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Phytoforensics: Soil and Groundwater Sampling Without Soil or Groundwater!

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PHYTOFORENSICS: SOIL AND GROUNDWATER SAMPLING WITHOUT SOIL OR GROUNDWATER!

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ABSTRACT

Plants directly interact with surrounding water, air, and soil, collecting and storing chemicals and elements from the surrounding environment. Tree coring methods have shown that groundwater contamination can be assessed without directly sampling the groundwater. In this work, two new and innovative sampling methods that place sampling devices inside the plant, i.e. "*in-planta*", were developed to access this valuable data that can direct and perhaps replace traditional methods for contaminated-site investigations. Traditional site assessments may be limited due to time, site access, and expense, resulting in incomplete understanding of the contaminated plumes and inefficient remedial approaches. The new techniques presented include placing established solid phase microextraction fibers (SPMEs) and newly developed solid phase samplers (SPSs) that have greater sensitivity and reproducibility and can also provide repeated sampling of the same trees with minimal damage, offering new possibilities in using plants to monitor contaminated sites as well as doing initial investigations. These methods are also much faster and can be accomplished with little of no property and ecological damage, and with amazing acceptance by property owners.

INTRODUCTION

Field site investigations using groundwater sampling can be very time consuming, expensive 'per sample' costs, and have big mobilization costs. As well, most of the time there is not enough information and direction for initial placement of groundwater wells. Methods to reduce labor, time, cost, and environmental disruption are needed. Studies using tree cores collected from contaminated sites have shown VOC concentrations in plants correlate with the groundwater and soil vapor concentration of VOCs. Previous research has proven that cores can be taken from the tree and analyzed using gas chromatography to determine contamination within the subsurface, particularly for chlorinated solvents (Vroblesky et al. 1999; Larsen et al. 2008, Struckhoff et al 2005). Previous research has also modeled partitioning coefficients from wood to water of contaminants to understand more accurately the correlation between concentrations of contaminants in cores to groundwater concentration (Baduru 2008). Although this modeling can be used, the heterogeneity of the cores leaves a range of unpredictability and error, and the sensitivity is not fully understood relative to environmental conditions. Vroblesky and colleagues clearly showed that a simulated rainfall event can lead to changes in tree core analysis results in a matter of hours (Vroblesky, et al 2004). In order to improve the use of plants for environmental assessment and monitoring, new breakthroughs in analytical chemistry can be implemented.

One of the new analytical methods that have promise uses Solid Phase Microextraction (SPME) sampling. SPME samplers consist of fibers of varying matrixes that have high affinities for different chemicals. SPME samplers passively extract the VOCs from a sample matrix and then can introduce the entire sample into a gas chromatograph for analysis (Skaates et al., 2005; Legind et al., 2007) or can be extracted into minute volumes of solvent for liquid chromatography. Using SPME fibers can also be very rapidly analyzed and used repeatedly. This can allow for sampling of mixed matrices as well. SPME fibers can sample water, air, slurries, and have even been used in plant sampling for food contamination (Lord 2004).

Another sampling method used for environmental monitoring is solid phase passive samplers. Semipermeable Membrane Device (SPMD) is a sampling device designed to sample hydrophobic semivolatile organic contaminants from water and air. The SPMD consists of a neutral, high molecular weight lipid such as triolein which is encased in a thin-walled polyethylene membrane tube. Another passive sampler uses Polydimethylsiloxane (PDMS) as the matrix to absorb the contaminant (Laak 2008). Using this concept of

passive samplers, a new sampling device and method was developed to sample contamination in trees.

In this research, novel analytical methods were brought into the trees, in the first *in-planta* sampling methods development. *In*-planta methods place a high affinity solid phase sampling device in the tree, rather than taking a small portion of the tree to the laboratory. Advantages herein reveal improved sensitivity and reproducibility. Additionally coring the tree results in damage to the trunk and frequent sampling is not possible without significantly damaging or causing the death of the trees (Gopalakrishnan 2007). The following results show there clearly is great potential for this application and the patent-pending technology may greatly increase the accuracy of Phase I site investigations and concurrently decrease costs and damage to property and the environment. Placing these sampling devices inside the trees on site, we can sample trees naturally occurring on a contaminated site or those planted in phytoremediation or redevelopment efforts, evaluate the plume size, and even monitor changes in concentration. These methods will have a minimal footprint and can be accomplished with little materials cost, time, or labor demands. These quick sampling techniques can provide an array of data within a short amount of time to help the efficiency in placement of groundwater monitoring wells, saving time and money as well as undue impact to the ecosystems at hand or personal property.

MATERIALS AND METHODS

Tree Coring The tree cores obtained during this project were taken with a 0.5 cm diameter increment borer manufactured by Forestry Services, Inc. Tree cores were taken either 30 cm above the ground surface or at breast height depending on the diameter of the tree. Tree cores were immediately stored in 20 mL headspace vials caped with Teflon coated septa and crimp tops until analysis. Cores were allowed to equilibrate for ~24 hours in all analyses. Headspace concentrations were then determined using headspace analysis using a Tekmar 7000 headspace autosampler and a HP 5890 gas chromatograph with electron capture detection.

Solid Phase Microextraction (SPME) Dilution vials of chloroethenes were made up using chloroethenes in PDMS stock solution of concentration of 1 g/L. The standards were made with a dilution rate of 10% in 25 mL glass vials containing 5 mL of PDMS. The vials were then capped with Teflon septa caps to seal off air exchange. Allowing the vials to equilibrate with the headspace overnight, the next day SPME-PDMS fibers were exposed for two minutes and run in the GC in duplicates.

SPS development and Testing A new sampling device, Solid Phase Sampler (SPS), consisting of PDMS tubing was designed for *in-planta* sampling. The tubing is permeable and absorbs the contaminant into its matrix. The mass of the tube is much greater than a SPME fiber; therefore, more contaminant can absorb into the tube allowing for detection levels higher than SPME.

SPSs were constructed and exposed to a steady concentration of PCE and TCE to evaluate absorption rates. SPSs were constructed using polydimethyl silicone (PDMS) tubing cut into sections with mass ~0.5g. Mass was accurately determined and recorded, and each section was placed on a threaded stainless steel #4, 1 ¼" bolt and secured with a nut, Figure 1. SPSs were placed in methanol for two days and allowed to dry under a hood to remove any contamination from production or shipping and storage. The SPS's were then placed in an incubator for 2 days at 100°C. The tubes were then cooled off and placed into a 100 mL beaker within a 300 mL screw top jar also containing a layer of PDMS oil dosed with PCE/TCE at a concentration of 10 ppm, Figure 2. This controlled the chemical activity (i.e. concentration) in the gas phase at low levels, without depleting the mass via absorption into the SPSs. There was no direct contact of SPSs with PDMS oil containing PCE/TCE. The tubes were placed within the PCE/TCE environment at the same time. To determine the uptake rates, one SPS was removed at varying times: 1 hour, 2 hour, 12 hour, 24 hr/1 day, 2 days, 3 days, 4.25 days, 7 days, 11 days, and 14 days. When a SPS was removed from the vial with tweezers, the tube was placed within a 20 mL headspace sampling vial and immediately capped then stored at 4 ˚C. Once all SPSs were removed, they were run at once in a headspace autosampler at 35 ˚C with direct injection to an HP 5890 GC with ECD for detection. The data was plotted versus exposure time.

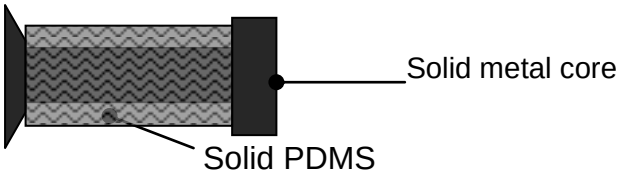

Figure 1. Solid Phase Sampler (SPS) assembly. PDMS mass was 0.5 g with a thickness of 3 mm and an outer diameter of 4 mm.

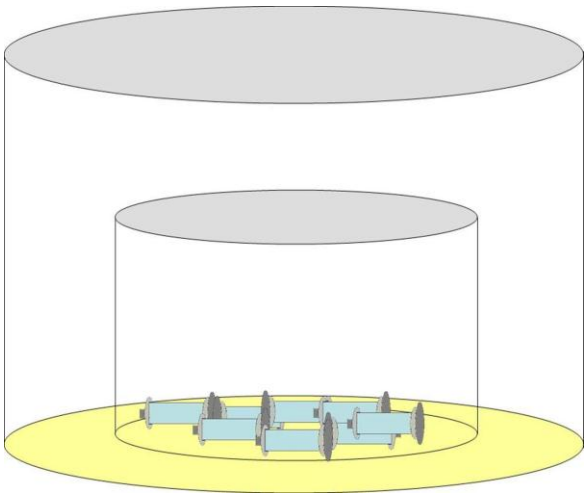

Figure 2 SPSs were placed in an open beaker inside a closed beaker containing PCE and TCE dosed PDMS oil.

Comparison of SPSs Versus Cores To compare the affinity of tree cores and the SPSs, the two materials were compared in side by side testing. As tree cores are highly variable in their collection and the chemical composition (Gopalakrishnan, et al. In Press) surrogate, uniform xylem tissue was used and constructed by cutting poplar dowel rods at a mass of ~0.5g, diameter 0.4 cm, and the mass of each was recorded. The SPSs and surrogate cores were placed in a 100 mL beaker, as noted above, with an added aluminum foil divider placed in the center to separate the cores from the SPSs, Figure 3. The SPSs and cores were exposed for 3 weeks to PCE and TCE at a concentration of 10 ppm allowing them both to come to equilibrium with the surrounding environment. Partitioning coefficients for the solvents and PDMS oil were determined in related studies and is shown in supporting information. The resulting vapor concentration was calculated using partitioning coefficients of 2000 for PCE and 1200 for TCE. SPSs and cores were removed using tweezers and placed into separate vials and capped for analysis as noted above.

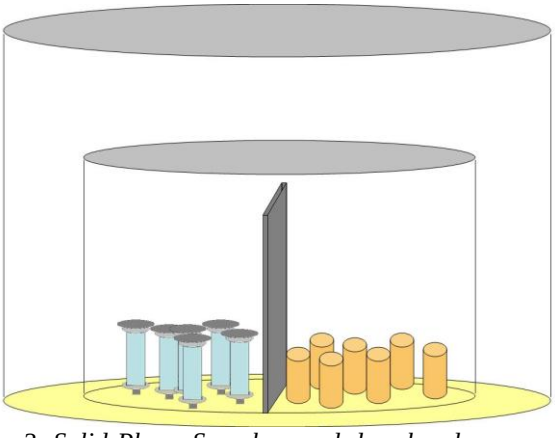

Figure 3. Solid Phase Samplers and dowel rods were placed in an open beaker inside a closed beaker containing PCE and TCE contaminated PDMS oil.

Comparison of PDMS-SPME, Carboxen-SPME, and Tree Cores To evaluate the relative sensitivity of different SPME methods, SPS analysis, and traditional tree coring methods, 4 methods were tested in the same contaminant activities. This testing also evaluates the linearity of the methods over a wide range of concentrations. Dilution vials of chloroethenes were made using chloroethenes in PDMS stock solution of concentration of 1 g/L. The standards were made with a dilution rate of 10% in 25 mL glass vials containing 5 mL of PDMS. The vials were then capped with Teflon septa caps to seal off air exchange.

Allowing the vials to equilibrate with the headspace overnight, the next day headspace analysis with a 1 mL air-tight syringe was performed on the vials in duplicates. After the initial headspace analysis, SPME-PDMS fibers were exposed for two minutes and run in the GC in duplicates. The inlet temperature was increased from 220°C to 250°C. This resolved the retention problem and results were obtained for the SPME-PDMS. Time-weighted average (TWA) analysis was then performed using a Carboxen fiber with z=5 mm for ten minutes. Next, multiple fibers were exposed at the same time in a large-mouthed glass vial with a Teflon septa cap. In order to compensate for more headspace, 25 mL of PDMS oil was used at the same concentrations as the original stock solutions. The fibers were exposed at 1, 2, 3, 4, 6, and 16 hours for 10 ppm concentration at z=5mm. One and two hour exposure times were also observed at concentration 100 ppm and 1 ppm.

Multiple Sampling of SPSs To evaluate the potential for multiple analyses of single SPS samplings, three SPSs were exposed to PCE and TCE in the environment using the method explained above (Figure 2.). After the SPSs had been allowed to equilibrate with the PCE/TCE environment, the SPS were removed and immediately vialed and capped. The tubes were then run with the GC in the autoheadspace sampler. Without removing the tubing from the vial, the tubes went through eight runs in the autosampler. The results were found using the mean value of peak area for the SPSs. The initial peak area was the baseline results. For every analysis, the percentage was found by dividing the peak area of a run by the baseline peak area.

Field Sampling Using SPME In New Haven, MO, PCE contaminated groundwater has impacted the city water supply and tree-core sampling was critical in delineating the sources on the contamination (Schumacher et al 2004). On the Kellwood Site (OU2) five trees were cored and then tested using *in-planta* SPME analysis. Cores were collected as previously described and in the borehole remaining, SPME analysis was conducted using time weighted average (TWA) methods using 100 μm Carboxen SPME fibers supplied by Supelco Analytical (Sigma-Aldrich Co., Bellafonte, Pennsylvania). The fibers were exposed in the trees at the New Haven Kellwood Site (OU2) site for 70 – 75 minutes, capped and transported to the Missouri S&T environmental engineering laboratory for analysis using an Agilent 6890 GC with ECD detection.

Field Sampling Using SPS Tygon (silicon) tubing was cut into pieces with a mass of 0.45g. The mass on the tubing was

limited by the length of the bolts to be used. The bolts used for this experiment were size #4, 1 ¼" length bolts. The SPSs were cleaned and assembled as mentioned previously. Each SPS was then individually wrapped in foil and placed into the oven for two hours at 100°C. Once the SPSs were removed from the oven, one SPS was removed from the foil and placed in a vial as a blank. The other SPSs remained individually wrapped within the foil. This foil was placed in a 1 L jar with a screw-on Teflon cap. This is to prevent any contamination of the SPSs.

On arrival at New Haven, one SPS was removed from its foil and placed into a vial and capped for a background analysis. Tree cores were taken and SPS was placed into all core holes. Tags were attached to the SPS for flagging on return trip to remove SPSs from trees. The SPS were unwrapped partially from its individual wrapping and then using the foil to hold onto the SPS, the SPS will be placed inside the core hole completely exposed. Then, a screw was placed in the hole to seal the headspace inside from the outside exposure.

Using gloves, the foil was removed from three SPSs and wire was wrapped around them. One SPS was then hung from each of the three trees from the wire to evaluate the background concentration and potential for cross contamination from the surrounding air at the VOC contaminated site. The SPSs were placed so it would not touch the tree. At the end of the sampling trip, a SPS was removed from the foil and placed into a vial as the trip background.

On the return trip to remove the SPSs from the trees, another SPS was removed from its foil and used as a third background. This was then vialed and capped. To remove the SPS from the tree, tweezers were used to extract the SPS from the tree hole. The SPSs were then immediately vialed and capped with the wire being cut from the tag. All of the samples were analyzed at the Missouri S&T environmental engineering laboratory using an Agilent 6890 GC with ECD detection.

RESULTS AND DISCUSSION

Sorption Rates for SPSs Results for the absorption rates showed a clear relationship for both PCE and TCE absorption, Figure 4. Absorption as measure by the mass transferred to the SPSs increased rapidly over the first 96 hours and then reached apparent equilibrium at approximately 10 days. Equilibrium was reached if the change was less than 1 % over 72 hours. A simple first order uptake model, Equation 1, was applied to each and fit to the data using sum of least squares. The first order uptake coefficients were determined to be 0.017 hr^{-1} and 0.024 hr^{-1} for PCE and TCE respectively.

$$A = A_{max} (1\text{-}e^{-kt}) \quad \text{(Equation 1)}$$

Where A = peak area, A_{max} = peak area at equilibrium, k = 1st order rate coefficient (hr^{-1}), t = time (hours).

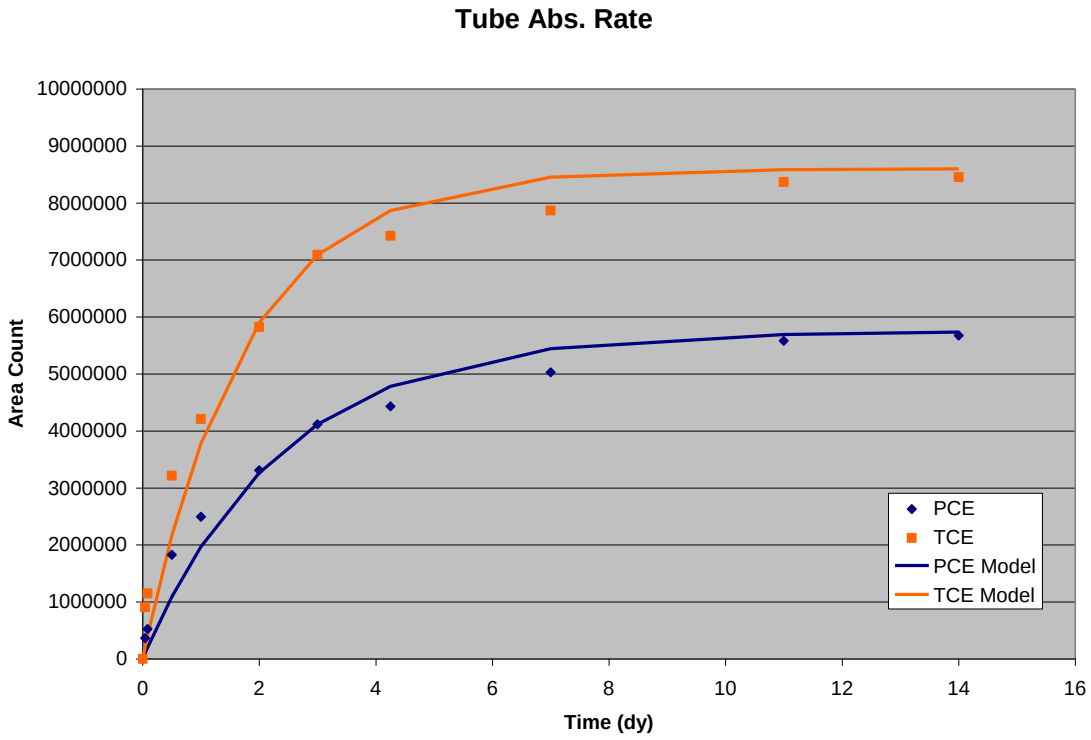

Figure 4. SPS-controlled absorption rate of PCE and TCE, showing equilibrium in approximately 10 days.

This experiment shows that the SPSs do take at least 8-10 days to equilibrate with their surroundings, assuming there are no limitations in the kinetics to supply the contaminants. This study also shows that while equilibrium may take many days, the predictable uptake can allow for rapid sampling after 1 or 2 days to get initial results, perhaps positive negative presence, and longer terms are needed for active equilibrium sampling with maximum sensitivity. While the sensitivity is beneficial for getting the lowest possible method detection limits, the predictability of the uptake lets short term sampling (24 hours) be extrapolated to actual equilibrium concentrations.

Comparison of SPS and Core Equilibrium Concentration The equilibrium comparison of cores and SPSs exposed to the same headspace concentration revealed that the SPSs were more sensitive for PCE while core and headspace analysis was slightly more sensitive for TCE, Figure 5. The SPS peak area response was 98% higher than the core analysis for PCE. The SPSs had lower variability for both PCE and TCE. As well, the SPSs were more reproducible. Although ten SPSs and dowel rods were dosed, only four are shown. The four dowel rods and SPSs shown are the four sets of samples that have a peak area closest to the mean peak area. All ten samples were analyzed for statistical findings. The average standard deviations for the peak area of the cores were 122428 and 84835 for PCE and TCE respectively. The average standard deviations for the peak area of the SPSs for PCE and TCE were 77987 and 20942 respectively. The 95% confidence interval was only 0.9% and 0.8% of the mean for SPS analysis of PCE and TCE respectively, where as these values were 2.7% and 2.4 % for the cores analyzed.

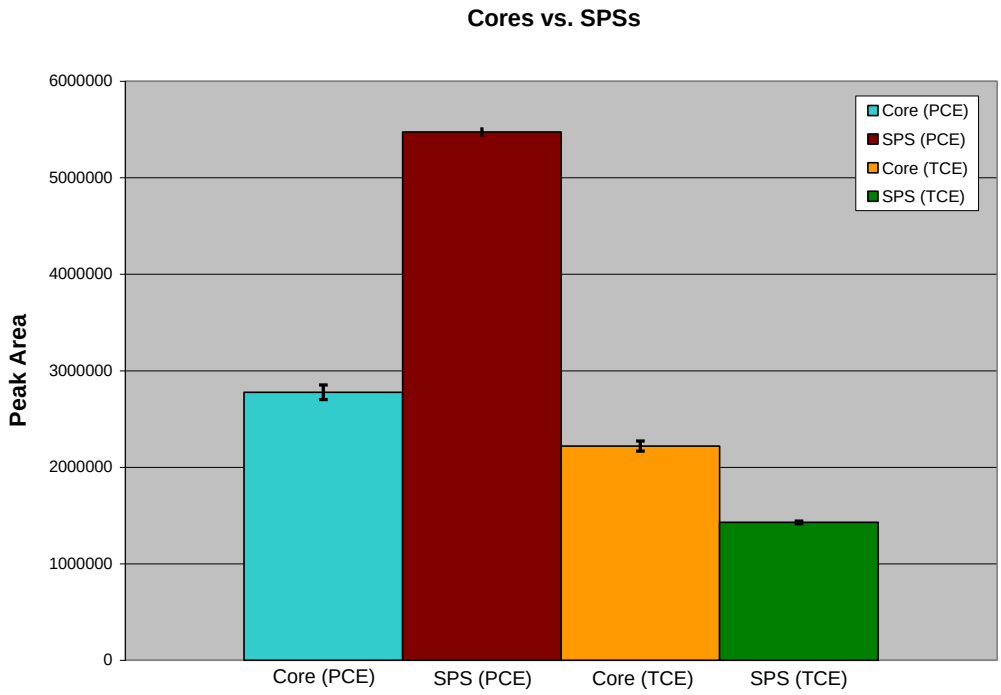

Figure 5. Ten samples of SPSs and Dowel Rods were averaged. When exposed to PCE and TCE under the same conditions, multiple replicates of SPSs have peak area sensitivity 96.8 % higher for PCE and 61% less for TCE than cores. For both PCE and TCE reproducibility was increased in SPSs compared to cores. SPSs had a variability of only 1.2% versus 4.9 % for the cores with PCE and 2.4% versus 7.2 % for the cores with TCE.

Comparison of SPME, SPME-TWA Analysis, and Tree Cores Comparison of Carboxen Time Weight Average (TWA) Analysis, SPME-PDMS analysis, and traditional headspace analysis resulted in the TWA analysis was much more sensitive to PCE and TCE, Figure 6. and Figure 7. respectively. If TWA analysis rules are adhered to, then as the time increases, the expected linear response will increase in sensitivity for these compounds (Sheehan 2009). The peak area response was close to four times higher for TWA for two hours exposure and had a slightly higher sensitivity for TWA for one hour exposure compared to headspace analysis. On the other hand SPME-PDMS had similar peak area sensitivity

compared to headspace analysis with TCE and more than twice the sensitivity in peak area with PCE. TWA analysis performed at a short time of 10 minute resulted in a peak area sensitivity of 22% lower compared to headspace analysis.

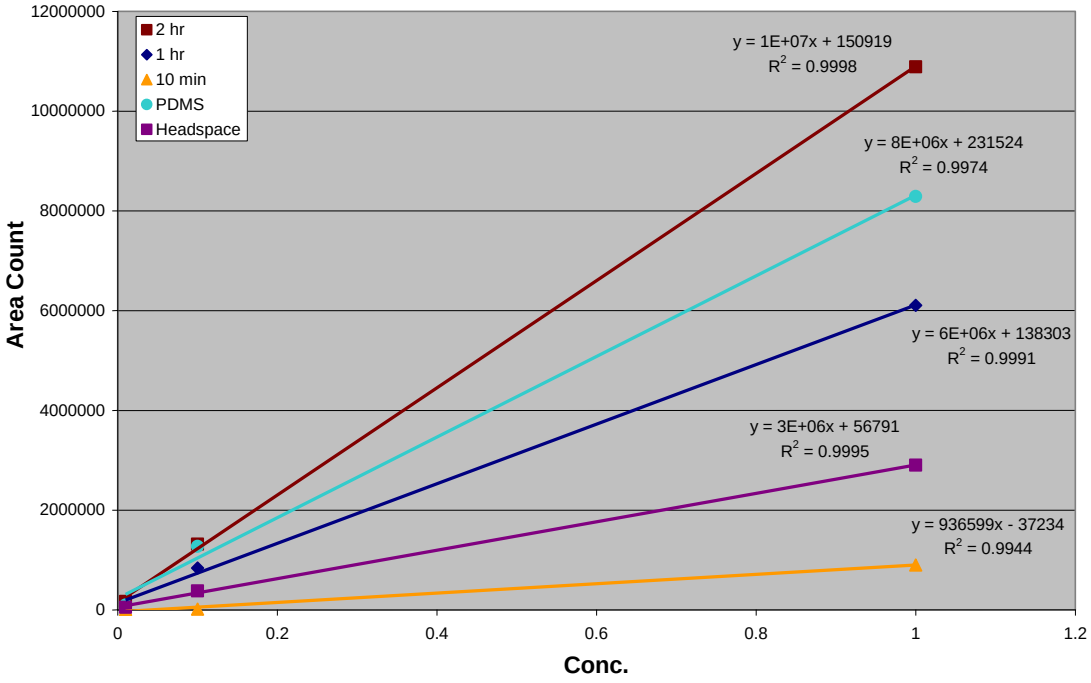

Figure 6. Comparison of Carboxen Time Weighted Average (TWA) Analysis, SPME-PDMS analysis, and traditional headspace analysis at different concentrations of PCE. TWA Analysis produces greater peak area sensitivity than SPME-PDMS and headspace analysis.

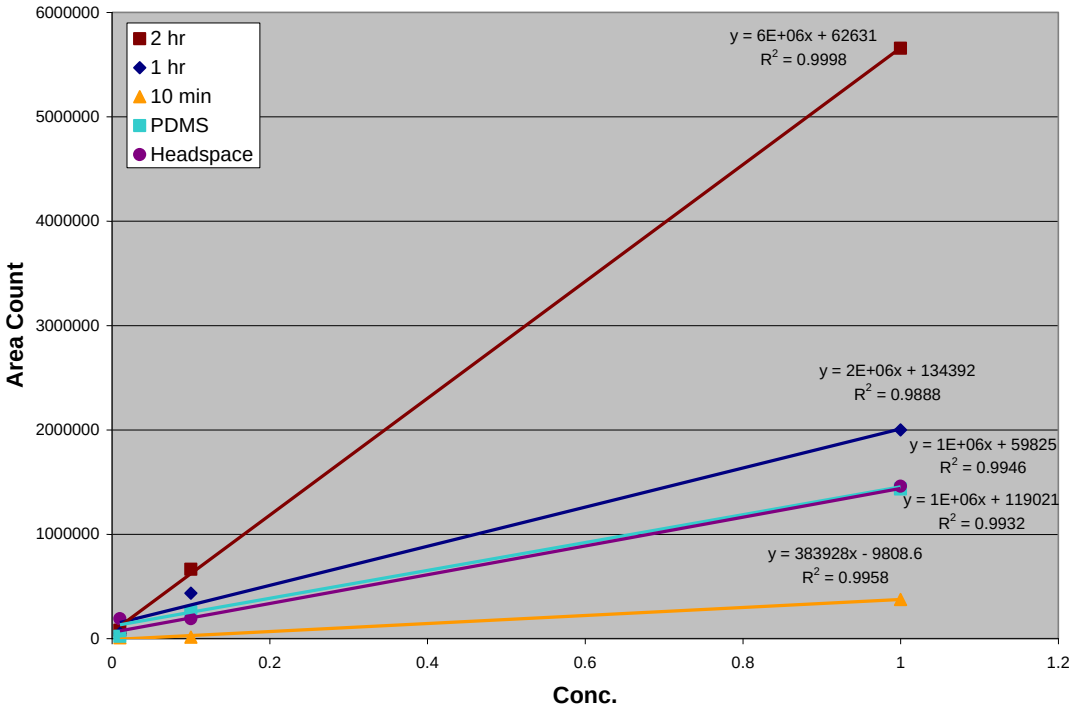

Figure 7. Comparison of TWA Analysis, SPME-PDMS analysis, and traditional headspace analysis at different concentrations of TCE. TWA Analysis produces greater peak area sensitivity than SPME-PDMS and headspace analysis.

Sequential Headspace Analysis of SPS Through repeat analysis of dosed SPSs, a set amount of PCE and TCE were removed after each sampling, Figure 8. After four runs, SPSs still contained over half of PCE and TCE within its matrix. This repeat analysis proves that even after an initial determination run, a known concentration was removed which allows for determination of initial concentration. This predictive decrease can help to determine analytical results under multiple analysis using different detectors. Standard deviation was found for PCE and TCE. The averaged standard deviation was found to be 3.4% for PCE and 3.9% for TCE.

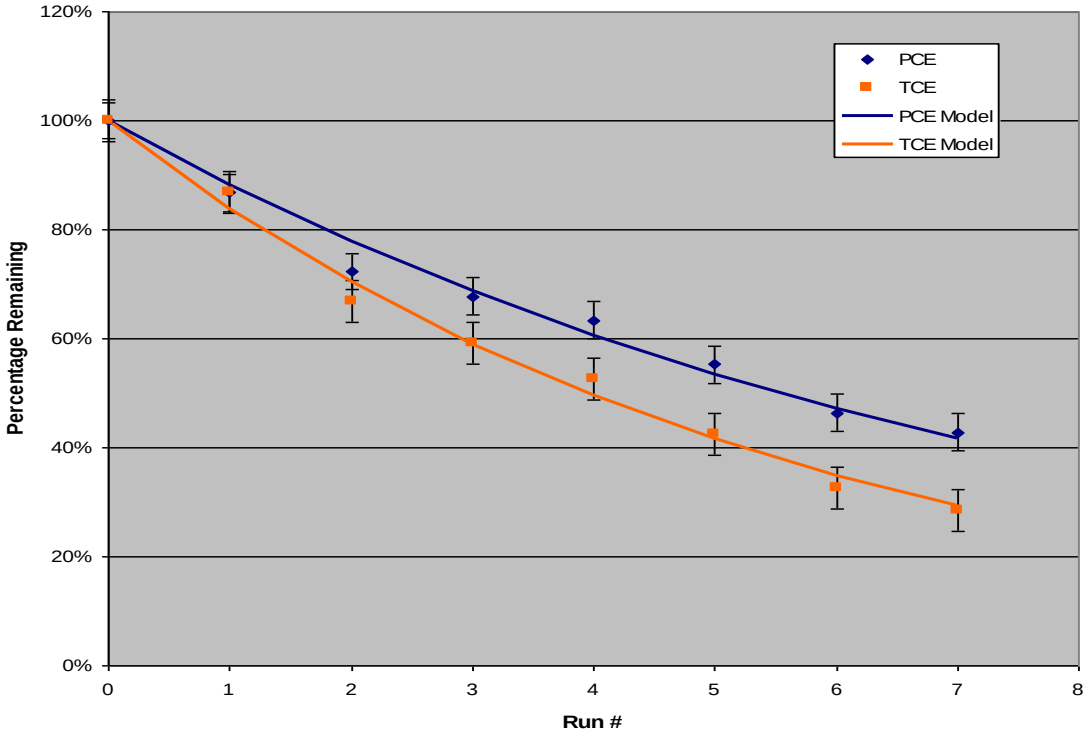

Figure 8. Repeat analysis of SPS analysis, showing that samples can be analyzed numerous times with predictable results. Standard Deviation for PCE is 3.4% and for TCE is 3.9%.

Field Comparison of *In-planta* SPME Methods, Tree Core Analysis, and SPS Methods Sampling of trees at the New Haven Kellwood Site (OU2) was conducted on 4 trees known to be contaminated from previous sampling. Results of tree core analysis using accepted methods revealed contamination of both TCE and PCE in the trees as well as the tree previously believe to be free of contamination, Figure 9. and Table 1. The *in-planta* SPME methods had peak areas 4 to 230 times higher using the same GC methods for analysis. Also, an average increase in the peak area of 13 times for TCE and 62 times greater for PCE was also detected. As well, SPSs used to sample reached similar results within the same log scale as the SPME fibers and resulted in higher sensitivity than tree cores. This analysis shows that SPME and SPS *in-planta* analysis have potential for providing improved method detection limits with similar variability in analysis. The SPME analysis also has the benefit of potentially rapid analysis.

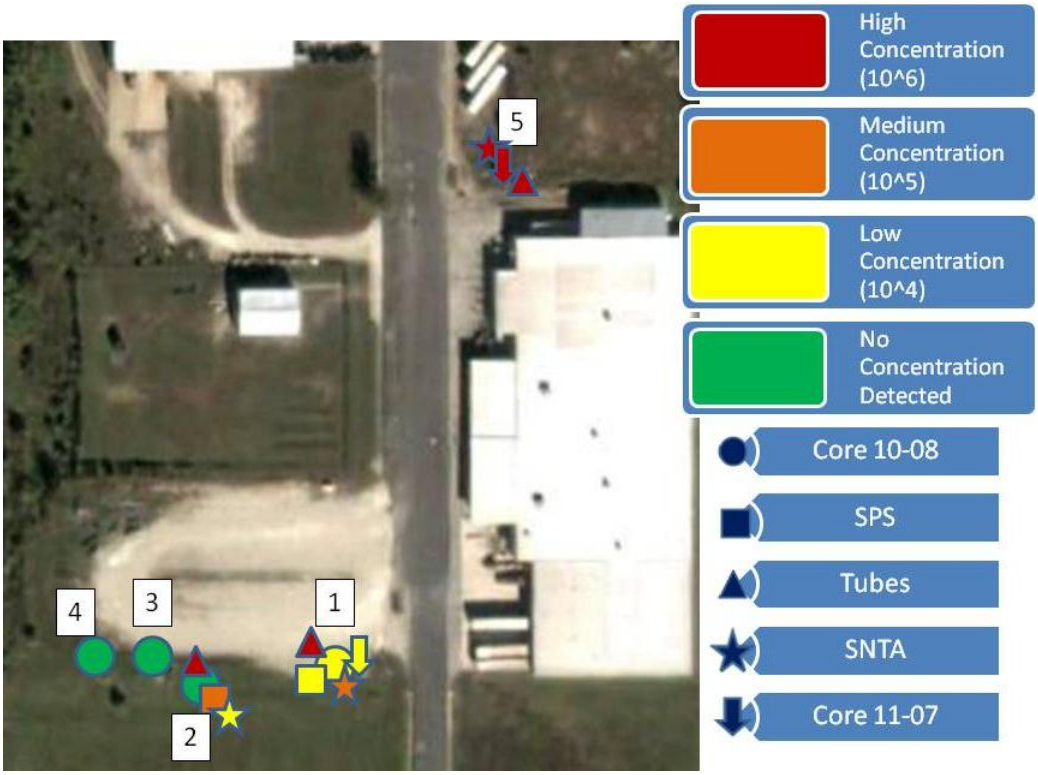

Figure 9. Site map of New Haven Kelwood Site (OU2) with repeat sampling information.

Table 1. Comparison of standard tree core, in-planta TWA, and in-planta SPS analysis. Values shown in peak area via GC-μECD.

Tree #	Cores-TCE	Cores-PCE	SPME-TCE	SPME-PCE	SPS-PCE
Tree 1	3.8×10^2	2.1×10^4	5.8×10^3	1.2×10^6	2.1×10^4
Tree 2	6.1×10^2	1.9×10^4	1.7×10^4	4.4×10^6	2.8×10^4
Tree 3	9.4×10^1	5.2×10^2	5.8×10^2	2.5×10^3	ND
Tree 4a	5.3×10^1	2.8×10^3	3.7×10^2	3.3×10^4	ND
Tree 4b	3.6×10^2	6.2×10^3	4.3×10^3	7.1×10^4	ND
Tree 5	ND	1.4×10^2	ND	7.2×10^3	7.7×10^5

FINDINGS

Using the SPME fibers and SPSs to sample trees in the field appears to have benefits relative to traditional tree coring analyses. These methods may improve the vegetation-sampling approaches that have great benefits for Phase I site assessments and also for monitoring groundwater concentrations at phytoremediation sites. Actual groundwater concentrations still require sampling groundwater wells, but these methods can give relative quantifications (Schumacher et. al. 2004, Ma 2002). Using plant sampling to gain relative quantifications, benefits can be gained that could not with groundwater monitoring such as minimal environmental or property disturbance as well as little materials cost. Sampling is accomplished with very little energy use or labor demands. As well, with the reproducibility of the SPME fiber and SPSs, groundwater monitoring can be replaced or become more efficient through these methods that are at the very infancy of development. Using these new methods, continuous groundwater sampling used in natural attenuation monitoring could also be replaced. This new approach is patent-pending and appears to have a bright future if optimized further.

ACKNOWLEDGEMENTS

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