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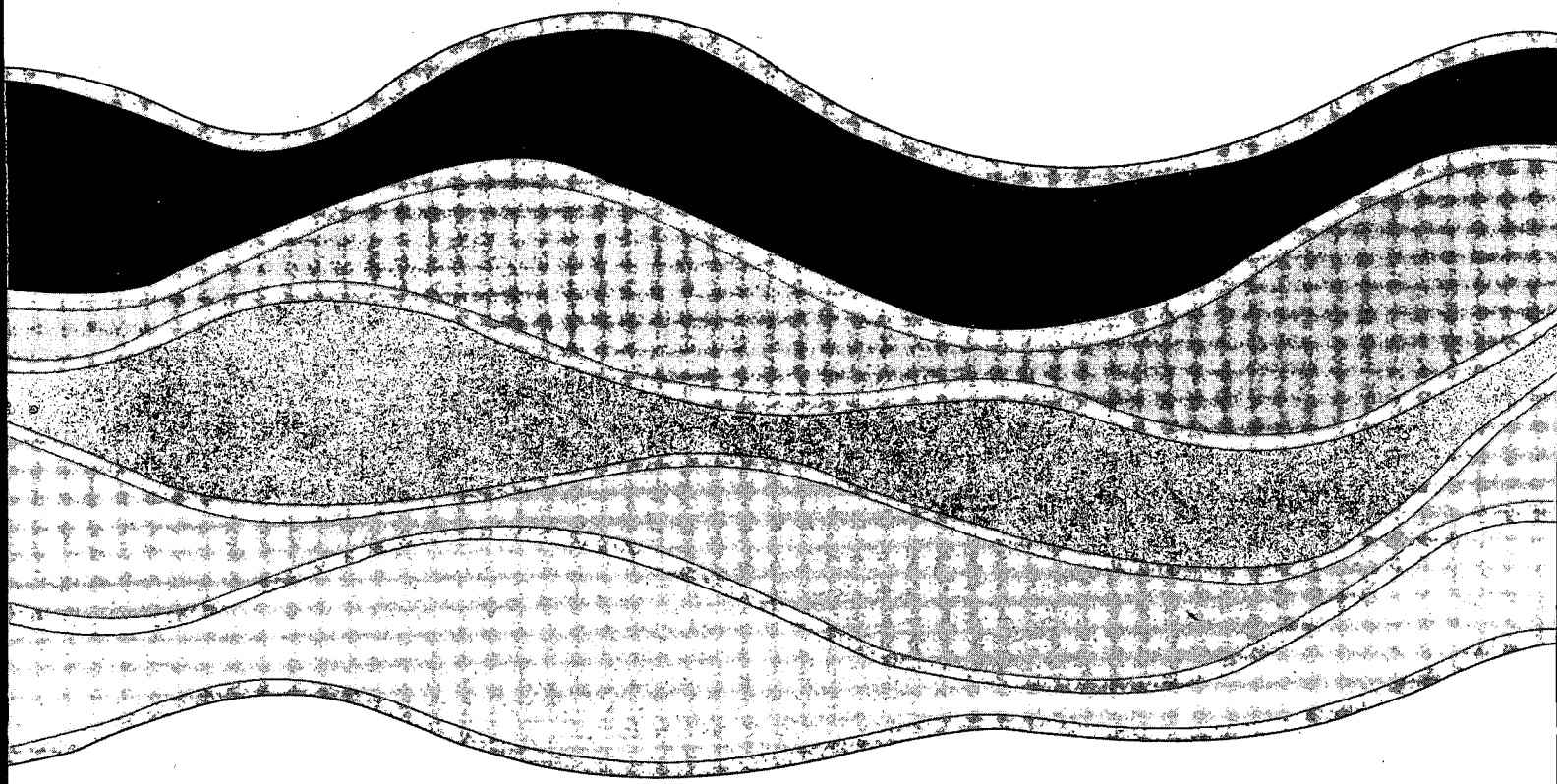

EXTRACTION OF ORGANIC COMPOUNDS FROM SEDIMENTS USING SFE

Bruno DeLuca and Michael E. Fox

NWRI Contribution No. 95-183

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Management Perspective

Extraction of organic compounds from sediments using SFE

by Bruno DeLuca[1] and Michael E. Fox[2]

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Organic contaminant analyses (PCBs etc.) are labour intensive and produce large quantities of toxic waste. New technologies are needed to improve laboratory economy and to cope with restrictions on the use of toxic solvents.

We tested recent advances in Supercritical Fluid Extraction (SFE) using liquid Carbon Dioxide combined with new automated equipment.

We validated the SFE technique to extract PCBs, petroleum hydrocarbons, and sterol from sediments of Hamilton Harbour and Bay of Quinte Remedial Action Plan sites.

The SFE technique resulted in a tenfold saving of time and materials; virtually no solvent waste was produced.

Work continues at NWRI to develop the SFE techniques for use on more classes of compounds with the aim of eliminating the use of toxic solvents.

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Abstract

An ISCO model SFX 2-10 supercritical fluid extractor, using supercritical CO_2, was used to extract analytes from sediments rather than using the customary large volume solvent extractions such as Soxhlet extractors. (1) Total Petroleum Hydrocarbons, or TPHs, were quantitatively extracted from polluted (Hamilton Harbour) and cleaner (Bay of Quinte) sediments using less than 10 mL of supercritical CO_2. Recoveries of TPH were superior to those achieved using a 5 hour extraction with dichloromethane. A range of temperatures (60 to 120°C) and pressures (300 to 450 atm) were examined. Recoveries of TPH were found to be heavily temperature dependant. Extractions performed at 120°C and 300 atm yielded 36% higher recoveries of TPH than the recommended US EPA supercritical extraction procedure. (2) Bay of Quinte sediments spiked with 6 PCB congeners, representing a wide range of chlorination, demonstrated very high recoveries. However, results suggest that spiked PCB recoveries do not accurately represent extraction of native PCBs. (3) Hamilton Harbour samples were also extracted and analysed for coprostanol using conditions suggested for TPH extractions. Recoveries of coprostanol from a 15 minute extraction exceeded those using a 2 x 24 hour Soxhlet extraction with toluene/methanol. A supplemental discussion is included. It focuses on wider theoretical and practical aspects of SFE with specific emphasis on complex environmental matrices.

1.0 Introduction

The extraction of organic contaminants from sediment matrices has been problematic in the past. Common extraction procedures, such as Soxhlet extraction, have shown to be efficient at extracting contaminants . However, they are far from ideal. Traditional methods are labour intensive and time consuming requiring many hours of extraction and cleanup. The final product usually requires concentration which results in large amounts of hazardous solvent waste.

Supercritical fluid technology has shown promise as a new method for extracting organic contaminants from environmental matrices. A fluid is considered supercritical when its temperature and pressure are raised above its critical point. At the critical point, the substance is neither a liquid or a gas. Supercritical fluids have solvating powers similar to that of liquid solvents, but have viscosities, low surface tensions, and rapid diffusion characteristics which liken them to a gasses (Myer *et al*, 1990).

Supercritical fluids are especially useful since their solvating powers can be varied. Solvent power is directly related to the density of the supercritical fluid. Density can be altered by adjusting the temperature and pressure conditions of the fluid. As the temperature of the supercritical fluid increases, its molecules absorb energy. The kinetic activity of the molecules increase resulting in lower density. Conversely, increased pressures raise the density of the supercritical fluid since the molecules become compressed.

The Hildebrand solubility coefficient is a semi-quantitative measure of a liquids solvent power. This coefficient is a function of fluid density. Typically, solvents with equal Hildebrand solubility parameters exhibit similar solvent properties (Tehrani 1993). This is an important concept since it explains how supercritical fluids can theoretically be used as a substitute for solvents in traditional extraction methods.

Supercritical extraction techniques boast many advantages over traditional methods. Due to the properties mentioned above, supercritical fluids are especially suitable to penetrate difficult matrices and remove organic contaminants (Erkey *et al*, 1993). Since supercritical fluids are gases at ambient conditions, there is no need for concentration of the analyte after the extraction is completed (Hawthorne, 1990). Also, one can optimize the extraction conditions and select a particular compound class thus reducing the interference from less favourable compounds (Hawthorne, 1990). Carbon dioxide is an excellent candidate for use as a supercritical fluid. It has a low critical temperature 31.1°C and pressure 72.8 atm (Shantz and Chesler 1986) . It is non-flammable and fairly inert. CO_2 is also inexpensive, non-toxic, and can be obtained at very high purity (Pipkin, 1990).

Supercritical fluids have been used for many years in the food and pharmaceutical industries to decaffeinate coffee and tea and to extract the essence of hops, spices, natural colours, and drugs (reviews in Rizori 1986, Larson 1986, Pellerin 1991). There are many examples in recent literature where supercritical techniques have been applied to environmental samples. Chlorophenols have been recovered from wood samples (Kapila *et al*, 1992). Dibenzofurans and dioxins have been extracted from fly ash and pulp and paper mill effluents (Alexandrou *et al*, 1992). PCBs and organochlorine pesticides have been extracted from fish, milk, and blood (Kapila *et al*, 1992) and animal feed (Torreti *et al*, 1992). Also, PAHs have been extracted from soils (Burford *et al*, 1993; Hills and Hill, 1993) and sediments (Lee and Peart, 1993).

Total Petroleum Hydrocarbons, or TPHs, are a combination of biodegradable animal and vegetable oils and less biodegradable mineral oils. Contamination of soil due to spillage of oil and grease has been difficult to assess using traditional methods (Lopez-Avilla *et al*, 1992). This has led to the speculation that supercritical fluid extraction may be ideal for the task (Wylie *et al*, 1994; Bicking *et al*, 1993). The results of these studies were encouraging and it was thought that extending this application to sediment TPH analysis may prove fruitful.

PCBs are toxic and persistent chemicals that accumulate in the sediments of natural waters. Lee and Peart (1994) have extracted native PCBs from sediment using supercritical CO_2 with good results. Using their study as a model for comparison, an attempt was made to optimize the extraction of spiked PCBs using the ISCO SFX 2-10 supercritical extractor. Coprostanol, a decomposition product of cholesterol, is an

important marker used to assess the contamination of water bodies by human waste waters. Current extraction techniques for coprostanol involves two 24 hour soxhlet extractions and an intensive clean-up. Experiments were performed in order to assess whether coprostanol is a suitable candidate for supercritical extraction.

This report deals with the supercritical CO_2 extraction of TPHs, PCBs, and coprostanol from natural sediments. Many obstacles were encountered when developing methods for the extraction of these persistent pollutants. Restrictor plugging, analyte loss, and extract contamination were issues that had to be dealt with. Optimising extraction conditions also proved difficult since the recoveries were heavily temperature dependant. In addition, spiked samples did not appear to behave in the same manner as samples that were analyzed for native contamination. Examples of experiments that demonstrate these observations and other problems associated with sediment extractions will be discussed.

2.0 Materials and Methods

2.1 Supercritical Extraction Apparatus

The ISCO SFX 2-10 supercritical fluid extractor was used for all of the sediment extractions (ISCO Inc. Lincoln, NE). The SFX 2-10 consists of a syringe pump module and a separate extraction module accepting hand tightened stainless steel extraction vessels. A stainless steel fixed flow restrictor (1.5 mL/minute) (ISCO Inc. Lincoln, NE) was used in conjunction with a hand fabricated restrictor heater. Typically, a 2.5 mL extraction vessel was prepared by adding 0.15 g of diatomaceous earth (Celite 545, Fisher Canada) before adding the sediment sample. Celite was then added again to top up the volume. The vessel was capped with Gelman GF/F glass fibre filters (1 um nominal pore size) before tightening the stainless steel frits and end caps. The analyte was collected in glass screwcap centrifuge vials containing 2 mL of solvent. Supercritical Fluid Extraction grade carbon dioxide was provided by Air Products Canada (Mississauga).

As illustrated in figure 1, the restrictor heater was constructed from three concentric Teflon sheathes. The two inner sheathes are necessary since they prevent the heating coils from contacting the restrictor. The outer sheath insulates the heating coils from the trapping solvent. The entire assembly was encased in a modified borosilicate Pasteur pipette in order to minimize the transfer of contaminantsfrom sample to sample. The tip was cemented with an epoxy. Heat was supplied by nichrome resistance wire that was wound around the inner Teflon sheath at 2 to 3 mm intervals. Voltage was adjusted using a variable voltage transformer which was usually operated at 20 to 30% voltage. Unfortunately, restrictor temperatures could not be monitored.

2.2 Supercritical Extraction Procedure

All extractions were performed with an initial 5 minute static extraction period so that the extraction vessel could equilibrate to the prescribed temperature and pressure conditions. The temperatures of the extractions ranged from 60 to 140°C while pressures ranged from 150 to 450 atm. The dynamic extraction proceeded until 4 volumes (10mL) of supercritical CO_2 had swept through the sample. The time required to complete the dynamic extraction was approximately 7 to 15 minutes depending on flow rates. The collection vial contained 2 mL of toluene (JT Baker, Ultra Resi-Analyzed). Loss of collection solvent was corrected for by diluting the sample back to 2 mL. No effort was made to concentrate or clean-up the analytes prior to GC analysis.

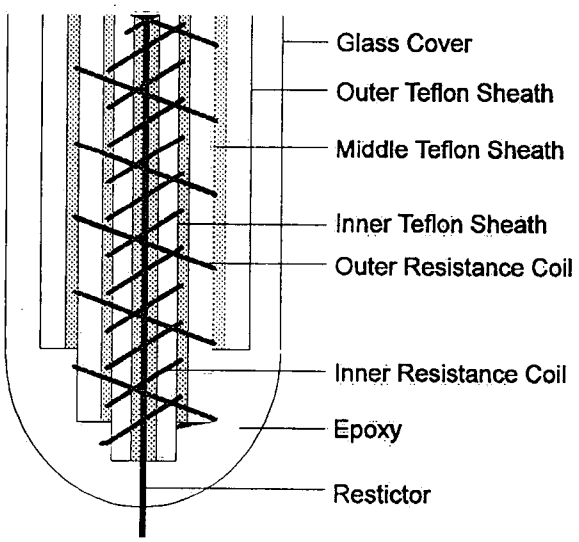

Figure 1. Hand fabricated restrictor heater

Table 1. Bay of Quinte sediments were prepared with 6 PCB congeners representing a wide range of chlorination.

IUPAC#	31	54	77	101	128	209
Chlorination	2,4',5	2,2',6,6'	3,3',4,4'	2,2',4,5,5'	2,2',3,3',4,4'	2,2',3,3',4,4',5,5',6,6'
PCB (ng/g)	308	327	318	302	289	356

2.3 Sediments

For most of the experiments, Bay of Quinte sediments were used. The Bay of Quinte is located near the north-eastern tip of Lake Ontario and is considered to have sediments that are fairly low in contamination. In contrast, Hamilton Harbour is adjacent to industrial areas in the south-west corner of Lake Ontario. Sediment samples from Hamilton Harbour are generally considered to be relatively more contaminated. Sediments were air dried and ground with a mortar and pestle. Large particles and biotic debris were removed using a 1 mm sieve.

2.4 Soxhlet Extraction

Sediments were mixed in a 1:4 ratio with prefired anhydrous sodium sulphate (BDH Inc. Toronto). The sample was placed in glass thimbles constructed with coarse porosity glass frits and soxhlet extracted for 12 hours using 250 mL of dichloromethane (DCM) (JT Baker, Ultra Resi-analyzed). The DCM was roto-evaporated to less than 1 mL and solvent exchanged with toluene.

2.5 Sparging Experiment

TPH (0.975 mg of Bunker C oil) was added to 2 mL of toluene trapping solvent. The spiked solvent was then sparged with clean CO_2 which had passed through an empty extraction vessel inside the supercritical fluid extractor. This experiment was performed in order to test for the loss of analytes via volatilization or escaping aerosols.

2.6 Spike Experiments

Bunker Oil was used for experiments involving the extraction of spiked TPHs from Bay of Quite sediment and from glass fibre filter papers. The Bunker oil standard (9.75 mg/mL) was diluted with DCM and added to these matrices using a glass syringe. Excess DCM was allowed to evaporate off before extraction.

As shown in table 1, six PCB congeners were used as the sediment spike (Supelco, Mississauga). The PCBs were mixed with 100 mL of methanol were added to 50 g Bay of Quinte sediment. The mixture was roto-evaporated in a round bottom flask until dry and then was left to equilibrate for two weeks before use. The final concentration of each PCB was roughly 300 ng/g.

2.7 GC Analysis

TPHs were analyzed on a HP5890 Series II gas chromatograph equipped with an HP7673 autoinjector and a Flame Ionization Detector set at 300°C. The 30 m DB5 microbore column had a 0.25 mm id and a 0.25 um film (J&W Scientific). The splitless 1 ul injection was held at the inlet at 200°C then purged after 1min. The oven temperature was steadily increased by 6°C/minute from 50 to 300°C for a program that lasted 41 minutes. The hydrogen carrier gas was held at a constant linear velocity of 38.5 cm/sec.

PCBs were analyzed using a dual column HP5890 Series II gas chromatograph equipped with an HP7673A autoinjector and dual Electron Capture Detectors set at 325°C. The analytical DB5 column (as above) was paralleled by an experimental 30 m HP50+ column with a 0.25 mm id and a 0.25 um film (obtained from Hewlett Packard). The HP50+ column has a comparatively polar film which led to different retention times allowing for peak confirmation. The splitless 2 uL injection was delivered to the inlet that was held at 230°C and purged after 1 minute. The 61 minute temperature program started at 70°C and rose to 150°C at 10°C/minute, then to 250°C at 2°C/minute, and finally to 280°C at 10°C/minute. The hydrogen carrier was kept at a constant linear velocity of 63.4 cm/sec.

Data was collected in real time using Hewlett Packard hardware (Missisauga). Chromatograms were integrated using HPChemStation software (Hewlett Packard). Quantification of TPHs was done using a Bunker Oil standard by establishing a nine point calibration curve relating area to concentration. PCBs were quantified using an external NOI standard. The standard is designed to reflect the concentrations of PCB congeners that are found in the natural environment.

3.0 Results

3.1 TPH

The calibration curve used to quantify TPH in sediment extractions is shown in figure 2. A dilution series was created with Bunker Oil and dichloromethane. It was plotted against the total integrated area determined by chromatographic analysis. The relationship is described by: (integrated area) = 201649(TPH mg/mL) with an r^2 of 0.998.

Preliminary experiments were conducted to investigate the time required to complete a 0.5g sediment extraction. Extractions were done at 80°C and 340 atm. Fractions were collected from two experiments at increasing intervals. The X axis in figure 3 is a measure of the amount of supercritical CO_2 that passes through the sample inside the extraction chamber. Supercritical CO_2 volume was used rather than time since restrictor flow (extraction time) can be variable between duplicate samples. Remarkably, 100% of the extractable TPHs were extracted with less than 2 mL of supercritical CO_2. This was established since where no more TPH peaks were found in subsequent extractions. This corresponds to approximately 7 minutes of extraction time; 5 minutes of static extraction when the analyte and matrix come to equilibrium with the supercritical CO_2, and then 2 minutes of dynamic extraction where the analyte leaves the extraction vessel.

An attempt was also made to asses loss of TPH from the toluene trapping solvent. Clean supercritical CO_2 passed through an empty extraction vessel and was decompressed into 2mL of toluene trapping solvent spiked with 0.975 mg of TPH. This procedure mimics a typical extraction except there is no sediment sample in the extraction vessel. As seen in figure 4, the decompression of CO_2 resulted in the sparging of the trapping solvent leading to loss of TPH as compared to the original spike concentration. Again, CO_2 was expressed on the X axis as the volume in its supercritical state in mL. Two

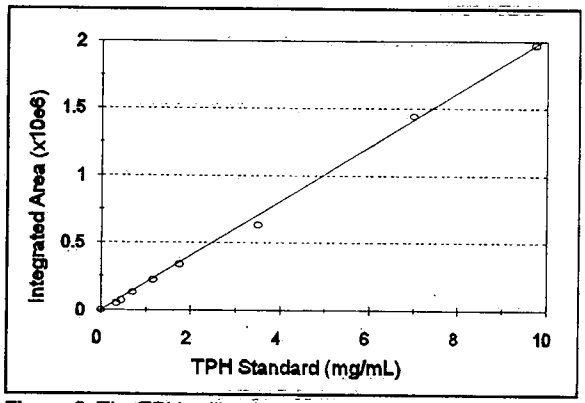

Figure 2. The TPH calibration curve created from 9 dilutions of Bunker Oil standard (Y=201649X, r^2=0..998)

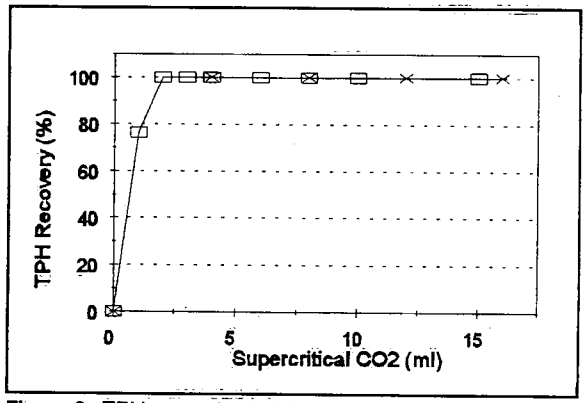

Figure 3. TPHs are extracted in about 7min using <2ml of supercritical CO_2 after a 5ml static extraction.

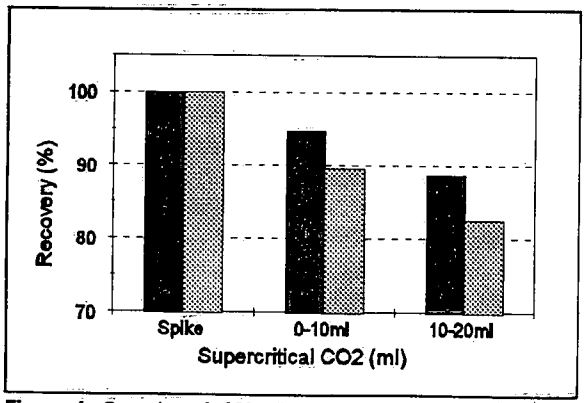

Figure 4. Sparging of -20°C (black bar) and ambient (grey bar) trapping solvent with supercritical CO_2 results in loss.

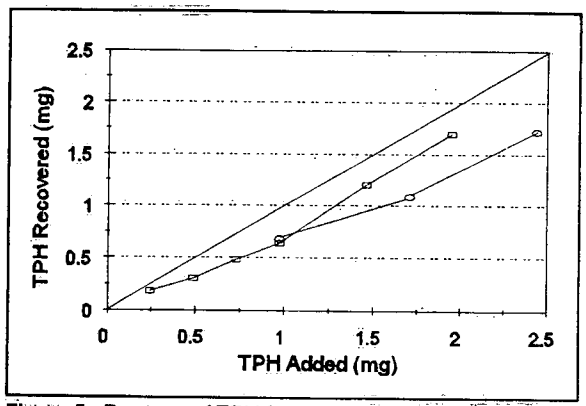

Figure 5. Recovery of TPH from glass fibre filters (boxes) and Bay of Quinte sediment (circles) relative to the 1:1 line

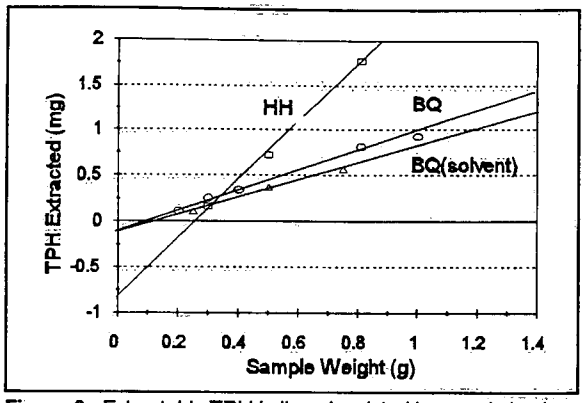

Figure 6. Extractable TPH is linearly related to sample loads. Hamilton Harbour (HH) and Bay of Quinte (BQ) supercritical extractions are contrasted to Bay of Quinte solvent extractions.

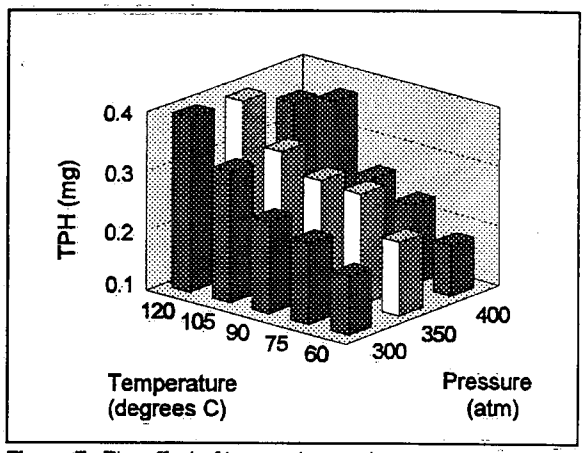

Figure 7. The effect of temperature and pressure on the extraction of native TPHs.

treatments were explored: toluene was either kept at -20°C before use, or at ambient temperatures. Cooling the trapping solvent before use led to higher retention of TPH (94.7% and 88.7% at 10 mL and 20 mL of CO_2 respectively) in comparison to the solvent at ambient temperature (89.5% and 82.4% at 10mL and 20mL of CO_2 respectively).

Recovery of spiked TPH from glass fibre filter paper and from Bay of Quinte sediment was assessed. Figure 5 demonstrates that recovery is approximately 65% at the lowest concentrations tested for both matrices as compared to the 1:1 line. The 1:1 line represents 100% recovery where the dose amount equals the amount recovered. The efficiency of the recovery appears to be bimodal since it improves as the magnitude of the spike increases. The recovery of TPHs from the filter papers approaches 90% at the highest concentrations tested, while recovery in sediment is approximately 70% at the highest concentrations tested. These extractions were performed at 340 atm and 80°C as suggested by the US Environmental Protection Agency (1986) and all data points are the mean of two experiments.

Subsequent experiments with Bay of Quinte and Hamilton Harbour sediments showed that native TPHs can be extracted in a dose dependant fashion over a wide range of sediment loads. Figure 6 shows a linear relationship for Bay of Quinte sediments which were extracted using supercritical ($y=1.08x-0.085$, $r^2=0.995$, $n=7$) and Soxhelet ($y=0.914x-0.104$, $r^2=.994$, $n=3$) methods. In a typical 0.5g extraction, the supercritical CO_2 method performed at 80°Cand 340 atm recovered 29% more TPH than the comparable solvent extraction method. It appears that there is about twice as much TPH in Hamilton Harbour sediments, however, further interpretation of the regression data ($y=3.20x-0.82$, $r^2=0.997$, $n=3$) suggests that this conclusion may be premature (see dicussion).

The final TPH experiments were performed in an effort to optimize the temperature and pressure conditions of the extraction. Figure 7 demonstrates that the extent of the extraction from 0.5 g of Bay of Quinte sediment is highly dependant on the temperature. The higher the temperature, the greater the extraction. Pressure exerts a weaker effect, perhaps due to the smaller range of values explored. The best extractions at 120°C (~0.4 mg) are approximately twice that at 60°C (~0.2 mg). Two extractions were done for each condition tested. The average standard deviation was 3.4%.

3.2 PCBs

The 6 PCB spike solution was prepared and calibrated against response factors that were calculated for our gas chromatograph. The calculated concentrations of PCBs in Bay of Quinte sediment for congeners 31, 54, 77, 101, 128, and 209 were 308, 327, 318, 302, 289, and 356 ng/g, respectively. The sum of 6 congeners totalled to 1900 ng/g. The spiked PCB peaks were sharp and free from any interference peaks originating from the natural sediment.

Recovery dynamics of the spiked PCBs from sediments are shown in figure 8. These extractions were performed at 80°C and 340 atm. Fractions of extract were monitored for PCBs after

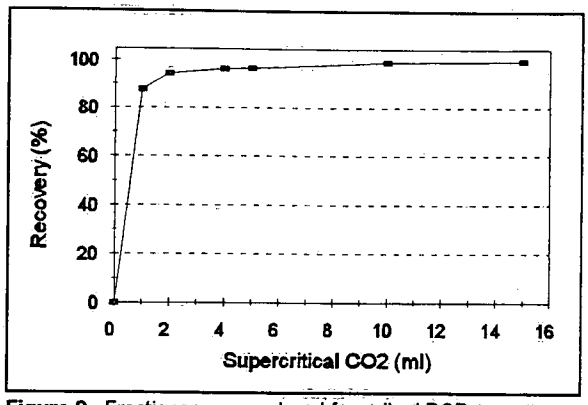

Figure 8. Fractions were analyzed for spiked PCB recovery. Data sum of two experiments.

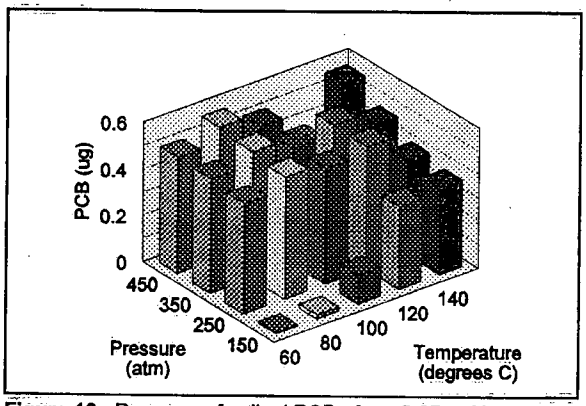

Figure 10. Recovery of spiked PCBs from 0.25g of Bay of Quinte sediment. Each bar is a mean of two extractions.

a 5 minute static extraction. After 2 mL of dynamic extraction, 94.2% of the 6 PCBs were extracted from the sample (in relation to the amount extracted at 15 mL). After 10 mL of dynamic extraction, 99.3% of the 6 PCBs were extracted. Consequently, all subsequent dynamic extractions were done with 10 mL of supercritical CO_2 which corresponded to about 12 minutes of extraction time (5 minutes static, 7 minutes dynamic).

The effect of the static extraction step was also explored. There appeared to be a 5% decrease in the recovery of spiked PCBs from sediment if the 5 minute static extraction step was omitted and the extraction was carried out in dynamic mode only. However, this difference was not significant (t-test, $p>0.05$). In spite of this, the 5 minute static extraction was employed in our experiments since it allowed the extraction vessel to reach equilibrium with the various temperature and pressure conditions tested.

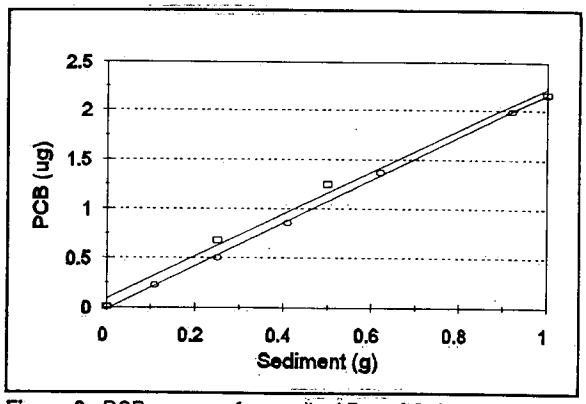

Figure 9. PCB recovery from spiked Bay of Quinte sediment using supercritical (circles) and soxhlet (squares) methods.

The recovery of the 6 PCBs from of spiked Bay of Quinte sediment using supercritical CO_2 extraction procedures were compared to Soxhlet extractions. Figure9 contrasts the recoveries for both methods in relation to the amount of sediment extracted. The equations of the best fit lines for the supercritical data ($Y=2.184X-0.0169$, $r^2=0.999$, $n=6$ and the Soxhlet data ($Y=2.137X+0.0916$, $r^2=0.9912$, $n=4$) show that the slopes are almost identicle. The constant, and thus the recovery, is slightly higher for the soxhlet data. Using the equations above, recovery of PCBs from 1 g of spiked sediment for supercritical and soxhlet techniques is 116% (2.201 ug) and 117% (2.229 ug) respectively, as compared to the dose amount (1.900 ug).Analysis of the individual congener patterns for the data in figure 9 showed that the percentage distribution of each PCB congener was unchanged as the amount of sediment extracted increased (data not shown). However, there were small differences in the individual congener distributions when contrasting supercritical and soxhlet methods (table 2). Congener 77 is extracted to a larger extent when using the soxhlet procedure (one tailed t-test, $p<0.05$, $n=6$), while congener 209 is extracted to a larger extent using the supercritical method (one tailed t-test, $p<0.05$, $n=6$).

An attempt to optimize temperature and pressure conditions for the extraction of the 6 spiked PCBs from Bay of Quinte sediment is shown in figure 10. A wider range of temperatures (60 to 140°C) and pressures (150 to 450 atm) were selected in comparison to the TPH extraction conditions tested. There seems to be little correlation between PCB recovery and pressure or

Table 2. Spiked PCB congener percent distribution after extraction of 0.25 g of Bay of Quinte sediment using supercritical and soxhlet methods (percent (SD), n=3)). Asterisk denotes a significant difference for the two treatments (one tailed t-test, $p<0.05$).

Congener	31	54	77*	101	128	209*
Supercritical	16.0 (0.44)	13.6 (0.79)	20.4 (0.32)	15.4 (0.42)	12.9 (0.37)	21.8 (0.29)
Soxhlet	16.9 (0.52)	12.6 (0.75)	23.9 (0.22)	15.2 (0.30)	12.4 (0.29)	19.1 (0.24)

temperature. Extremely low PCB recovery was experienced at 150 atm of pressure when using low temperatures.

3.3 Coprostanol

An NWRI method for TPH extraction was used in this study as a comparison in order to evaluate the use of supercritical CO_2 for the extraction of coprostanol from sediments (Leenheer *et al*, 1984). Coprostanol was extracted statically for 5 minutes then dynamically using 10 mL of supercritical CO_2 at 80°C and 340 atm of pressure. As shown in figure 11, recovery of coprostanol using the test supercritical method was 107% in comparison to the corresponding Soxhlet method. The supercritical method took less than 20 minutes and used 2mL of collection solvent while the Soxhlet extractions took over 24 hours and used several hundred mLs of solvent.

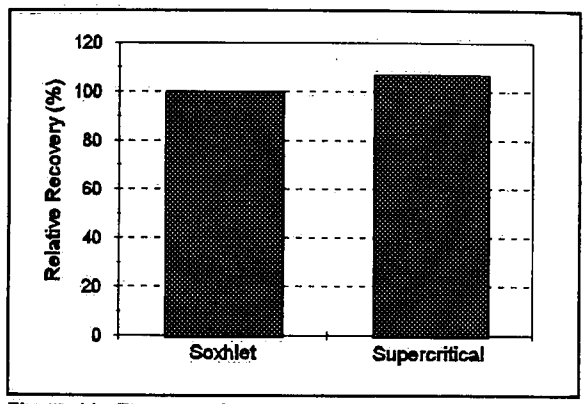

Figure 11. Recovery of coprostanol from sediment is 7% higher when using the non-optimized supercritical method (n=3) in contrast with a 24 hour Soxhlet method.

4.0 Discussion

4.1 Solvent Collection

Figure 4 illustrates the problem of TPH loss in relation to the duration of dynamic extractions. Two strategies were employed in order to improve recovery: cryogenic trapping and shortened dynamic extraction times.

The first involves cooling the trapping solvent at -20°C before the extraction starts. It is thought that cooling may reduce loss by inhibiting volatilization of high vapour pressure analytes. In figure 4, one can see a that the cold solvent recovery is 6 and 7% greater after 10 and 20 mL of sparging respectively, in comparison to ambient recovery. Interestingly, after the dynamic extraction begins, the decompressing CO_2 absorbs heat and causes a further byproduct cooling effect. This phenomena has been noted in other works (Porter *et al*, 1992; Burford *et al*, 1992). The solvent stays sub-zero for the duration of the experiment as indicated by the build-up of frost on the outside of the glassware.

Another way to reduce loss of analytes during solvent trapping is by reducing the time of the dynamic extraction. Studies by Hartonen *et al* (1994) with DCM trapping solvent has shown that recovery decreased to 60 or 70% for most of the analytes tested after 30 minutes of extraction time. Aerosols may form during CO_2 depressurization and may escape the trapping solvent, especially when supercritical CO_2 flow exceeds 1 mL/minute (Ashraf-Khorassani *et al*, 1992).

A balance must be reached between loss and recovery. Evidence seems to suggest that TPH is recovered quite quickly suggesting that short extraction times may be applicable. Figure 3 demonstrates that 100% of the extractable TPHs are removed from sediment within the first 2 mL of dynamic extraction. No peaks were detected in the following fractions (up to 16 mLs of supercritical CO_2). This result is comparable to the recovery of spiked PCBs observed in figure 9, where 94% is recovered at 2 mL. Theoretically, a short dynamic extraction volume would result in optimum recovery since losses due to volatilization or aerosol formation would be minimized.

The rapid recovery kinetics observed in this study may be a result of the 5 minute static

extraction that was performed before the dynamic extraction. During the static extraction, the sediment matrix is allowed to equilibrate with the supercritical CO_2 as the temperatures and pressures reach their experimental levels. TPHs are liberated as they partition into the supercritical CO_2 bath prior to removal from the extraction chamber via dynamic extraction. As a consequence, only a small dynamic extraction volume is needed.

Kinetic models of supercritical extraction support this finding, suggesting that only two void volumes (<5 mL) of supercritical solvent should be necessary for quantitative extraction of analytes from simple matrices (Pawliszyn, 1993). Although sediments are not simple matrices, it was decided that four volumes of supercritical CO_2 (10 mL) would be exhaustive enough to recover most of the extractable TPHs from sediments.

Miller *et al* (1993) experimented with short dynamic extraction bursts (3-30 seconds) in an effort to reduce the loss of analytes. Collection efficiencies of PCBs, PAHs, gasoline, and diesel fuel were greater than 90%. Their study supports findings in this report which suggest that static extractions combined with short dynamic extractions will maximize recovery while minimizing loss.

4.2 Recovery of spiked analytes

Recovery of spiked TPHs from simple matrices like glass fibre filters was not as complete as one may have anticipated (figure 5). Recovery of spiked TPHs approached 90% for filter papers and 70% for Bay of Quinte sediments. Recovery of spiked TPHs from sediment were consistently less, suggesting that sediment may have higher affinity binding sites as compared to the filter paper. Studies using less complex matrices like diatomaceous earth (Bicking *et al,* 1993)and filter papers and clays (Emery *et al*, 1992) often report excellent TPH recoveries since there is very little for the analyte to adhere. Also, it was observed that spiked TPH recovery increased as the amount of the spike increased. Higher spike concentrations may oversaturate the available binding sites and lead to the easy removal of TPHs.

Sub-optimal recovery of TPHs may be due to the method of sediment spiking. TPH was added to the surface of 0.5 g of sediment inside the extraction vessel using a syringe. This localized addition may poorly mimic actual contamination since the TPH molecules may agglomerate with each other and may resist solubilization into the supercritical CO_2. TPH molecules may also associate with high affinity sites in the sediment since it has to travel through the length of the sample before it can leave the extraction vessel.

The disappointing recovery of spiked TPH from sediment may also be due to poor selection of extraction conditions. This study used temperature and pressure conditions suggested by the US EPA for the removal of TPHs from soil. As will be discussed, increasing the temperature and pressure conditions may significantly increase the recovery of spiked TPHs.

Bay of Quinte sediment spiked with 6 PCB congeners was used to compare Soxhlet and supercritical extractions (figure 9). The slopes of the regression lines are almost identical, 2.137 and 2.184 respectively, indicating that the extraction efficiencies are approximately equal for both treatments. Deriving any useful conclusions from this observation is difficult since spiked contaminants have been reported to behave quite differently as compared to native contaminants. A study conducted by Burford *et al* (1993) on sediment spiked with PAHs demonstrated that extraction rates were up to 10 fold higher as compared to native PAHs. Other studies suggest that conditions that quantitatively extract spiked analytes may recover less than 10% of the same analytes in real world samples (Hawthorn *et al*, 1993).

Intuitively, it is reasonable to suggest that spiking leads to non-specific binding of analytes. This may result in weak analyte/matrix interactions and effortless removal. This is especially true if the spike concentrations are high, as they are in this study (300 ng/g). In order to better mimic native sediment contamination, slow roto-evaporation of PCBs onto the sediment and longer aging times (>14 days) were used. However, figure 10 demonstrates that recovery of spiked PCBs do not vary with temperature and pressure conditions. Except for extreme conditions, it appears as though the recovery hits a ceiling at about 0.5 ug. This value is comparable to the actual amount added, 0.475 ug per sample. Even though supercritical extractions compare well with soxhlet extractions, it is doubtful that this is an honest comparison since spiked analytes are so easily recovered.

PCB congener analysis of both the supercritical and soxhlet extracts reveal that there is very little difference in their ability to extract the six congeners (table 2). Congener 77 was extracted slightly better using the soxhlet technique, while congener 209 was extracted slightly better using the supercritical technique. The amounts of congeners 77 and 209 were significantly different (one-tailed t-test, $p<0.05$) between the two methods, although the total amount of spiked PCBs extracted remained similar for each method.

4.3 Extraction of native analytes

Extraction of native TPHs from Bay of Quinte and Hamilton Harbour sediments was investigated (figure 6). It is reassuring to be able to quantifiably extract TPHs over a wide range of sediment loads. The relationship is linear for all three treatments ($r^2>0.994$). By comparing the slopes of the regression equations one can observe that recovery of TPHs from Bay of Quinte sediment using the supercritical technique was consistently better than that for the Soxhlet technique over the sample loads tested. At 0.5g, the recovery of TPHs was 29% higher using the non-optimized supercritical technique.

The regression equations suggest that the recovery efficiency of native TPHs is less than 100%. The efficiency of the recovery of native TPHs can be effected by such factors as loss during collection. If the recovery of natural TPH was 100%, the y-intercept should be zero because as the weight of the sediment sample decreases, the amount of extractable TPH should approach zero. The relationships should also be linear since a doubling of the sediment load should result in a doubling in the extractable TPH.

The y-intercepts for the Bay of Quinte supercritical and solvent regressions are very close to zero, -0.085 and -0.104 mg of TPH respectively, suggesting only slightly less than perfect recovery (figure 6). The y-intercept for Hamilton Harbour is much less, -0.82 mg of TPH. One explanation may be that the efficiency of the TPH extraction is dependant on the amount of sediment used (ie. the relationship is not linear and loss of analyte is dependant on sample load). However, there is no reason to believe that this is so. The problem seems most likely to originate from the integration of the chromatograms from large sediment samples. The samples with large sediment loads were heavily contaminated, peaks merged, and the baselines were assigned in such a manner as to overestimate the total area under the curve. This effect was especially strong in Hamilton Harbour samples since they are generally more contaminated and contain interference from sulfur related compounds.

Optimizing the recovery of TPHs revealed that the US EPA method 3560 (1986) falls short of maximum recovery. Figure 7 illustrates the extraction of native TPH in 0.25 g of Bay of Quinte sediment when varying temperature and pressure conditions. It shows that TPH recovery is highly dependant on temperature and varies less with pressure. Extractions done at 400 atm had a slight negative impact on recoveries. When the data at 400 atm are eliminated, a Two-way ANOVA shows that both temperature and pressure is correlated to recovery ($p<0.05$). A multiple regression analysis of the 300 and 350 atm data showed that the percent of TPH recovery = 0.713(temperature) + 0.107(pressure) -25.18 (r^2 =0.891). In this study, 100% recovery was the maximum recovery, which was observed when the conditions were set at at 120°C and 300 atm . The recovery achieved at these conditions is 2x that observed using the EPA recommended method. These results suggest that higher temperatures result in higher recoveries, while extreme pressures may inhibit recovery.

Current research supports this finding (Hawthorne *et al*, 1994). Studies involving the extraction of PCBs and PAHs from standard reference materials agree that temperature is more important than pressure for achieving high extraction efficiencies when interactions between pollutant molecules and sample matrices are strong (Langenfeld *et al*, 1993). Langenfeld *et al* (1995) suggests that high temperature conditions are required to thermally decouple the analyte/matrix complex. This would result in both improved recoveries and faster extraction kinetics. In addition, high temperatures may have a matrix altering effect which can also improve extraction efficiency (Langenfeld *et al*,1993). These benefits are independent of the solvent properties that can be described using solubility parameters.

4.4 Native TPH extractions in contrast to literature.

Although no supercritical extractions have been performed for TPHs in sediment, several studies have described extractions using soil

matrices. Bicking *et al* (1993) modelled hexadecane recovery from Celite over a similar temperature range. In contrast to our TPH study, increasing temperatures resulted in significantly decreased recoveries of hexadecane when similar pressures are compared (300 to 350 atm). Using this preliminary data, subsequent experiments involving the extraction of native TPHs from soil were performed at their observed optimal conditions: 55°C and 290 atm. However, there are several problems with the determined optima: (a) they never establish that hexadecane is a valid surrogate for the entire TPH group of contaminants, (b) it has been shown that spiked analytes do not respond in the same manner as native analytes, and (c) it is assumed that the Celite matrix is a representative model for natural soil matrices when in actual fact they are very different. According to the regression analysis performed for our data, the extraction conditions recommended by Bicking *et al* (1993) would result in 45% recovery in comparison to our highest recovery observed at 120°C and 300 atm.

Lopez-Avila *et al* (1992) examined various soil samples for TPH contamination. Extraction times were significantly longer (30 to 120 minutes) in comparison to this report (<20 minutes). This discrepancy can be explained since (a) they used a much larger 3g sediment sample, (b) no static extraction phase was routinely included, and (c) the infrared detection procedure required an extra sample clean-up step. They performed supercritical CO_2 extractions using US EPA suggested extraction conditions on 17 different soil matrices and standard reference materials. Resulting recoveries were generally 80% or better in comparison to Soxhlet extractions. Our best recoveries were 136% in comparison to recoveries from extractions employing US EPA extraction conditions. In light of these results, it can be hypothesized that their recoveries could approach or surpass 100% just by raising the temperature of extraction. Their method had a average relative standard deviation of 20%, while results presented in figure 7 demonstrated a tighter average relative standard deviation of 3.4%.

A study by Hawthorne *et al* (1993) focussed on evaluating the field performance of the US EPA supercritical method for TPH extractions. Again, Soxhlet extractions were reported to be 20% greater than 30 minute supercritical extractions. If the soil matrices they tested behave the same way as Bay of Quinte sediment, perhaps recovery can be improved by increasing extraction temperatures. Field trials using shorter extraction times (10 minutes) were consistent with a relative standard deviation typically less than 10%. However, recoveries were poor. It would have been interesting to test if a static extraction step could have improved the rate of dynamic recovery thus allowing the short field extraction method to be more efficient.

The extraction of native coprostanol from wastewater contaminated sediments would be a significant contribution since traditional Soxhlet methods are so time consuming and labour intensive. Recoveries of coprostanol using sub-optimal supercritical extraction conditions were on average 7% greater than Soxhlet recoveries. Although the results presented in this paper are only preliminary, it is reasonable to suggest that supercritical extraction of coprostanol is both quantitative and quick. Further study in this area may prove to be most fruitful.

Contamination of the pressurized CO_2 was a problem. The size of the contaminant peaks are directly related to the volume of supercritical CO_2 used in the extraction. The relationship is represented in the equation (contamination in mg) = 0.0101(supercritical CO_2 in mL) - 0.03 having an r^2= 0.95 with an n of 4 (data not shown). As a result, a 10 mL supercritical extraction will result in approximately 0.1 mg overestimation of TPH concentration, since TPH analysis involves summing the peak areas of all the peaks in the chromatogram (as determined by an FID detector). Checks confirmed that the contamination was coming from the CO_2 cylinder itself and not some other apparatus. Fortunately, these contaminant peaks were discrete and thus the chromatograms for the contaminated samples could be edited and re-integrated.

This study can be criticized since no modifiers were used. Modifiers can be used as co-solvents to alter the polarity of the supercritical solvent. Studies have shown that modifier use may increase the extraction efficiency of some target analytes (Myer *et al*, 1990). Although the ISCO SFX 2-10 is capable of modifier addition, it was thought that use of a modifier would unnecessarily complicate the extraction procedure. Modifier-free extractions are advantageous for

many reasons. As reported in this paper, effects of extraction conditions on extraction kinetics or recovery can be singularly attributed to the state of the supercritical CO_2. Solvent collection efficiencies are not complicated by the diluting effects of modifier depressurization (Lopez-Avila *et al*, 1992). Modifier fluids can be contaminated and thus increase the risk of chromatographic interference. Also, using a modifier requires two cumbersome solvent pumps, making future field applications impractical (Hawthorne *et al*, 1993).

Since extractions performed with modifier-free CO_2 resulted in high recoveries in comparison to solvent techniques, one may argue that this base study is necessary and that modifier addition may only slightly enhance the recoveries demonstrated here. Lopez-Avila *et al* (1992) evaluated several candidate modifiers for the extraction of TPH from soil matrices and found no significant advantage over extractions using supercritical CO_2 alone. However, it may be necessary to use modifiers for more difficult analytes, such as PAHs. One should not assume that modifier use will be ineffective in extracting TPHs from sediment and future work should be done to investigate this possibility.

5.0 Conclusion

The use of spiked analytes is a poor substitute for native analytes when developing methods for supercritical extractions. Consequently, quantitative analysis of sediment spiked with 6 PCB congeners was futile. However, spiked analytes are useful for calibrating the extraction efficiency and determining loss in the system. Losses of TPH due to volatilization and aerosol formation is minimized when a short dynamic extraction time is used and when the trapping solvent is cooled during collection. These two techniques result in an estimated loss of approximately 5%.

The extraction of native TPHs from Bay of Quinte sediment was quantitative, although no optima was reached. Recovery of TPHs from a 0.5g sediment sample using the US EPA supercritical method was 29% greater than that for solvent extractions. Raising the temperature from 80°C to 120°C resulted in a further 36% increase in TPH extraction. Recovery was correlated strongly with temperature and less dependant on the pressure conditions tested. Successful extraction of TPHs did not result from mimicking the the solubility characteristics of traditional solvents. Preliminary tests with coprostanol suggest that recoveries greater than 100% (vs Soxhlet) are possible.

Subsequent supercritical experiments should test the envelope of temperature and pressure conditions required to optimize TPH and coprostanol extraction from sediments. Further investigation of PCBs in sediments should involve the use of native analytes and larger sediment loads. Incorporating modifiers and specialized trapping solvent configurations may also improve recoveries and these options should be investigated in the future.

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Supplementary Discussion: Theoretical and practical considerations affecting the extraction of organic compounds from complex environmental matrices using supercritical CO_2

A. Conceptual models of analyte extraction

A fundamental understanding of the interaction of the matrix/analyte complex with supercritical fluid is necessary in order to optimize both SFE equipment and methods. SFE is a relatively young technique and theories concerning the dynamics of analyte extraction have been developing over the past 10 years. Early studies of the SFE technique were focused on the optimization of analyte solubility in the supercritical fluid. The work was done by chemical engineers who thought that extraction of analytes could be maximized primarily by altering pressure, and thus density conditions, of the supercritical CO_2.

This idea worked well for bulk extractions such as the extraction of fat from meat, or caffeine from coffee. All that was needed was for the scientist to match a solubility parameter of the supercritical fluid with that for the analyte. One such solubility parameter that was commonly used was the Hildebrand solubility coefficient (δ), where

$$\delta = P_c^{1/2}(\rho/\rho_l).$$

Here, the coefficient is related to the critical pressure of the fluid (P_c), and the density of the fluid in its supercritical (ρ) and liquid (ρ_l) states (Hawthorne 1990). From this relation, one can see that the solubility coefficient of the supercritical fluid is determined primarily by the density of the fluid. Both the temperature and pressure of the supercritical fluid can be regulated to modify its density, and therefore its solubility coefficient. Early work concentrated on the use of pressure to control the density of the supercritical fluid and little attention was paid to temperature.

Problems arose when the SFE technique was modified for environmental applications, especially soil and sediment samples. Discrepancies were noticed between theoretical extraction efficiency and experimental results. The reason was that the environmental analytes are found at trace levels, in contrast to original engineering applications where the analyte often constituted more than 1% of the matrix (Pawliszyn 1993). It was found that maximizing the solubility parameters of the supercritical fluid was not enough to extract trace environmental analytes. Another model was needed that would take into account the strong matrix effect experienced when attempting to SFE analytes at ppm and ppb levels from complex matrices.

The effect of complex matices

Recent studies suggest that partitioning of the analyte from the matrix to the supercritical fluid has historically been oversimplified. A more complex model is necessary since environmental matrices are very heterogeneous. Soils and sediments, as an example, consist of many phases which differ in their ability to retain analytes.

Current models hypothesize that thermal and kinetic barriers exist, barriers which are independent of the solubility of the analyte in the supercritical fluid. Erkey *et al* (1993) suggests a two phase model where the analyte is either deposited on the surface of the particle or adsorbed into it. They suggest that the analyte in the deposited phase is removed simply by dissolution in the supercritical fluid. However, unlike previous models, they recognize that the analyte in the adsorbed phase requires more energy to be extracted and is controlled by adsorption/desorption equilibrium kinetics.

Pawliszyn (1993) further describes these interactions by reducing the matrices to unit particles. A particle consists of two phases, an inert core and an organic outer coating. The particle core is solid, by definition, and the analyte is only able to adsorb onto its surface. The organic outer coating is discontinuous and varies in thickness. For the analyte to leave the particle during the SFE, it must be desorbed from the surface of the solid core, diffuse through the organic layer, desorb from the surface of the organic layer, and dissolve in the supercritical fluid.

Another level of complexity exists. Samples may differ in the shape and porosity of the unit particle. A sample whose particles are convoluted with many deep pockets will experience slower extraction dynamics since the supercritical fluid will not flow directly through the

sample. The Pawliszyn (1993) model accommodates for tortuosity of the flow path of the supercritical fluid as well as and the diffusion of the analyte through the fluid from areas where the fluid is stagnant to areas where it is flushing.

One can see that increasing the thermal energy of the analyte would increase its activity so that it may be more easily swept away by the supercritical fluid. Pawliszyn's (1993) model is analogous to what happens to an analyte during gas chromatography; a carrier gas sweeping analytes through a film coated column.

The analyte/matrix complex needs to be thermally decoupled. Increasing the temperature of the extraction will cause the analyte/matrix complex to destabilize due to the net increase in the energy and activity of the molecules. In addition to this thermal advantage, increasing SFE temperatures will kinetically activate the analyte molecules. Partitioning of the analyte into the supercritical fluid will be quicker. As a result, extractions will require much less time and resources.

The supercritical fluid itself becomes activated at high temperatures. As a result, the overall resistance of the matrix decreases since the fluid becomes more penetrative and pervasive. The fluid molecules also become more competitive for analyte binding sites. The overall advantage is faster and higher recoveries of analytes. This study, and others (Langenfeld *et al*, 1995; Hawthorne *et al*, 1994) have demonstrated that increasing temperatures improve the rate and efficiency of extraction of organic contaminants.

However, one must keep in mind that analyte solubility is still a priority. Temperature increases result in decreased density and changes in supercritical fluid solubility. Suitable solubility ranges can be achieved by readjusting the pressure so that the density of the fluid remains optimal.

B. Complications arising from using environmental matrices

Early SFE test work has been done using simple matrices like glass beads, filter papers, sand, or Florisil. These diagnostic experiments were necessary in order to understand and develop the SFE technique for use with naturally contaminated environmental matrices. It has been difficult to transfer the methods developed for these surrogates, since naturally contaminated samples are not comparable.

Natural sample matrices contain varied amounts of organic mater. The presence of organic matter increases the particles capacity to bind contaminants. Native analytes form many complex physio-chemical associations with heterogeneous binding sites located in many phases of a matrix particle. Studies using artificial matrices with no organic matter are fundamentally incapable of mimicking naturally contaminated matrices and they should not be used as substitutes for environmental samples when developing methods.

SFE of complex environmental matrices often results in the collection of much more than the analyte. Coextractives such as non-target organic compounds and sulphur compounds can impair restrictor performance. Coextractives can accumulate at the pinched end of the restrictor, the bottle-neck point at which the fluid flow is regulated. Extracted material may also precipitate along the length of the restrictor where the supercritical fluid cools and depressurizes. This may result in slow fluid flow rates and inefficient extractions. However, in the worst case, the sample may be lost altogether if the restrictor becomes clogged. Restrictor plugging will be discussed further below. It remains as one of the major obstacles to successful SFE.

Large amounts of coextracted matter may also interfere with chromatographic resolution of the analyte. Peaks from sulphur containing compounds and non-target organic compounds may co-elute with the analyte. In addition, baseline noise and drift can occur. These interference problems can be appreciable when looking for trace organics.

One must also control for water content of the natural soil or sediment since it may vary from sample to sample. Water acts as a modifier. Depending on the analyte, it has been known to both inhibit and enhance extraction efficiency. It is suspected that the extraction of non-polar analytes may be inhibited by water by interfering with the solubility of the analyte and prevent the penetration of the supercritical fluid.

Water can also reversibly slow or stop restrictor flow by the formation of ice particles. Ice particles can collect along the length of the restrictor or at the tip. There are several approaches suggested (Levy *et al,* 1995) to perform SFE on wet matrices: (a) use of an absorbent, (b) removal of water by freeze drying, heating or air drying, (c) prextraction of water at low solvent densities, and (d) insert an in line secondary effluent absorbent filter. All of these methods result in some degree of analyte loss, either thought volatilization or secondary adsorption.

As one can see, environmental matrices differ from simple experimental matrices. Another facet of this argument concerns the method of contamination. Spiked analytes are commonly used as surrogates for native organic contaminants. However, spiked analytes do not associate with complex environmental matrices in the same manner as native analytes. Spiked analytes are only weakly associated with sample matrices and are removed relatively easily. This phenomena has been demonstrated in this report as well as in others (Burford *et al,* 1993; Hawthorne *et al,* 1993; Langenfeld *et al,* 1995). Although spiked analytes are useful for assessing the efficiency of the collection method (Hartonen *et al,* 1994), they should never be used as a surrogate for native analytes when developing SFE methods.

C. Practical considerations

Restrictors

Restrictor plugging can be a major obstacle when extracting analytes from soil or sediment matrices. Analyte transfer between the sample vessel and the end of the restrictor has also been identified as a site of loss (Thomson and Chesney 1991). Recent studies suggest heating the length of the restrictor from 50 to 250°C (Porter *et al,* 1992; Burford *et al,* 1992). Heating the restrictor prevents the precipitation of extracted material and water and promotes constant flow rates. The design of the heated restrictor presented in the accompanying paper was functionally successful as well as being fairly simple and inexpensive to fabricate.

Heat is supplied to the whole length of the restrictor, except for 5 mm of the end which penetrates the surface of the trapping solvent. The apparatus is made of flexible Teflon which made it heat resistant and easy to handle. The layered design concentrated heat near the restrictor thus decreasing heating of the trapping solvent and consequently reducing volatilization. A glass cover protects the last 8 cm of the heater and prevents cross-contamination between samples since it can be efficiently rinsed. The epoxy seal prevents backflow of trapping solvent. Initial tests confirmed that residues from the epoxy quickly leaches and the epoxy soon becomes inert. The fixed flow stainless steel restrictor employed in this study worked comfortably between 0.7 to 1.0 mL/minute of supercritical CO_2.

The newest restrictors on the market are coaxially heated in a similar manner as above. They can be purchased with a fixed flow rates or with a manually controlled variable flow rate valves (Levy *et al,* 1995). Care must be taken to ensure that the temperature of the restrictor does not vary greatly from the temperature of the extraction vessel because the analyte may precipitate out of the supercritical fluid if its solubility properties are greatly changed.

Analyte collection

Depressurization of analyte into a solvent is an easy way to collect an analyte after SFE. It has many advantages over other collection strategies (Thomson and Chesney 1991). The trapping solvent can be evaporated to obtain a more concentrated product. It can be subjected to various cleanup procedures if analyte selectivity is a concern. Also, analytical flexibility is maximized since the solvent product can be examined repeatedly using one or more different instruments.

To avoid an additional concentration of the final solvent product, it is beneficial to use low volumes of trapping solvent. However, one must use enough solvent so that the path length of the depressurizing gas is adequate to trap the analyte. There are several helpful strategies that lead to minimized solvent use and maximized collection efficiency.

Resting the tip of the restrictor against the bottom of the collection vial results in gas flow resistance, a reduction of the its path length, and highly vigorous frothing. Inserting the tip of the restrictor into the meniscus of the trapping solvent is comparatively more efficient. The path length of the depressurized gas doubles since it has to travel

both down through the solvent and back up. Analytes are trapped more efficiently (Porter *et al*, 1992). Frothing and aerosol emission is minimised since the speed of the depressurised gas is dissipated more effectively. However, caution must be exercised. As the experiment progresses, solvent is inevitably lost and the tip of the restrictor must be adjusted so that it remains below the meniscus of the solvent.

Minimizing aerosol formation is important since the aerosols are capable of carrying analyte out of the trapping vial. Again, longer path lengths inhibit aerosol formation. Larger solvent volumes increase the path length of the depressurizing gas. It also dilutes the sample so that the concentration of the analyte on the aerosol is reduced. It is also important to choose a collection vial wide enough to prevent the propulsion of aerosol and/or solvent up and out between the restrictor apparatus and the collection vial.

Experiments involving cryogenic cooling of the trapping solvent have demonstrated comparatively better recoveries for some compounds (Porter *et al*, 1992; and this study). This may be due to decreased volatilization of high vapour pressure analytes. *In situ* cooling occurs as the supercritical fluid decompresses, but this process depends on minimum flow rates which are difficult to maintain. External cryogenic cooling is recommended but is fairly cumbersome.

Minimizing the time of the dynamic extraction phase will also reduce loss of analytes. Partially replacing some of the dynamic extraction phase with an initial static phase serves two purposes: (a) it allows time for the analyte to interact with and dissolve into the supercritical fluid in the extraction chamber and (b) there is a reduction in aerosol formation and volatilization since the sparging time of the collection solvent by the supercritical fluid during the dynamic phase is decreased.

Miller *et al* (1993) has experimented with short dynamic extraction bursts (3-30 seconds) in an effort to reduce the loss of analytes. Collection efficiencies of PCBs, PAHs, gasoline, and diesel fuel were greater than 90%. Their study supports findings in this report which suggest that static extractions combined with short dynamic extractions will maximize recovery while minimizing loss.

Although solvent collection is the most common analyte trapping technique, other methods exist. Solventless trapping involves expelling the supercritical CO_2 into an empty container. This generally results in poor recovery (Ashraf-Khorassani *et al*, 1992). However, solventless trapping experiments using rapid depressurization, such as that employed by Miller *et al* (1993), have resulted in high analyte recoveries.

The analyte can also be trapped using a solid phase column. The solid phase is then eluted with an appropriate solvent. Efficient recoveries have been demonstrated using a solid phase especially when the trap is cryogenically cooled (Levy and Houck, 1993; Ashraf-Khorassani *et al*, 1992). However, use of modifier often complicates the solid phase trapping because it collects on the solid phase and may prematurely elute the analyte (Hawthorne *et al*, 1993). Also, regular use of a single solid phase trap may result in the cross-contamination of samples with both analyte and interference substances.

In contrast to these off-line collection procedures, on-line collection involves diverting the pressurized extract directly to an analytical instrument. Depressurization of analytes onto the inlet ports of HRGC (Onuska and Terry, 1989), HPLC (Janda *et al*, 1993), and ICPMS (Blake *et al*, 1994) have been described. Off-line analysis is highly sensitive, however, there is no opportunity for the sample to be cleaned-up or archived.

D. Optimization vs Standardization

When optimizing an SFE method, one must take into account both the properties of the analyte and the properties of the matrix. The physico-chemical properties of the analyte never change. However, the nature of an environmental matrix, in this case soil or sediment, changes from sample to sample. The problem then arises that if one optimizes an SFE method for the extraction of PCBs (as an example) from site A sediment, will that method for that analyte be equally effective for sediment from site B?

Traditional extraction methods (Soxhlet, sonication, ect.) focus on the optimal solvent that would maximize the extraction of an analyte or analyte class. The large volumes of solvent and long extraction times allow for a greater variation in

the physico-chemical properties of the matrix. However, not all sediments are similar. The sensitivity of SFE to the properties of a matrix has yet to be explored. Porosity, organic and water content, particle size, void volume, and biotic content are some factors that influence the extractability of analytes from sediment. It is suspected that the matrix may have a substantial effect on the efficiency of the extraction of the analyte and that one extraction procedure may be inadequate for all such matrices.

If this is true, it will be difficult to standardize one set of SFE conditions for one analyte. This will make it difficult to compare samples from different sites. Compounding this problem is the fact that equipment differences have not yet been adequately assessed. SFE efficiency is very sensitive to extraction cell dimensions and volume, restrictor performance, and the analyte collection mechanism. This is unlike traditional solvent extractions where equipment variations do not have significant effects on extraction efficiency.

Conclusions

Current theoretical understanding of SFE has enabled us to develop mathematical models that can predict the recovery kinetics of analytes from well defined complex matrices. The model suggests that high temperature extractions are advantageous in order to thermally decouple the analyte/matrix complex and accelerate the kinetics of the extraction.

These theoretical advances are plagued by technical problems. However, it is expected that restrictor performance and analyte collection techniques will eventually improve to the point where quantitative extractions can be done even on the most difficult matrices.

The largest obstacle to wide scale use of SFE technology may be method standardization. Unlike traditional extraction methods, SFE is especially sensitive to matrix properties, instrument design, and operator experience. However, as in all new areas of research, time is needed for scientists to develop and advance new methods and equipment.

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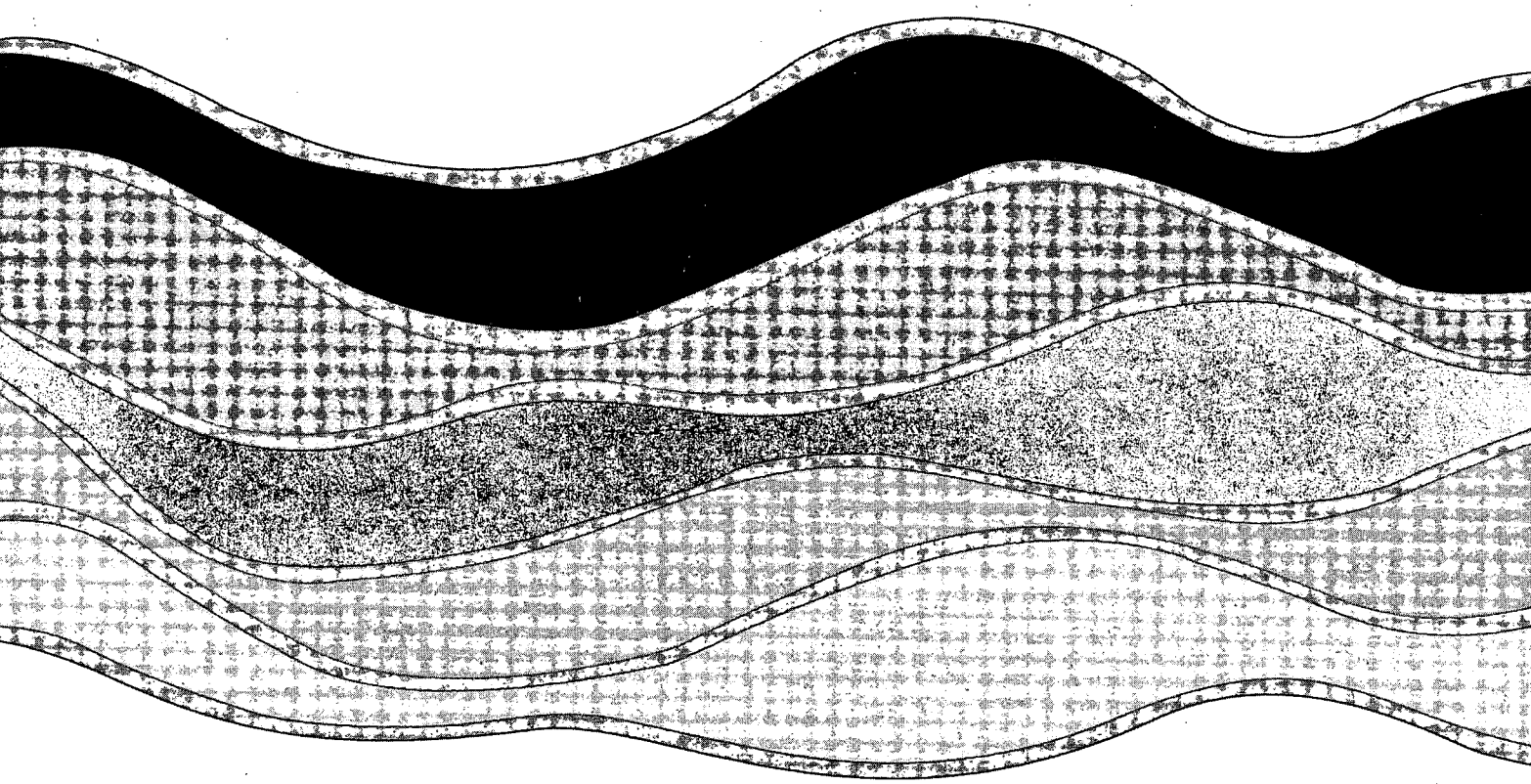

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