 (v/v) THF/acetonitrile reconstitution solvent was flushed through the trap at a rate of 1.0 mL/min, compensating for a void volume compensation of 1.0 mL; the final volume dispensed is 0.8 mL.

- Step 2:

Extraction: The extraction chamber pressure was raised to and maintained at a pressure of 338 bar (4900 psi) at 120 °C. The extraction fluid composition was 95:1:4 CO_2/methanol/water at a density of 0.63 g/mL; the flow rate was 4.0 mL/min. The modifier was introduced by a separate pump and mixed with the CO_2 prior to introduction to the extraction thimble. The static equilibration time was set at 10 minutes, and the dynamic extraction time at 30 minutes.

Collection: The trap and nozzle temperatures were both set to 80 °C.

Reconstitution: No reconstitution of the analytes was performed in this step; analytes were maintained on the trap.

- Step 3:

Extraction: The extraction chamber pressure and temperature were set to 338 bar (4900 psi) and 120 °C, respectively. The density of extraction fluid, 100% CO_2, was maintained at 0.63 g/mL at a flow rate of 4.0 mL/min. This step involved only 5 minutes of static equilibration time and 10 minutes of dynamic extraction time.

Collection: The trap and nozzle temperatures were set to 80 °C.

Reconstitution: The trap temperature was maintained at 80 °C, and the nozzle temperature was lowered to 45 °C. The 1:1 (v/v) THF/acetonitrile rinse solvent was pumped through the trap at a rate of 1.0 mL/min; 0.8 mL was dispensed into the same collection vial used in Step 1, resulting in a final volume of 1.6 mL.

Aliquots of the final extracts were pipetted into 0.25 mL glass inserts for the HPLC autosampler vials.

4.4.2 Modifier B - Methanol

This method used all of the exact same parameters- temperatures, pressures, flow rates, etc.- as Method A, described in the previous section (4.4.1); the only exception is that the modifier was pure methanol (MeOH). Thus, the extraction fluid composition in Step 2 of the method is 95:5 (v/v) CO_2/MeOH.

4.4.3 Fraction Determinations

Aside from varying the modifier, any further experimentation with altering other method parameters would require an evaluation of which analytes were swept from the trap in each of the two fractions collected.

To do this, single extractions each were performed using the exact temperature, pressure, flow, and density conditions in both Method A and Method B modifier experiments. The initial 0.8 mL fraction at the end of Step 1 was collected, then followed by a 1.0 mL rinse

into another vial. Any analytes present in this samples would indicate the initial rinse was not clearing the trap. Another trap rinse of 5 mL, five times the void volume, was used to remove any remaining analytes; this was collected in a waste bottle. Finally, an additional 1.0 mL rinse was collected to determine of the trap was indeed free of contamination before the second extraction step. Step 2 involved no trap rinses. After Step 3, the 0.8 mL sample fraction was collected, and followed by the same rinse procedures performed after Step 1. A 1.0 mL rinse was collected, a 5.0 mL rinse was sent to a waste bottle, and another 1.0 mL fraction was collected; again, this checked the effectiveness of the 0.8 mL trap rinses used in the method, as well as the trap condition before the next extraction.

4.5 High Performance Liquid Chromatography Analysis of Extracts

4.5.1 Establishing the Instrument Method

In using the Millennium software program to control the HPLC components, an instrument method had to be established first. Throughout the analyses, the solvent reservoirs were sparged with helium at a rate of 50 mL/min. The temperature of the water bath circulating through the column jacket was maintained at 17 °C.

Prior HPLC analysis of PAHs in our laboratory had been performed using a gradient elution; those conditions are given in Table 2. Each injection was 10 μL. The run time for each injection was 40 minutes; the 2-fluorobiphenyl and 16 PAHs chosen all elute by this point. Injections were spaced at 17 minute intervals; this was in order for the column pressure to stabilize after returning to the initial percent compositions of the mobile phase, and to ensure the column had been adequately flushed before the next sample was injected.

Table 2 HPLC Gradient Conditions - PAH Analysis

Time (min)	Flow (mL)	% THF[a]	% ACN[b]	% H_2O	Curve[c]
0	0.8	0	50	50	0
6	0.8	0	50	50	6
15	0.8	0	100	0	6
25	0.8	5	95	0	6
40	0.8	5	95	0	6
41	0.8	0	100	0	6
42	0.8	0	50	50	6
90	0.8	0	50	50	6
91	0.0	0	50	50	6

[a]Tetrahydrofuran (THF). [b]Acetonitrile (ACN). [c]A value of 6 indicates a linear change in mobile phase composition over the time interval of change.

To conserve the lamp and stop the flow when each sample set was complete, the method was programmed to shut off the lamp after 90 minutes, return the mobile phase to 50:50 v/v acetonitrile and water, and steadily decrease the flow to zero.

As none of these PAHs elute before approximately 10 minutes, a conservative 6 minutes was chosen for the time at which the instrument would begin integration of the detected peaks. Peaks were integrated from the baseline at the beginning of a peak to the baseline at the end of the peak. Scanning of the peaks was performed over the range of 215 nm to 350 nm; peaks were extracted at 254 nm. The spectrum was scanned once a minute at a resolution of 1.2 nm.

Before each sample set was run, the PDA detector lamp was turned on and allowed to warm up for a minimum of one hour. The appropriate solvent reservoirs were set to initial conditions (50:50 acetonitrile/water), the flow rate was brought to 0.8 mL/min (initial), and the solvent lines and pump heads were primed while the column pressure was monitored. The typical pressure range of the initial mobile phase was from 2900 to 3000 psi or slightly greater. Both a purge of the autosampler system and needle wash were performed prior to each analysis; these maintenance procedures are ran from the autosampler menus.

Sample sets were set up with appropriate sample names, vial numbers, method sets containing the instrument and processing methods, run times, injection volumes, etc. Once the sample set was saved, the instruments were set up through the software and the baseline was monitored. When it was steady and zeroed, the sample set was started.

In addition to the method for acquiring data from the extracts, a method for cleaning the column was used; a vial of acetonitrile was injected at the end of each sample set, and a stronger gradient with acetonitrile and THF was run. The conditions for the column cleanup method are listed in Table 3.

Table 3 HPLC Gradient Conditions - Column Cleanup

Time (min)	Flow (mL)	% THF[a]	% ACN[b]	% H_2O	Curve[c]
0	0.8	0	50	50	0
3	0.8	0	100	0	6
18	0.8	50	50	0	6
48	0.8	50	50	0	6
51	0.8	0	100	0	6
54	0.8	0	100	0	6
59	0.8	0	50	50	6
60	0.0	0	50	50	6

[a]Tetrahydrofuran (THF). [b]Acetonitrile (ACN). [c]A value of 6 indicates a linear change in mobile phase composition over the time interval of change.

4.5.2 Data Processing Method

The PDA processing options followed the instrument method in recognizing the spectrum scanned from 215-350 nm, and peaks extracted at 254 nm. Other information gathered included the peak retention time, the tentative peak identification, confirmed peak identification by library matching, the purity of the peak, and how well its spectrum corresponded with that of the library reference spectrum.

The library used for the analysis of all samples in this study was established using the same instrument conditions. Peaks which appeared in the result table without a confirmed match, but appeared at retention times characteristic of a PAH anticipated to be in that sample, were compared to the library spectra individually. If it was determined that the peak

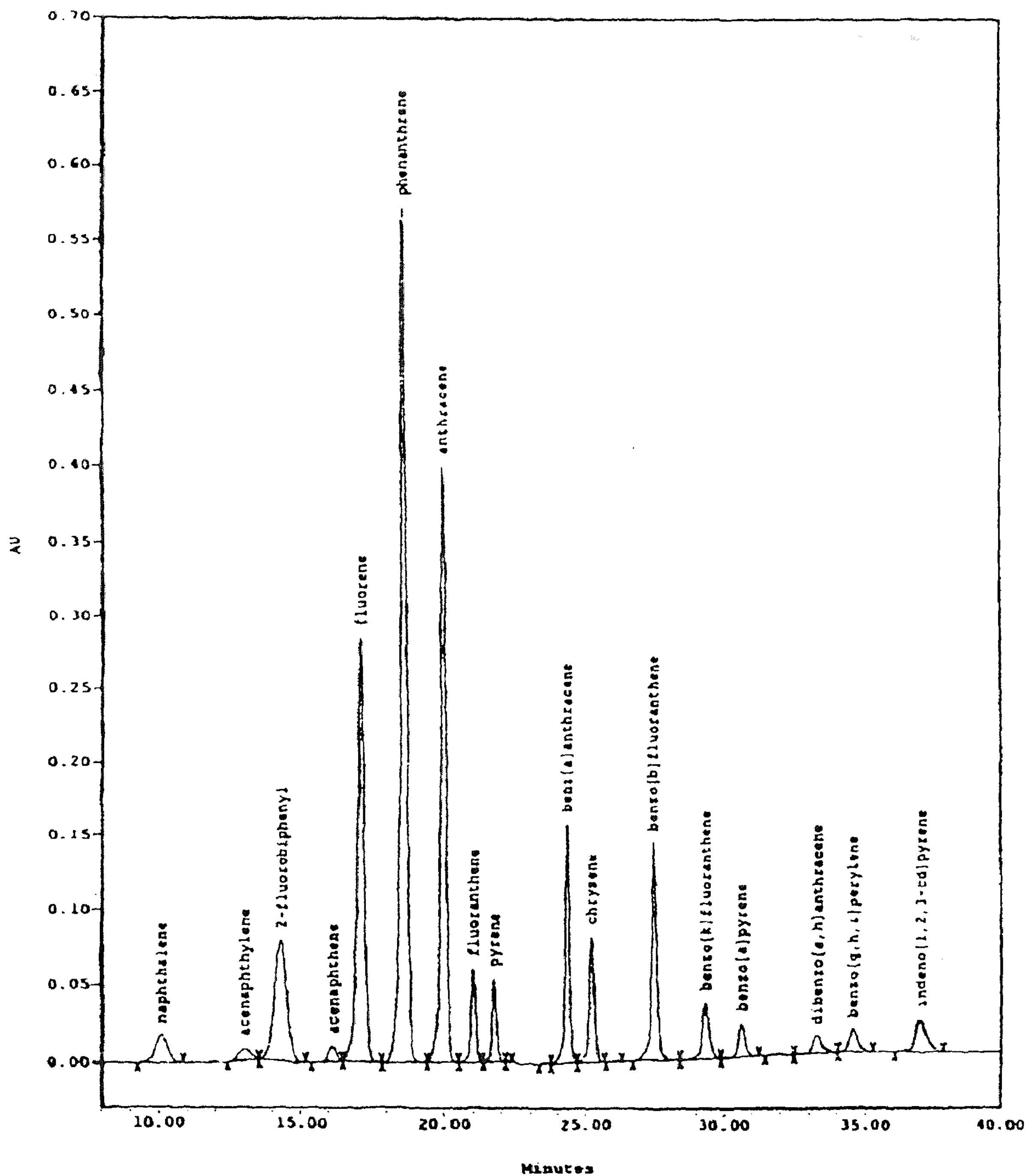


Figure 3 Chromatogram from the HPLC Analysis of a PAH Standard

shapes were close matches, it was concluded that the analytes were present. In some cases, the lack of confirmation by the software is due to extremely low concentrations, even if the peak is quite pure. This is evident when a sample peak is compared to the library peak of the anticipated PAH; the sample peak may appear as a flat line until normalized to the library peak, at which point it may be a nearly perfect match.

4.5.3 Calibration Curves

Each of the seven calibration standard concentration levels, Level 1 through Level 7, were run in quadruplicate using the gradient and processing methods described in Sections 4.5.1 and 4.5.2. The results were then processed by the Millennium software program to generate calibration curves for each PAH; the retention times of each PAH and the correlation coefficients of their calibration curves are given in Table 4. Figure 3 is a sample chromatogram of a Level 5 standard, showing the order of elution of the PAHs.

Table 4 PAH Retention Times and Correlation Coefficients from Calibration Curves

Component	Retention Time (minutes)	Correlation Coefficient (r^2)
naphthalene	10.032	0.999975
acenaphthylene	12.965	0.999929
2-fluorobiphenyl	14.265	0.999996
acenaphthene	16.115	0.999809
fluorene	17.115	0.999995
phenanthrene	18.598	0.999903
anthracene	19.982	0.999293
fluoranthene	21.015	0.999988
pyrene	21.765	0.999994
benz[a]anthracene	24.365	0.999941
chrysene	25.265	0.999992
benzo[b]fluoranthene	27.515	0.999988
benzo[k]fluoranthene	29.348	0.999971
benzo[a]pyrene	30.632	0.991511
dibenz[a,h]anthracene	33.248	0.996436
benzo[g,h,i]perylene	34.515	0.999025
indeno[1,2,3-cd]pyrene	36.932	0.999465

4.5.4 Instrument Detection Limits for the Extraction Methods

A series of ten standards at concentration Level 5 were analyzed; the mean concentration and standard deviations were calculated for each PAH. These standard deviations were then multiplied by a factor of three, and adjusted for the approximate sample weights and final volumes used for Soxhlet and supercritical fluid extractions.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Instrument Detection Limits

The instrument detection limits (IDLs) for the HPLC with a PDA detector using both Soxhlet and supercritical fluid extraction methods are given in Table 5. The final results represent the approximate minimum concentrations of the analytes that must be present in the soil for the PDA detector to recognize and detect their signals. They are considered approximate because the standard deviation was multiplied by a recommended factor of three; this is said to give a confidence level of 89% or greater.[17]

The advantage of the Soxhlet extraction here is two-fold; first, the amount of soil extracted is twice that of the SFE method, approximately 10 grams as opposed to 5 grams. Secondly, the final volume of the Soxhlet extraction is 1.0 mL, compared to 1.6 mL for the SFE. Twice as much material is being extracted, and concentrated in a smaller extract volume; this is especially helpful in being able to detect analytes present in low concentrations.

However, being able to detect a signal at these concentrations does not preclude the HPLC software from neglecting to identify a peak. Aside from the retention time of a component, the purity of the peak and the degree to which it matches the spectra of a standard peak within the software library are important considerations; each peak is generally assigned a purity angle and threshold, as well as a match angle and threshold. The thresholds are user-defined or software-derived values which account for solvent impurities and baseline noise encountered during analysis. Ideally, the angles should be lower than the threshold

Table 5 HPLC Instrument Detection Limits for Soxhlet and SFE Methods

Component	Mean Conc. (n=10)[a]	SD[b]	3SD	IDL (Soxhlet)[c]	IDL (SFE)[d]
naphthalene	32.724	0.057	0.171	0.017	0.055
acenaphthylene	30.107	0.633	1.900	0.190	0.608
acenaphthene	31.362	0.496	1.488	0.149	0.476
fluorene	64.271	0.184	0.552	0.055	0.177
phenanthrene	48.406	0.102	0.305	0.030	0.098
anthracene	16.609	0.149	0.448	0.045	0.143
fluoranthene	16.133	0.040	0.121	0.012	0.039
pyrene	16.056	0.052	0.156	0.016	0.050
benz[a]anthracene	16.061	0.049	0.147	0.015	0.047
chrysene	6.522	0.012	0.037	0.004	0.012
benzo[b]fluoranthene	16.022	0.043	0.129	0.013	0.041
benzo[k]fluoranthene	8.073	0.059	0.178	0.018	0.057
benzo[a]pyrene	5.614	0.263	0.789	0.079	0.252
dibenz[a,h]anthracene	8.063	0.137	0.410	0.041	0.131
benzo[g,h,i]perylene	9.602	0.187	0.560	0.056	0.179
indeno[1,2,3-cd]pyrene	6.347	0.082	0.247	0.025	0.079

All values given are in mg of the component per kg of dry soil (ppm). [a]Mean concentration of each PAH determined by averaging values obtained for ten injections. [b]Standard deviation. [c]IDL for Soxhlet extractions; based on 10 g samples, 1.0 mL final volume. [d]IDL for SFE, based on 5 g samples, 1.6 mL final volume.

values. The lower the purity angle is with respect to the purity threshold, the greater the peak purity; the lower the match angle is with respect to the match threshold, the better the match of the sample peak to a library peak. An interfering species may have the same retention time of an analyte of interest, but it may have a very different absorption spectra; in such a

case, the library match will decrease in certainty. This uncertainty is measured as the match angle; when it significantly exceeds the match threshold, the peak will not be labeled as the analyte of interest even if it is present. The same holds true for the purity angle and threshold; if the amount of an interfering species is large enough to cause the purity angle to exceed the purity threshold, the peak is considered too impure to justify naming it a single compound.

For these reasons, it is important to remember that a PAH of interest may indeed be present in the sample in a quantity detectable by the instrument, but background contamination or interferences may make qualification or quantitation difficult. In the event a peak elutes at a retention time characteristic of a PAH expected to be present in the sample, it is possible to extract the chromatogram at a particular wavelength (254 nm here, for PAHs), and compare the PDA spectra of that peak to those stored in the library. The peak shapes and characteristic maxima can be looked at to determine if they are indeed a match; this requires the analysts best judgement of what should be accepted or rejected.

5.2 Soxhlet Extractions

5.2.1 Certified Reference Material

The reference values and prediction intervals for the reference soil are given in Table 6; the results obtained for each of four Soxhlet extractions of this soil are given in Table 7, along with the mean concentrations determined, standards deviations, and percent recoveries based on the reference values provided. While the value of 1.67 ppm for anthracene in Sample 4 is within the prediction interval of 0.08-2.80, this value failed a Q-test with 90% confidence ($0.98 > 0.76$ for Q_{90}, $n=4$), so it was omitted from the mean calculation.

Table 6 Certified Reference Material - Reference Values and Prediction Intervals

Component	Reference Values[a]	Prediction Intervals[b]
phenanthrene	5.79	2.11-9.48
anthracene	1.44	0.08-2.80
fluoranthene	24.60	4.53-44.6
pyrene	15.00	0.0-30.7
chrysene	8.60	3.39-13.8

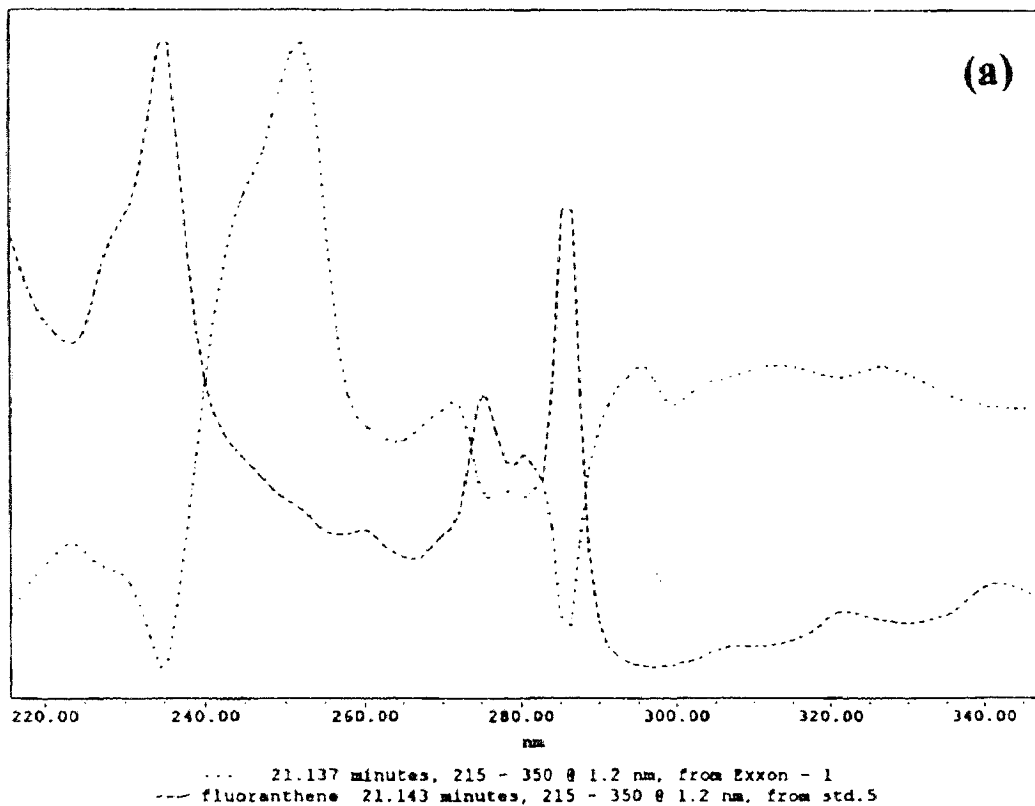
[a]Expressed in mg/kg (ppm) on a dry weight basis, as determined by Soxhlet extraction and GC/MS (USEPA SW846, 3rd edition, Methods 3540 and 8270A, respectively).
[b]Prediction Intervals are the 95% P.I. around the reported reference values.

Table 7 PAH Concentrations Determined for the CRM; Soxhlet Extraction

Component	S 1	S 2	S 3	S 4[a]	Mean	SD	% CV[b]
phenanthrene	5.28	4.29	5.36	5.56	5.12	0.49	88.4
anthracene	0.13	0.10	0.13	1.67	0.12	0.67	8.4
fluoranthene	33.25	29.08	34.36	30.64	31.83	2.09	129.4
pyrene	9.41	8.30	9.72	9.59	9.26	0.56	61.7
chrysene	8.85	8.07	9.22	8.20	8.59	0.47	99.8

All values, except percentages, are given are in mg/kg of the component in dry soil (ppm). [a]The value for anthracene in this sample was not included in the mean (Q-test). [b]Percent recoveries based on the Certified Values, Table 6.

In comparing these results to the certified values in Table 6; all of the values obtained for each of the PAHs in all four samples falls within the range of predicted concentration intervals. The recovery of anthracene at a mere 8% is of some concern; the Soxhlet extraction and cleanup procedure may severely underestimate the actual concentration in the experimental soil if this value truly reflects the recovery of anthracene from the CRM.



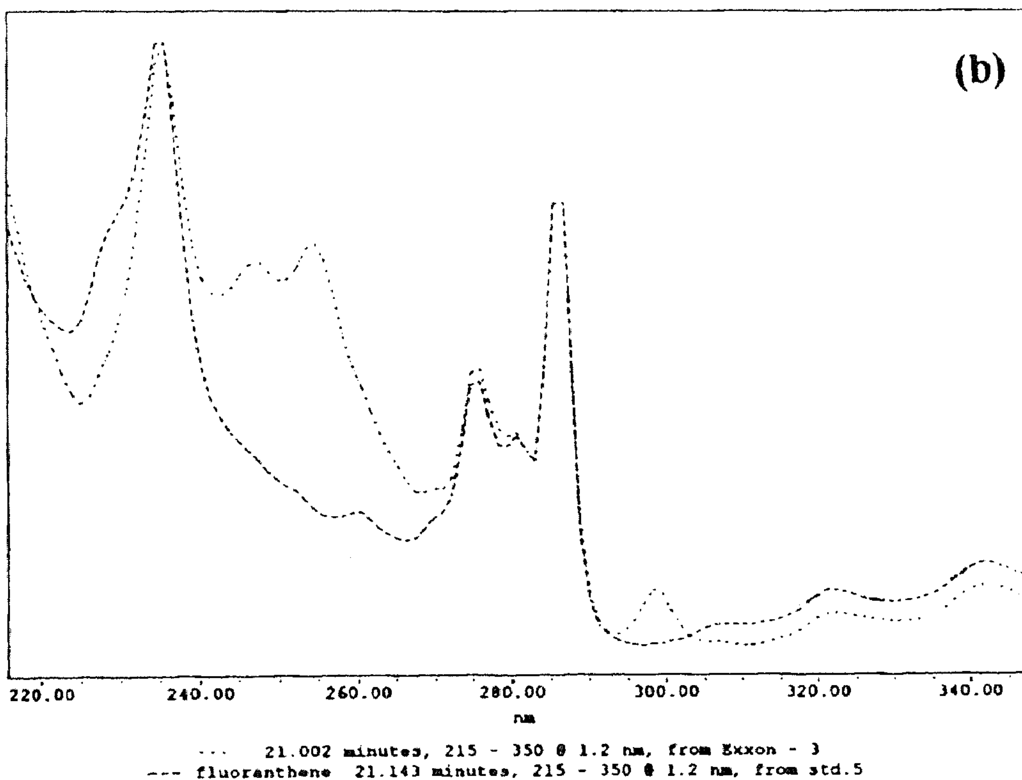


Figure 4 Sample and Library Spectra of Fluoranthene for Soxhlet Extracted Experimental Soil
(a) No match; absence of fluoranthene (b) Match; presence of fluoranthene

5.2.2 Experimental Soil

Three Soxhlet extractions were performed on the experimental soil; the results, mean recoveries, and standard deviations are given in Table 8. In all samples, the results are above the IDLs for the respective PAHs.

Table 8 PAH Concentrations Determined for the Experimental Soil; Soxhlet Extraction

Component	S 1	S 2	S 3	Mean	SD
phenanthrene	1.31	1.23	1.51	1.35	0.12
anthracene	0.12	0.13	0.18	0.14	0.02
fluoranthene	ND	ND	4.65	----	----
pyrene	0.16	0.21	0.29	0.22	0.05
chrysene	1.33	1.26	1.43	1.34	0.07

All values given are in mg of the component per kg of dry soil (ppm). ND = No detection.

While the recoveries for Sample 3 are generally higher overall, no explanation could be found for the significant recovery of fluoranthene from this sample, compared to no recoveries for either of the other samples. Samples 1 and 2 did not have any peaks matching the spectrum of fluoranthene. Figure 4a shows a peak closes to the retention time of fluoranthene for Sample 1; the spectra clearly do not match. This was also representative of Sample 2. The identity of the peak for Sample 3 was confirmed by comparison with the library spectrum of fluoranthene, as seen in Figure 4b. However, the peak purity was well above the threshold; the actual concentration of fluoranthene would be expected to be lower.

In referring to the Soxhlet extraction of the CRM, it appears fluoranthene is recoverable using the Soxhlet extraction and silica gel cleanup procedure. This is also suggested by the spectra in Figures 4a and 4b; however, it is always possible that Sample 3 was somehow contaminated with fluoranthene after the extraction. It should be noted that the blank sample showed no contamination. If the recovery of anthracene from the experimental soil was similar to that of the certified reference material, the actual concentration of anthracene in the experimental soil is likely to be significantly higher.

At this point, the baseline concentrations of the PAHs in our experimental soil based on these Soxhlet extractions are 1.35 ppm for phenanthrene, 0.14 ppm for anthracene, 0.22 ppm for pyrene, and 1.34 ppm for chrysene; the concentration of fluoranthene can not be determined with confidence at this point, based on a single recovery.

5.3 Supercritical Fluid Extraction with Modifier A

5.3.1 Certified Reference Material

Individual sample and mean concentrations, standard deviations, and percent recoveries for four extractions, based on both the reference values and Soxhlet recoveries reported in Section 5.2.1, are listed in Table 9.

The recovery of fluoranthene was higher, and that of anthracene was markedly improved from 8.4% to 26.5% of the certified values. Phenanthrene, pyrene, and chrysene had comparable recoveries using the certified values, differing by no more than 6%; of these, SFE obtained higher recoveries for pyrene. Much more anthracene was recovered here than by the Soxhlet, suggesting possible losses using the Soxhlet extraction and silica gel cleanup.

Table 9 PAH Concentrations Determined for the CRM; SFE, Modifier A

Component	S 1	S 2	S 3	S 4	Mean	SD	% CV[a]	% Sox.[b]
phenanthrene	4.96	5.03	5.01	4.80	4.95	0.09	85.5	96.7
anthracene	0.39	0.40	0.39	0.35	0.38	0.02	26.5	315.7
fluoranthene	35.19	36.47	35.24	34.79	35.42	0.63	144.0	111.3
pyrene	9.92	10.25	9.48	9.66	9.83	0.29	65.5	106.2
chrysene	7.99	8.40	7.92	8.03	8.08	0.19	94.0	94.2

All values given are in mg of the component per kg of dry soil (ppm). [a]Based on the Certified Values. [b]Based on the Soxhlet extractions performed in this study.

methods. All recoveries based on the results of the Soxhlet extraction were 94% or greater; based on these findings, this SFE method remained a candidate for use in the project.

5.3.2 Experimental Soil

Six supercritical fluid extractions were performed on the experimental soil, along with one instrument blank. Additional trap rinses were collected after each extraction. The original samples were then re-extracted to determine the success of the first extraction in removing PAHs from the soil, at least to levels below the HPLC detection limits. Because the high pressures of the SFE method pack the soil towards the exit end of the extraction thimbles, and the modifier moistens the sample, it was necessary to reload each thimble after the samples were removed and dried before the second extraction. Minimal loss of some soil occurred during the transfers between thimbles; aluminum pans were used for drying the samples in a desiccator. In the event the original filters in each thimble retained some of the PAHs, these were included in the thimbles, along with new filters at each end, for the second extraction.

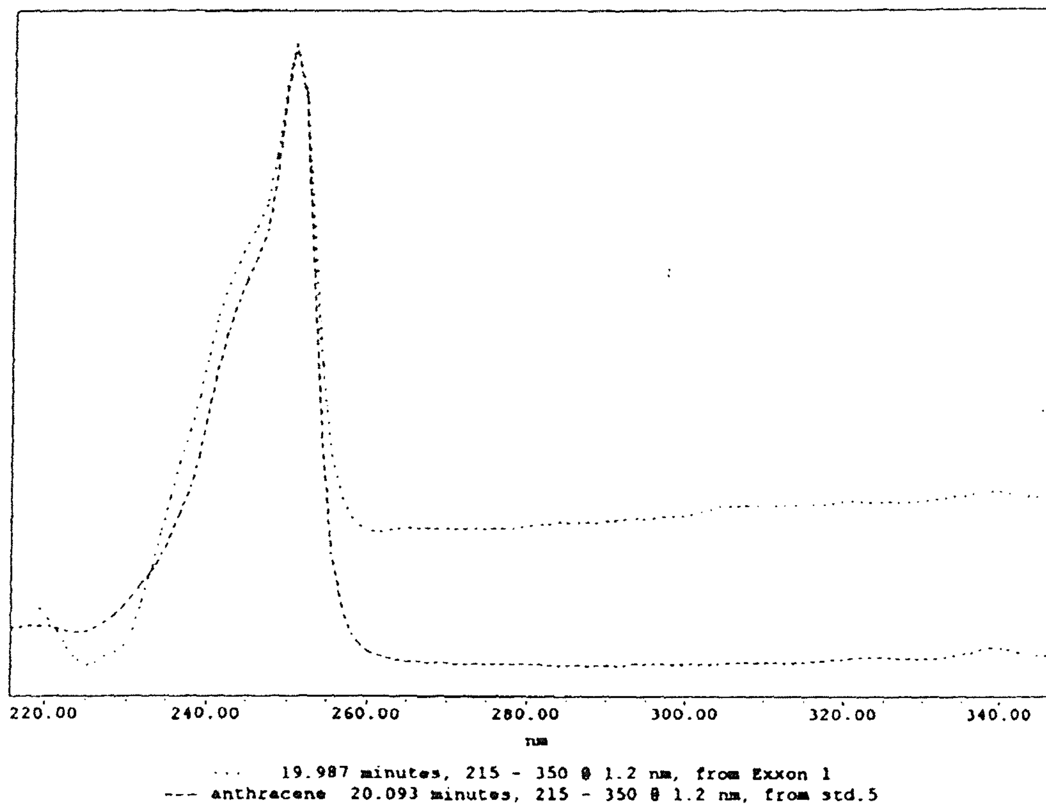


Figure 5 Sample and Library Spectra of Anthracene from Experimental Soil; SFE, Modifier A

The initial extractions of the soil yielded the recoveries given in Table 10, along with their mean, standard deviation, and recovery based on the Soxhlet results. Though the recoveries listed for anthracene are below the IDL of 0.143 for this method, a review of the spectra of each peak tentatively identified as anthracene confirmed its presence. The amounts given were calculated from peak areas, and are given as estimated values only. Figure 5 is a representative spectrum comparison of a sample peak and library peak for anthracene; the sample peak is normalized to the library peak for comparison. The recovery here compared to the Soxhlet recovery is high at 146.9%, but the purity of the peaks in the analysis of the SFE extracts were consistently above the threshold. Only Sample 3 showed any recovery of pyrene; though the calculated value is well below the IDL of 0.05, again, spectrum matching confirmed the peak identity as seen in Figure 6. Fluoranthene was not recovered in any measurable quantity. The recovery for phenanthrene and chrysene in the initial extracts were determined to be 53.3% and 42.5%, respectively, based on the Soxhlet recoveries.

Table 10 PAH Concentrations Determined for the Experimental Soil Samples; SFE, Modifier A

Component[a]	S 1	S 2	S 3	S 4	S 5	S 6	Mean	SD	% Soxhlet[b]
phenanthrene	0.73	0.63	0.75	0.65	0.79	0.77	0.72	0.06	53.3
anthracene	0.02	0.02	0.02	0.02	0.02	0.03	0.02	<0.00	----
fluoranthene	ND	ND	ND	ND	ND	ND	----	----	----
pyrene	ND	ND	<0.00	ND	ND	ND	----	----	----
chrysene	0.57	0.55	0.58	0.55	0.58	0.58	0.57	0.01	42.5

All values given are in mg of the component per kg of dry soil (ppm). ND = No detection. [a]Values for anthracene given as estimates only. [b]Based on the Soxhlet extractions performed in this study.

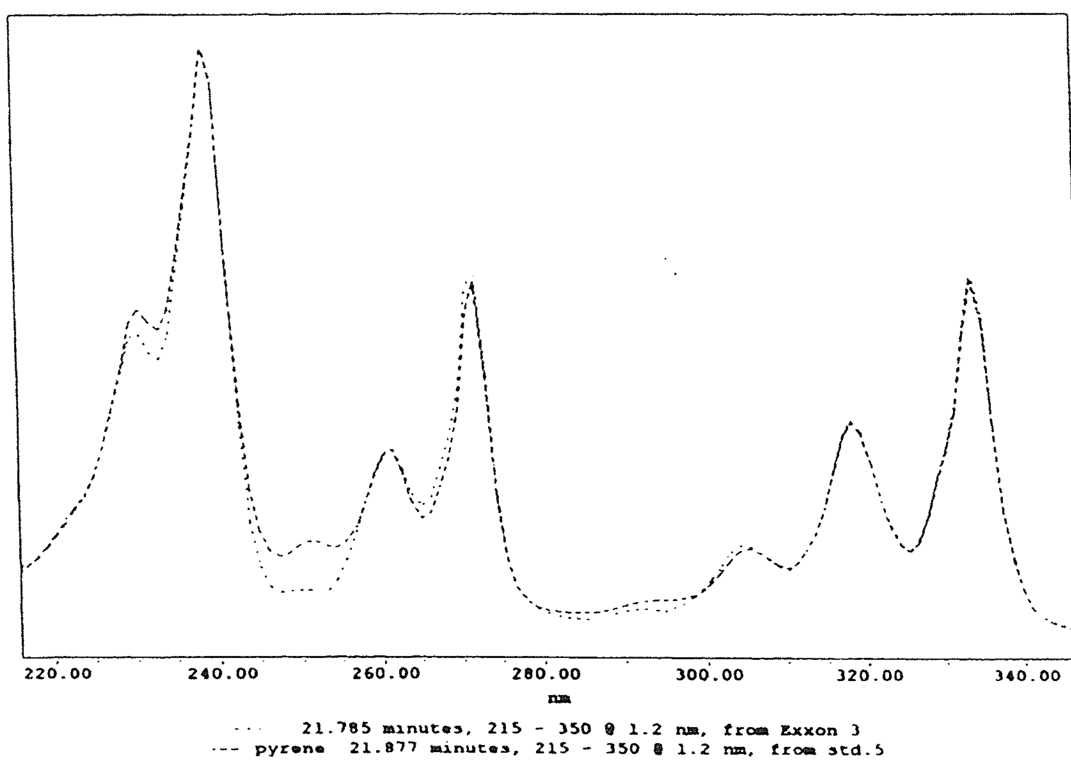


Figure 6 Sample and Library Spectra of Pyrene from Experimental Soil; SFE, Modifier A

The results of the additional trap rinses after each extraction are in Table 11; with the exception of the rinse between the first and second samples, only trace quantities of phenanthrene and chrysene were recovered from other post-sample rinses. The significant recoveries of fluoranthene, pyrene, and chrysene from the first post-extraction rinse are unaccounted for. Though fluoranthene and pyrene were not recovered from the original sample, one possible explanation for a recovery in this instance would be the inadequacy of the trap rinse provided for in the method. These PAHs may have been extracted from the soil, but left behind on the trap. However, it is believed that contamination of the sample occurred outside the SFE system, since no other post-extraction rinse showed such significant recoveries.

Table 11 PAH Concentrations Determined for the Experimental Soil Trap Rinses; SFE, Modifier A

Component	S 1	S 2	S 3	S 4	S 5	S 6	Mean
phenanthrene	Trace	Trace	Trace	Trace	Trace	Trace	----
anthracene	ND	ND	ND	ND	ND	ND	----
fluoranthene	0.259	ND	ND	ND	ND	ND	----
pyrene	0.256	ND	ND	ND	ND	ND	----
chrysene	0.269	ND	Trace	ND	ND	Trace	----

All values given are in mg of the component per kg of dry soil (ppm), based on the sizes of the samples preceding the rinses. ND = No detection.

The recoveries of PAHs from second extractions of the original samples are given in Table 12; Sample 1 was unable to be analyzed due to loss of the sample in the preparation process. Trace and measurable quantities were recovered for phenanthrene, but chrysene was recovered from every sample. From these results, it can be seen that certain PAHs such as fluoranthene and chrysene may be either difficult to initially extract from the soil, sweep from the extraction thimble, or adequately rinse from the trap.

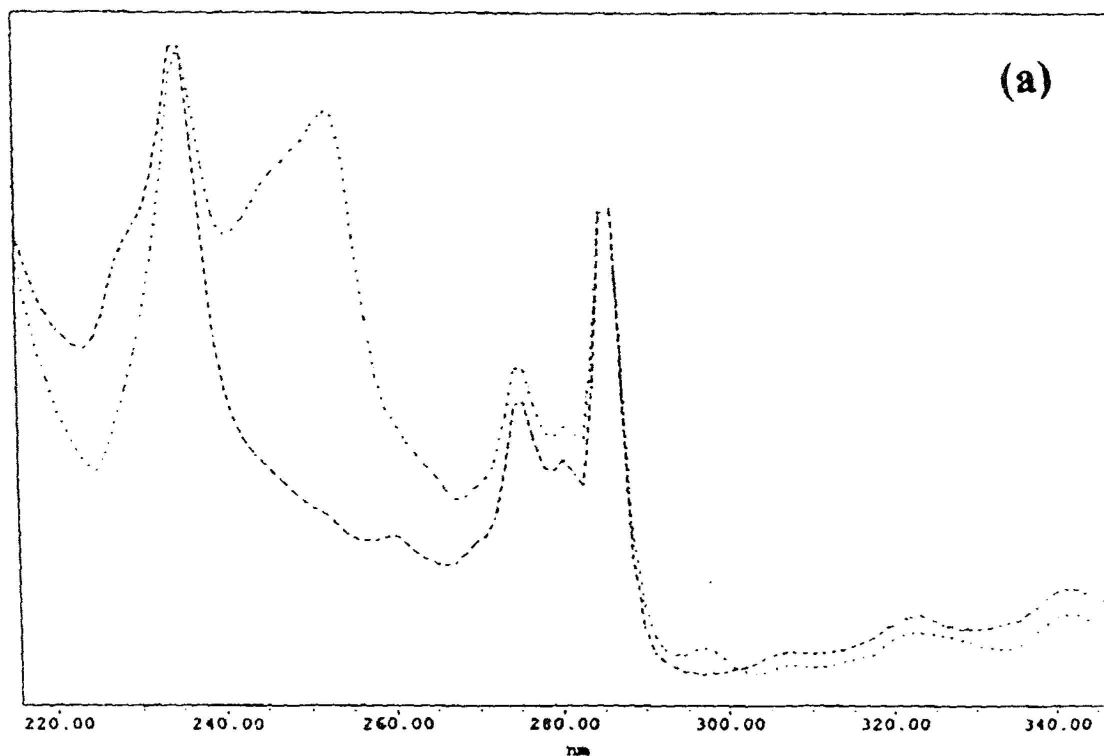
Table 12 PAH Concentrations Determined for the Experimental Soil Repeat Extractions; SFE, Modifier A

Component	S 1	S 2	S 3	S 4	S 5	S 6	Mean	SD
phenanthrene	----	Trace	Trace	0.009	Trace	0.055	----	----
anthracene	----	Trace	Trace	Trace	Trace	Trace	----	----
fluoranthene	----	ND	ND	ND	ND	ND	----	----
pyrene	----	ND	ND	ND	ND	ND	----	----
chrysene	----	0.039	0.102	0.100	0.014	0.121	0.075	0.040

All values given are in mg of the component per kg of dry soil (ppm), based on the original sample sizes.
ND = No detection.

5.3.3 Fraction Determinations

Experimental parameters can be varied to help improve recoveries, but as multiple extraction steps are used in this method, the step requiring adjustment needs to be identified first. Table 13 summarizes a qualitative analysis of a single trial sample, as described in Section 4.4.3. Small amounts of phenanthrene and anthracene were recovered from the first fraction of a soil sample extracted using this method; again, the first extraction step is aimed at recovering the



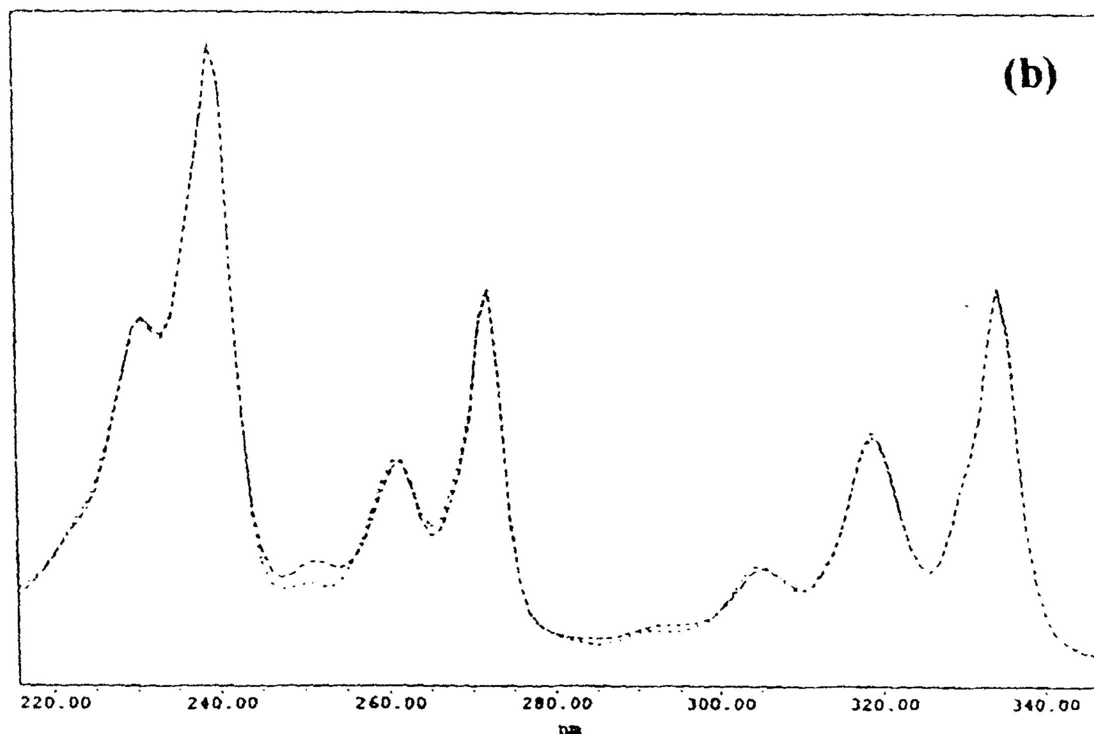


Figure 7 Sample and Library Spectra of Fluoranthene and Pyrene from Experimental Soil; SFE, Modifier A, Fraction 2
(a) Match; presence of fluoranthene (b) Match; presence of pyrene

Table 13 PAH Fraction Determinations for the Experimental Soil; SFE, Modifier A

Component	Fraction 1	Fraction 2
phenanthrene	Trace	Yes
anthracene	Trace	Trace
fluoranthene	ND	Trace
pyrene	ND	Trace
chrysene	Trace	Yes
2-fluorobiphenyl	Yes	Trace

ND = No detection.

more volatile PAHs. Trace quantities of chrysene were also recovered, as determined by spectrum matching. Pyrene and fluoranthene were not recovered. The 2-fluorobiphenyl surrogate standard for HPLC analysis was also collected in this fraction. The fraction collected before the second extraction step proceeded was free of detectable contamination. Aside from extracting the 2-fluorobiphenyl and perhaps initially aiding the desorption of the PAHs from the soil, the first extraction step does not seem to contribute much to the overall method.

The second fraction contained trace amounts of the surrogate standard, anthracene, fluoranthene, and pyrene. Of these, fluoranthene had not been recovered from these soil samples using SFE; pyrene had only been recovered from one sample in trace quantities. However, Figure 7 shows the spectra of the peaks identified as these components match very well with the spectra of the known PAHs; they are certainly extractable with this method, though the recoveries need improvement. Phenanthrene and chrysene were both recovered

in this fraction, and in greater quantities than the first. The recoveries of some PAHs in both fractions suggests a variety of possible explanations. It is possible they do not completely desorb from the sample matrix in the duration of the first extraction step. If they do, the volume of extraction fluid used in the first step may not be enough to sweep them all out of the extraction vessel and to the trap. In either case, this single sample experiment suggests the two main extraction steps in this method are not suitable for use in fractioning the sample. The extraction steps would need to be modified to either selectively extract PAHs from the soil, or selectively desorb them from the SFE trap. To obtain more complete recoveries of all extracted analytes, especially those present in low concentrations, the fractions should be combined; if the extracts are treated additively, small quantities in one fraction or another can be accounted for.

5.4 Supercritical Fluid Extraction with Modifier B

5.4.1 Certified Reference Material

A greater number of samples would have been preferred in this experiment, but some interesting observations can be made from when the pure methanol modifier was used. Table 14 presents the values obtained, their mean values, the standard deviations, and the percent recoveries based on the certified values and Soxhlet recoveries.

In comparing the results of this method to those obtained by Soxhlet extraction, phenanthrene and chrysene recoveries are similar; the Soxhlet recoveries are higher by 1.1% and 4.4%, respectively, basing the recoveries for each extraction method on the certified values. The recovery of pyrene is lower at 46.0%, compared to the Soxhlet recovery of

Table 14 PAH Concentrations Determined for the CRM; SFE, Modifier B

Component	S 1	S 2	Mean	SD	% CV[a]	% Soxhlet[b]
phenanthrene	5.051	5.053	5.052	<0.00	87.3	98.7
anthracene	1.297	1.323	1.310	0.01	91.0	1082.6
fluoranthene	2.553	2.608	2 .581	0.03	10.5	8.1
pyrene	6.847	6.945	6.896	0.05	46.0	74.5
chrysene	8.195	8.212	8.204	0.01	95.4	95.6

All values given are in mg of the component per kg of dry soil (ppm). [a]Based on the Certified Values. [b]Based on the Soxhlet extractions performed in this study.

61.7%. Anthracene was recovered in much greater quantities; the percent recovery based on the reference values at 91.0% indicates the 5% MeOH extraction modifier was superior to both Soxhlet extraction, and SFE with the original modifier. Whereas good recoveries of fluoranthene were obtained for the CRM using Soxhlet extraction or SFE with the original modifier, these results indicate using the 5% MeOH modifier may not be suitable for recovering fluoranthene.

5.4.2 Experimental Soil

Three supercritical fluid extractions were performed on the experimental soil, along with one instrument blank, using the 5% MeOH modifier. The recoveries are given in Table 15 along with their mean, standard deviation, and recovery based on the Soxhlet results for this soil. Trace recoveries of anthracene were found; pyrene was not detected, as was the general case using the original modifier. Figure 8 is a representative spectrum comparison of a sample

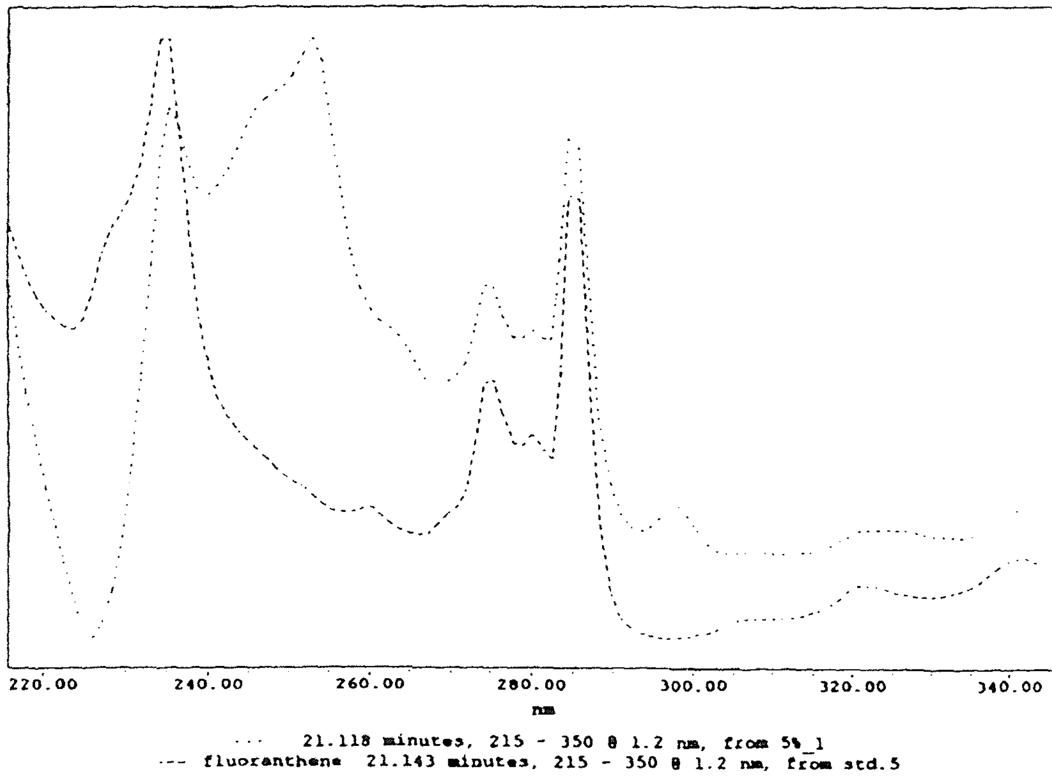


Figure 8 Sample and Library Spectra of Fluoranthene from Experimental Soil; SFE, Modifier B

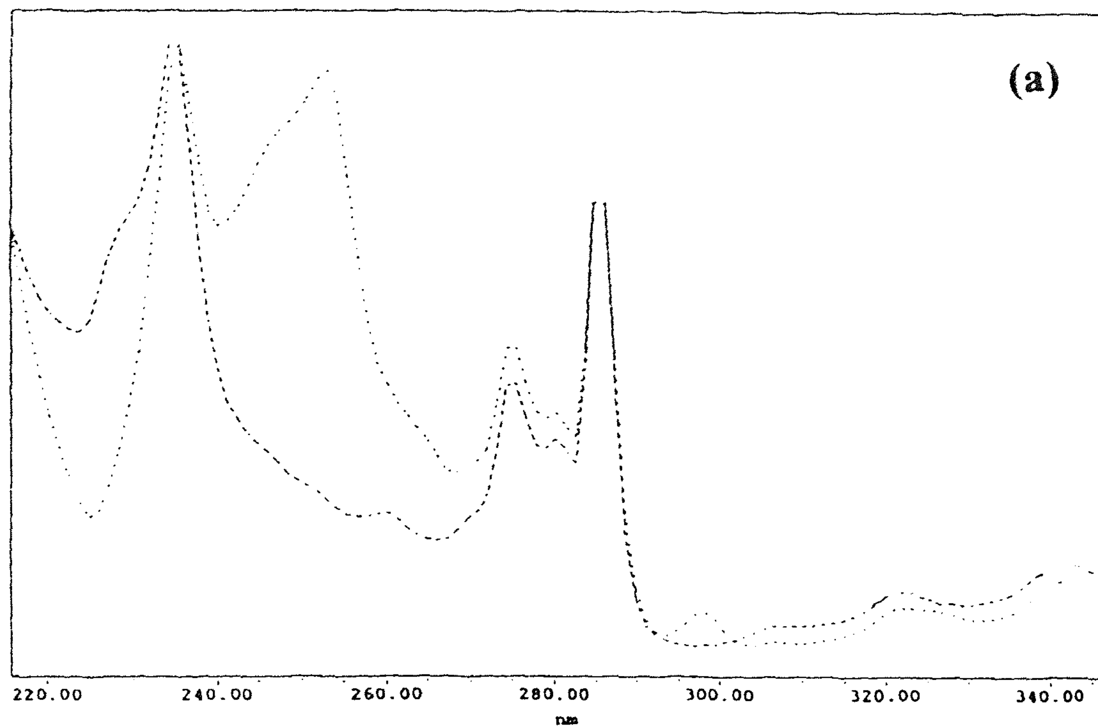
Table 15 PAH Concentrations Determined for the Experimental Soil Samples; SFE, Modifier B

Component	S 1	S 2	S 3	Mean	SD	% Soxhlet[a]
phenanthrene	0.37	0.61	0.55	0.51	0.10	38.1
anthracene	Trace	Trace	Trace	----	----	----
fluoranthene	1.66	2.37	2.22	2.08	0.31	----
pyrene	ND	ND	ND	----	----	----
chrysene	0.52	0.60	0.59	0.57	0.04	42.5

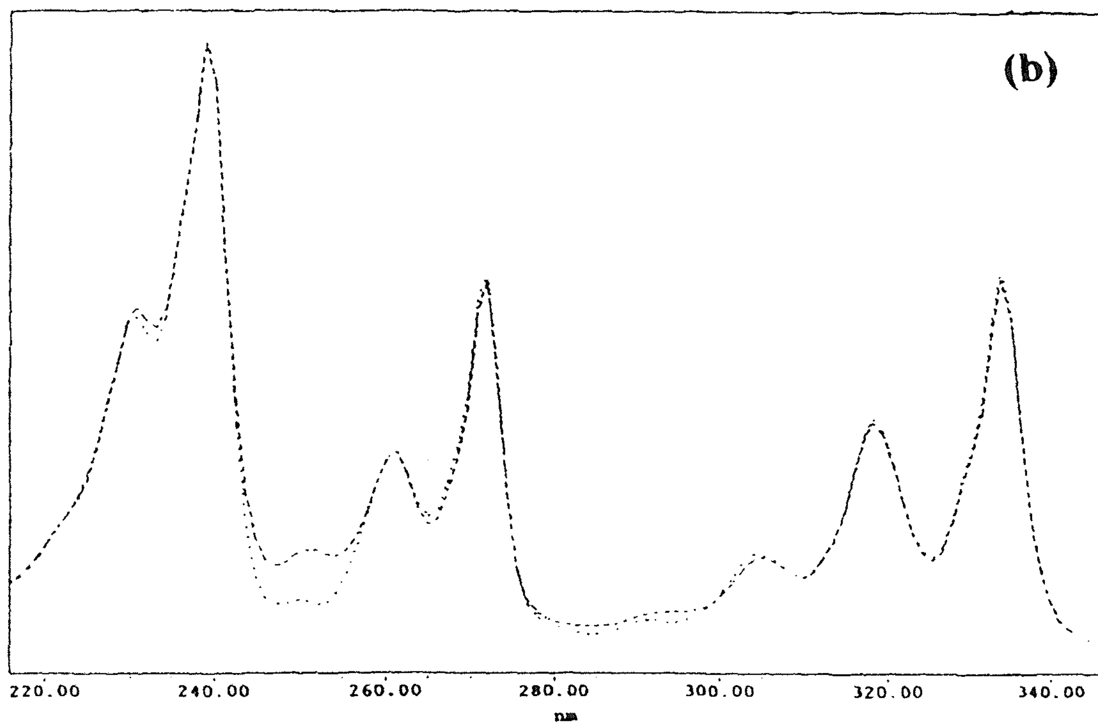
All values given are in mg of the component per kg of dry soil (ppm). ND = No detection. [a]Based on the Soxhlet extractions performed in this study.

peak and library peak for fluoranthene; this PAH was not recovered using Soxhlet extraction or SFE with Modifier A, but is clearly present in the extracts here with Modifier B. This was unexpected, since the recoveries of fluoranthene from the CRM were extremely low. This illustrates that while a reference soil may be useful in that its reference values have been determined, similar samples may respond differently to the same extraction methods.

Chrysene recoveries were essentially the same using both modifiers, 42.5%, but the standard deviation for the results obtained using this modifier was higher (0.04 compared to 0.01). The phenanthrene recoveries and reproducibility were also better using the original modifier; here, the recovery at 38.1% of the Soxhlet extraction is lower than the 53.3% recovered using the original modifier.



··· 21.123 minutes, 215 - 350 @ 1.2 nm, from FRAC2
--- fluoranthene 21.143 minutes, 215 - 350 @ 1.2 nm, from std.5



··· 21.840 minutes, 215 - 350 @ 1.2 nm, from FRAC2
--- pyrene 21.877 minutes, 215 - 350 @ 1.2 nm, from std.5

Figure 9 Sample and Library Spectra of Fluoranthene and Pyrene from Experimental Soil; SFE Modifier B, Fraction 2
(a) Match; presence of fluoranthene (b) Match; presence of pyrene

5.4.3 Fraction Determinations

As with the original modifier, a single sample extract was collected in two separate fractions for the pure methanol modifier; the results are presented in Table 16. This modifier also recovered trace amounts of phenanthrene and anthracene in the first fraction, but no chrysene. The significant fluoranthene recovery in the second fraction was expected, based on the results of the multiple experimental soil extractions. Evidence of pyrene within the second fraction can be seen from Figure 9; as blank and additional trap rinse samples did not indicate pyrene contamination, it is likely that pyrene is being extracted, but not well enough to generate a quantifiable peak upon analysis of the extract.

Table 16 PAH Fraction Determinations for the Experimental Soil; SFE, Modifier B

Component	Fraction 1	Fraction 2
phenanthrene	Trace	Yes
anthracene	Trace	Trace
fluoranthene	ND	Yes
pyrene	ND	Trace
chrysene	ND	Yes
2-fluorobiphenyl	Yes	ND

ND = No detection.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The results of this study demonstrate that while supercritical fluid extraction can successfully recover PAHs from the soil being used for the treatability study, the Soxhlet extraction still achieved higher recoveries of all the PAHs, with the exception of fluoranthene. The results are summarized in Table 17.

Table 17 Summary of Mean Recovery Results for the Experimental Soil

Component	Soxhlet Extraction	SFE with 5% Modifier A	SFE with 5% Modifier B
phenanthrene	1.35	0.72	0.51
anthracene	0.14	0.02	----
fluoranthene	----	----	2.08
pyrene	0.22	----	----
chrysene	1.34	0.57	0.57

All values given are in mg of the component per kg of dry soil (ppm).

Neither modifier recovered all of the PAHs, but the 5% methanol modifier appears to be the more promising of the two. Out of the three extraction methods applied to the soil, only this method and modifier recovered fluoranthene. Chrysene was equally extracted, and anthracene and pyrene were both extracted in marginal or trace quantities, using either modifier. While it did not recover as much phenanthrene, recoveries from the 5% methanol modifier may be improved by simple modifications of the extraction conditions. Since it was

shown to extract at least trace quantities of each of the PAHs, better recoveries may be achieved by extending the static/equilibration time in the second extraction step. This will allow the modifier greater contact time with the soil, and should aid the desorption of the PAHs. This experiment can also be performed with the original modifier for comparison.

Another advantage of this modifier is that it contains no water, so soils with low enough moisture contents may not need to be dried before analysis; the required sample preparation will depend in part on the experimental design of the soil microcosms in the treatability study. Additional experiments at different moisture levels can be performed to find an optimum; soil samples can be adjusted to this moisture content through drying or the addition of water. However, this will have to be done so that any microbial activity is suppressed, so that the concentrations determined for the PAHs more accurately reflect the concentrations at the time of sampling.

If the extraction time in the second step is increased, it is possible that one or both of the first and third extraction steps can be eliminated. This can be simply determined by performing a set of extraction with and without the first step for comparison. The higher temperatures of the second extraction step may cause the loss of more volatile analytes, however, unless the temperatures of the trap and nozzle are adjusted accordingly. If the recoveries are approximately equal or improved, the extraction method can be condensed into one or two steps; aside from the analytes of interest, the recovery of the surrogate standard should also be checked in the event another standard needs to be selected.

Further study aimed at increasing the recoveries of these PAHs should first repeat a solvent based extraction method for comparison to the Soxhlet results reported here. As the

PAH concentrations in this soil have been determined to be relatively low, so SFE experiments should consider incorporating longer static/equilibration times in the second extraction step. Drying the soil to lower moisture contents may also be useful, but this should be done in a desiccator at room temperature to prevent the loss of more volatile PAHs. Sets of extractions performed on treated soil should also include an extraction on untreated soil, to avoid assuming the baseline concentrations determined by SFE in this study will remain constant.

REFERENCES

1. Bjørseth, Alf. *Handbook of Polycyclic Aromatic Hydrocarbons.* v.1 New York: Marcel Dekker, Inc., 1983.

2. Reindl, S., and F. Höfler. "Optimization of the Parameters in Supercritical Fluid Extraction of Polynuclear Aromatic Hydrocarbons from Soil Samples." *Anal. Chem.* 66 (1994): 1808-1816.

3. Hawthorne, S. B., and D. J. Miller. "Extraction and Recovery of Polycyclic Aromatic Hydrocarbons from Environmental Solids Using Supercritical Fluids." *Anal. Chem.* 59 (1987): 1705-1708.

4. Bjørseth, Alf, and Thomas Ramdahl. *Handbook of Polycyclic Aromatic Hydrocarbons.* v.2 New York: Marcel Dekker, Inc., 1985.

5. Mahro, B., G. Schaefer, and M. Kästner. "Pathways of Microbial Degradation of Polycyclic Aromatic Hydrocarbons in Soil." *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds.* Boca Raton, Florida: CRC Press, Inc., 1994.

6. Cerniglia, C. "Biodegradation of Polycyclic Aromatic Hydrocarbons." *Biodegradation* 3 (1992): 351-368.

7. Luthy, R. G., D. Dzombak, C. Peters, S. Roy, A. Ramaswami, D. Nakles, and B. Nott. "Remediating Tar-Contaminated Soils at Manufactured Gas Plant Sites." *Environ. Sci. Technol.* 28 (1994): 266A-276A.

8. Boldrin, B., A. Thiehm, and C. Fritzsche. "Degradation of Phenanthrene, Fluorene, Fluoranthene, and Pyrene by a *Mycobacterium* sp." *Appl. Environ. Microbiol.* 59 (1993): 1927-1930.

REFERENCES
(Continued)

9. Tiehm, A. "Degradation of Polycyclic Aromatic Hydrocarbons in the Presence of Synthetic Surfactants." *Appl. Environ. Microbiol.* 60 (1994): 258-263.

10. Guerin, W. F., and G. E. Jones. "Mineralization of Phenanthrene by a *Mycobacterium* sp." *Appl. Environ. Microbiol.* 54 (1988): 937-944.

11. Westwood, S. A. *Supercritical Fluid Extraction and its Use in Chromatographic Sample Preparation.* Boca Raton, Florida: CRC Press, Inc., 1993.

12. Charpentier, Bonnie A., and Michael R. Sevenants. *Supercritical Fluid Extraction and Chromatography.* American Chemical Society Symposium Series no. 366. Washington, D.C.: American Chemical Society, 1988.

13. Barnabas, I. J., J. R. Dean, W. R. Tomlinson, and S. P. Owen. "Experimental Design Approach for the Extraction of Polycyclic Aromatic Hydrocarbons from Soil Using Supercritical Carbon Dioxide." *Anal. Chem.* 67 (1995): 2064-2069.

14. Hawthorne, S. B., J. J. Langenfeld, D. J. Miller, and M. D. Burford. "Comparison of Supercritical $CHClF_2$, N_2O, and CO_2 for the Extraction of Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons." *Anal. Chem.* 64 (1992): 1614-1622.

15. Hawthorne, S. B., A. B. Galy, V. O. Schmitt, and D. J. Miller. "Effect of SFE Flow Rate on Extraction Rates: Classifying Sample Extraction Behavior." *Anal. Chem.* 67 (1995): 2723-2732.

REFERENCES
(Continued)

16. Langenfeld, J. J., S. B. Hawthorne, D. J. Miller, and J. Pawliszyn. "Effects of Temperature and Pressure on Supercritical Fluid Extraction Efficiencies of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls." *Anal. Chem.* 65 (1993): 338-344.

17. Skoog, D., and J. Leary. *Principles of Instrumental Analysis.* Fourth ed. New York: Saunders College Publishing, 1992.

18. Langenfeld, J. J., S. B. Hawthorne, D. J. Miller, and J. Pawliszyn. "Kinetic Study of Supercritical Fluid Extraction of Organic Contaminants from Heterogeneous Environmental Samples with Carbon Dioxide and Elevated Temperatures." *Anal. Chem.* 67 (1995): 1727-1736.

19. Yang, Y., A. Gharaibeh, S. B. Hawthorne, and D. J. Miller. "Combined Temperature/Modifier Effects on Supercritical CO_2 Extraction Efficiencies of Polycyclic Aromatic Hydrocarbons from Environmental Samples." *Anal. Chem.* 67 (1995): 641-646.

20. Miller, D. J., and S. B. Hawthorne. "Solubility of Polycyclic Aromatic Hydrocarbons in Supercritical Carbon Dioxide from 313 K to 523 K and Pressures from 100 bar to 450 bar." *J. Chem. Eng. Data* 41 (1996): 779-786.

21. Barna, L., J. Blanchard, E. Rauzy, and C. Berro. "Solubility of Fluoranthene, Chrysene, and Triphenylene in Supercritical Carbon Dioxide." *J. Chem. Eng. Data* 41 (1996): 1466-1469.

22. Burford, M. D., S. B. Hawthorne, and D. J. Miller. "Extraction Rates of Spiked versus Native PAHs from Heterogeneous Environmental Samples Using Supercritical Fluid Extraction and Sonication in Methylene Chloride." *Anal. Chem.* 65 (1993): 1497-1505.