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Determination of Chlorophenols in Soils Using Accelerated Solvent Extraction Combined with Solid-Phase Microextraction

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A method for the determination of chlorophenols in soil samples using accelerated solvent extraction (ASE) with water as the solvent combined with solid-phase microextraction (SPME) and GC/MS has been developed. Important ASE parameters, such as extraction temperature and time, were optimized using a spiked wetland soil. The effect of small amounts of organic modifiers on the extraction yields was studied. An extraction temperature of 125 °C and 10 min extractions performed three times proved optimal. Two ASE-SPME procedures without and with an organic modifier (5% acetonitrile) were evaluated with respect to precision and detection limits (LOD). The reproducibility of replicate water extractions/SPME determinations ($n = 6$) was in the range 7−20% relative standard deviation for the nine chlorophenols investigated. LOD values in the low-ppb range were achieved for all chlorophenols. The ASE-SPME procedure presented here was applied to the determination of chlorophenols in soil samples taken from contaminated areas near Bitterfeld, Germany.

Chlorinated phenols (CPs) belong to the most important contaminants in the environment (aqueous systems and soils) as a result of their widespread use in industry and agriculture and for domestic purposes for more than 50 years. It is well-known that chlorophenols are toxic at low levels. The more highly chlorinated phenols such as trichlorophenols and pentachlorophenol are also persistent.[1] Five of the chlorophenols (2-chlorophenol, 2,4-dichlorophenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol, and pentachlorophenol) have been classified as priority pollutants by the U.S. Environmental Protection Agency (EPA).[2]

Determining chlorophenols in soil samples usually involves various methods of liquid−solid extractions, e.g. Soxhlet,[3,4] microwave- or ultrasonic-assisted,[5,6] and accelerated solvent (ASE)[7] extraction, with an organic solvent or solvent mixture followed by both cleanup and preconcentration procedures.

Efforts are being made to reduce both the use of organic solvents and the time-consuming cleanup and preconcentration steps. One such approach is to apply supercritical fluid extraction (SFE)[8] with carbon dioxide as the extraction medium for sample preparation.

Hawthorne and co-workers[9−12] utilized subcritical water ("hot water under enough pressure to maintain the liquid state" [12]) as the extraction solvent. The significantly improved solvency of water at higher temperatures (up to 250 °C) for nonpolar and moderately polar organics is based on its reduced polarity, surface tension, and viscosity under these conditions. The authors utilized these changes in water's properties to extract several classes of organic pollutants from model sorbents[9] and various environmental solids,[10−12] such as contaminated soils, sludges, and particulate matter. In these studies, the subcritical water extractions were performed in high-pressure reaction tubes (stainless steel) filled with defined amounts of contaminated soil and HPLC grade water in a gas chromatographic oven. After heating, the reaction cells were cooled and the supernate was subsequently analyzed using solid-phase microextraction and gas chromatography (GC).

This extraction method has the disadvantage that much of the solvated organic pollutants can repatriate to the soil after cooling.

The aim of the present work was to investigate the capabilities of coupling accelerated solvent extraction with water as the extraction solvent and solid-phase microextraction to determine chlorophenols in polluted soils. Subcritical water extraction was performed using a commercially available accelerated solvent extractor. This system solves the problem of the analytes partitioning back to the soil matrix because the aqueous phase and the soil are separated under the extraction conditions.

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Table 1. ASE-SPME of Chlorophenols in Soils: Relative Standard Deviations (RSD) and Detection Limits (LOD) Using Procedures A and B

		proc A		proc B	
compd	abbrev	RSD[a] (%)	LOD (μg kg^{-1})	RSD[a] (%)	LOD (μg kg^{-1})
2-chlorophenol	2-CP	6.7	5.4	9.3	1.1
4-chlorophenol	4-CP	8.4	7.9	14.9	2.2
4-chloro-3-methylphenol	4-C-3-MP	11.8	6.0	18.6	6.7
2,4-dichlorophenol	2,4-DCP	11.3	8.9	20.0	2.2
2,3,5-trichlorophenol	2,3,5-TCP	11.7	6.6	15.6	4.4
2,4,6-trichlorophenol	2,4,6-TCP	10.9	8.0	18.1	1.6
2,3,4-trichlorophenol	2,3,4-TCP	16.9	8.9	17.4	4.5
2,3,4,6-tetrachlorophenol	2,3,4,6-TeCP	16.7	6.0	19.8	3.9
pentachlorophenol	PCP	11.6	6.7	20.3	5.7

[a] $n = 6$.

EXPERIMENTAL SECTION

Reagents. The chlorophenols (see Table 1) were obtained from Supelco (Bellefonte, PA). Water, acetonitrile, and methanol (HPLC grade) were purchased from Baker (Deventer, The Netherlands). Acetone and *n*-hexane (for organic trace analysis) were supplied by Merck (Darmstadt, Germany), as were hydrochloric and phosphoric acids (Suprapur) and sodium chloride (analytical grade).

Chlorophenol stock solutions in methanol were prepared with concentration levels of 10 mg mL^{-1} for each compound.

Isotope-labeled ($^{13}C_6$) chlorophenol standards (4-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol) dissolved in methanol (100 μg mL^{-1}) were purchased from Promochem (Wesel, Germany).

Soil Samples. Five soil samples collected in the Bitterfeld region (Saxony-Anhalt, Germany) were investigated for their contamination with chlorophenols. The sampling sites K1, K2, and K3 are located in the flood plain of the stream Spittelgraben (which flows into the Mulde River and was used for several decades as a wastewater channel for the chemical industry) at various distances from the stream. The soil samples WS1, WS2, and WS3 were taken at different polluted locations in the flood plain of the Mulde River near Bitterfeld.

The soils were air-dried at room temperature, sieved to a grain size of <2 mm, homogenized, and stored at 4 °C.

All studies to optimize the extraction procedure were performed using the lowly polluted wetland soil WS3, a sandy loamy silt with a total carbon content of 2.41% (humus content 4.14%).

Sample Preparation. For the extraction experiments, the WS3 soil was spiked at concentration levels of 1000, 100, 10, and 1 μg kg^{-1} of each chlorophenol. The spiking procedure was as follows:

Briefly, 100 g of dried soil is mixed in a porcelain dish with 90 mL of a solution containing the relevant amount of the chlorophenols in acetone. Subsequently, the solvent is slowly evaporated at room temperature under frequent homogenization.

ASE with Water. The extractions were carried out using a Dionex (Sunnyvale, CA) ASE 200 accelerated solvent extractor equipped with 11 mL stainless steel extraction cells. The extaction solvents were water and aqueous solutions with 3 or 5% (v/v) of an organic modifier, such as methanol, acetone, or acetonitrile. Additionally, diluted phosphoric acid (3%, w/v) was applied. The HPLC grade water used had previously been purged with clean nitrogen for about 2 h to remove dissolved oxygen.

The extraction procedure was optimized with respect to extraction temperature and extraction time using 10 g of dried and spiked WS3 soil samples. For all ASE experiments, an extraction pressure of 10 MPa was chosen. The flush volume amounted to 60% of the extraction cell volume. The volume of the resulting extracts was about 13 mL.

SPME and GC/MS Procedures. The chlorophenols in the aqueous extracts were determined using SPME and gas chromatography/mass spectrometry (GC/MS).

GC/MS analysis was performed on a Hewlett-Packard (Palo Alto, CA) model 5971 GC-MSD system equipped with a split/splitless injector. The temperature of both the injector and the transfer line was 280 °C. The injector fitted out with a deactivated glass insert (2 mm i.d.) was used in the splitless mode with a splitless time of 4 min. A Supelco PTE-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) was employed with the following temperature program: 40 °C, 4 min isothermal, 12 °C/min to 200 °C, then 20 °C/min to the final temperature of 250 °C, held for 2.5 min. The carrier gas was helium with a column head pressure of 1 bar. The transfer line temperature of 280 °C set the ion source temperature of the directly coupled mass spectrometer to 200 °C. The ionization was performed with a kinetic energy of the impacting electrons of 70 eV. The detector voltage was 1800 V. The single-ion-monitoring (SIM) mode was selected for the sensitive and selective detection of chlorophenols in the matrix-loaded aqueous soil extracts.

The SPME experiments were done using a manual SPME device from Supelco. For chlorophenol extraction 85 μm polyacrylate fibers were chosen. The SPME procedure[13] was as follows:

A 3 mL portion of the aqueous soil extract (sodium chloride saturated and adjusted to pH 2 with concentrated hydrochloric acid) is poured into a 5 mL sample vial. The polyacrylate fiber is exposed for 20 min to the aqueous solution while being stirred at 1000 rpm. The SPME fiber is immersed in HPLC grade water for about 1 s to clean it after exposition (to avoid sodium chloride crystals being deposited on the fiber during desorption). The extracted chlorophenols are desorbed inside the GC injector for

(13) *Supelco Application Note 17*; Supelco: Bellefonte, PA, 1997.

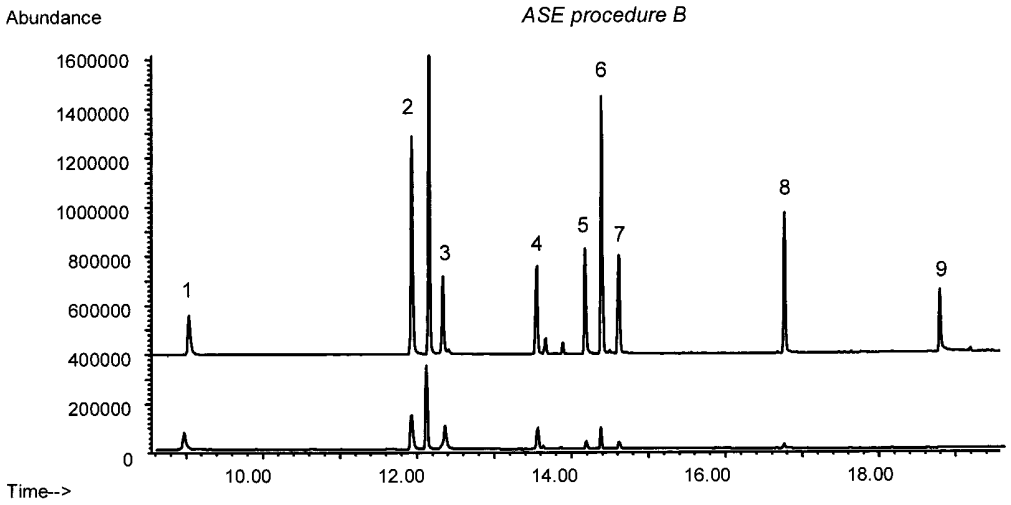

Figure 1. TIC chromatograms of chlorophenols extracted from a spiked soil (100 μg kg^{-1} of each compound) using ASE-SPME: upper chromatogram, procedure B; lower chromatogram, ASE conditions of water, 150 °C, 15 min. Peak identifications: (1) 2-CP; (2) 2,4-DCP; (3) 4-CP; (4) 4-C-3-MP; (5) 2,3,5-TCP; (6) 2,4,6-TCP; (7) 2,3,4-TCP; (8) 2,3,4,6-TeCP; (9) PCP (for abbreviations, see Table 1).

4 min. GC/MS(SIM) analysis of the desorbed analytes is carried out under the conditions mentioned above.

RESULTS AND DISCUSSION

Optimization of the ASE Parameters. Nowadays, accelerated solvent extraction using different organic solvents or solvent mixtures is a well-established technique for the efficient extraction of a variety of organic pollutants from soil samples. However, the use of water as an ASE solvent has not been reported as far as we know. The parameters which significantly affect the yield of the subcritical water extraction are the extraction temperature and time, whereas the influence of the extraction pressure is minor.[12]

At first, the extraction yield was investigated in three subsequent runs of aqueous extractions of a spiked soil (1 mg kg^{-1}) applying an extraction temperature of 150 °C and a static extraction time of 15 min. Figure 1 shows the total ion current (TIC) chromatogram of the first extract. In the following calculation, the yield of the three extractions as the sum of the peak areas was set to 100%. Under the chosen extraction conditions, all compounds were extracted by more than 50% in the first run. With increasing chlorine substitution and decreasing water solubility, a reduced extraction yield in the first extract was observed (Figure 2). This means combining the three extracts would lead to the undesirable dilution of the sample. Therefore, in further investigations, only the first extract was analyzed using SPME.

The influence of the temperature on the extraction efficiency was studied (Figure 3) in the range 75−200 °C (200 °C being the upper temperature limit of the accelerated solvent extractor used). As expected, the extraction yields of particularly the more hydrophobic chlorophenols such as 2,3,4,6-TeCP and PCP (for abbreviations see Table 1) increased with temperature. However, both the chlorophenols and the soluble organic soil matter have comparable solubility characteristics, resulting in increasingly darker extracts with increasing temperature. Consequently, the subsequent SPME procedure is influenced by relatively high amounts of dissolved organic matter (DOM).[14] The values given in Figure 3 reflect these contrary effects. We found the favorable

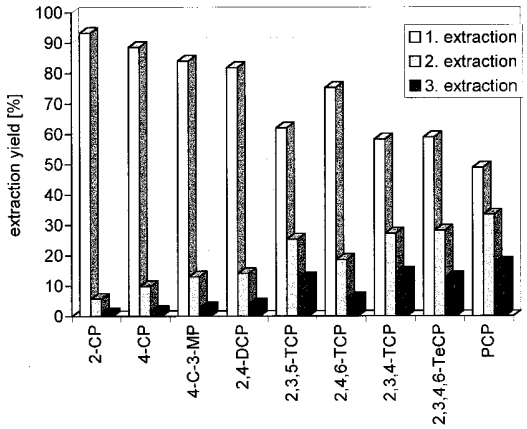

Figure 2. Extraction yields of chlorophenols in three sequential aqueous ASE runs of a spiked soil (1 mg kg^{-1} of each compound). ASE conditions: 150 °C, 15 min.

extraction temperature for most of the analytes investigated to be 150 °C. Because PCP is of particular interest for toxicological reasons, an extraction temperature of 125 °C (peak area of PCP 3 times greater compared to that at 150 °C) was chosen for the following experiments.

The extraction time was varied in the range from 15 to 45 min (Figure 4), with 30 min appearing optimal. Ten-minute extractions performed three times with partial solvent exchange (working with three extraction cycles) proved to be more suitable. This enabled the extraction yields to improve for the tri-, tetra-, and pentachlorinated phenols by factors of 1.7−6.7.

The suitable conditions for subcritical water extraction (ASE procedure A) can be summarized as follows: temperature, 125 °C; pressure, 100 bar; time, 3 × 10 min (three cycles).

Addition of Modifiers. In further experiments, the effect on the extraction efficiency of various organic modifiers (methanol, acetone, and acetonitrile) and phosphoric acid added to the

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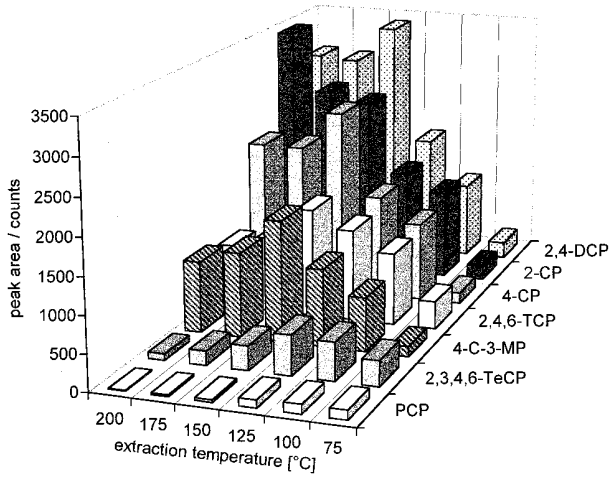

Figure 3. Effect of the extraction temperature on the signal intensity of chlorophenols (100 μg kg^{-1} of each compound; extraction time 15 min).

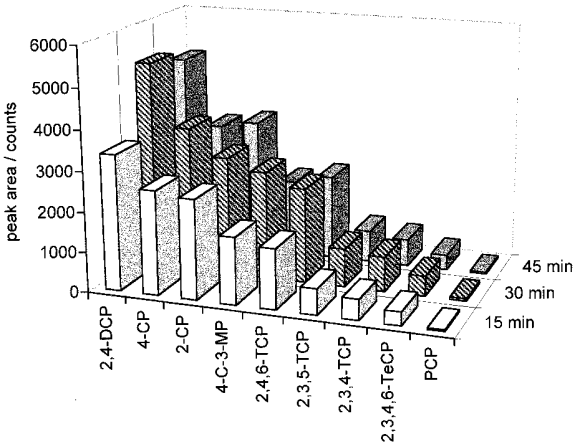

Figure 4. Influence of the extraction time on the signal intensity of chlorophenols (100 μg kg^{-1} of each compound; extraction temperature 150 °C).

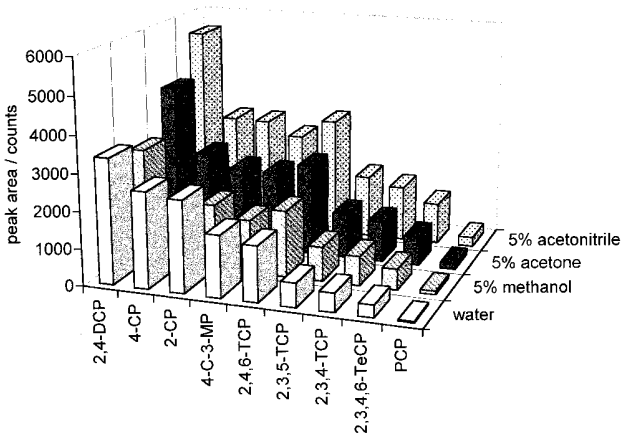

Figure 5. Effect of different organic modifiers on the signal intensity of chlorophenols from a spiked soil (100 μg kg^{-1} of each compound). ASE conditions: 150 °C, 15 min).

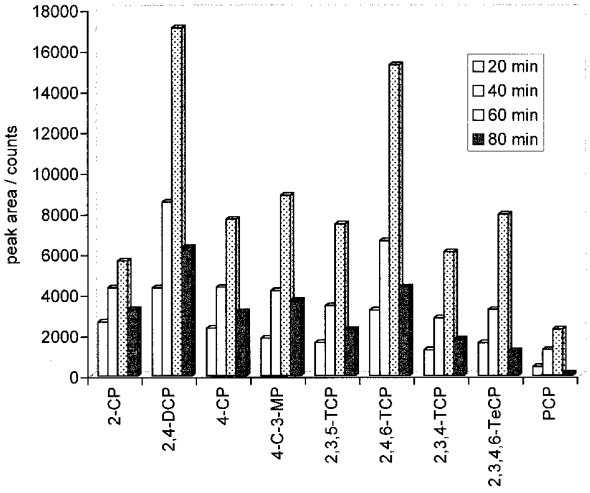

Figure 6. Dependence of signal intensity on SPME exposure time (ASE procedure B).

Table 2. ASE Extraction Yields (%)

compd	proc A	proc B	compd	proc A	proc B
4-CP	72	82	2,4,6-TCP	44	42
2,4-DCP	62	68	PCP	32	45

extraction medium was studied. Because in SPME both the distribution of the analytes between fiber and liquid phase and the long-term stability of the fiber are negatively affected by the addition of organic solvents, only small amounts of solvents (3 and 5%, v/v) were applied. Selected results of these investigations are given in Figure 5. The addition of organic solvents, especially acetone and acetonitrile, improves the extraction efficiency significantly, with the highest yields being obtained by adding 5% acetonitrile. Therefore, a water−acetonitrile mixture (95:5, v/v) was subsequently used as the extraction medium (ASE procedure B). The other ASE parameters were the same as those given in procedure A (without any additive).

Using diluted phosphoric acid (3%, w/v) as an extraction solvent for the ASE was not successful. The extraction yields were not improved in comparison to those obtained with the application of pure water.

As already known, the solubility of chlorophenols in water improves with increasing pH values. Because under these conditions considerable quantities of the organic soil matter (humic matter) are also dissolved, we dispensed with the water extractions at basic pH values (compare preceding paragraph, influence of extraction temperature). Additionally, the use of strong bases, like sodium or potassium hydroxide, is discouraged.[18]

Supelco[13] recommends exposing the polyacrylate fiber for 20 min. As already explained, the aqueous soil extracts contain a relatively high amount of dissolved organic matter. However, the presence of humic acids leads to a significant decrease in chlorophenol recoveries in the SMPE procedure as shown by Möder et al.[14] As reported there, this decrease was partly compensated by extending the exposure time to 70 min. There-

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Table 3. Concentrations of Chlorophenols in Soil Samples Taken from the Bitterfeld Region (in μg kg^{-1}; Mean Values of $n = 2$): Comparison of the Results Using ASE-SPME Procedure B and the "Classical" ASE Procedure

	WS1		WS2		K1		K2		K3	
compd	proc B	ASE	proc B	ASE	proc B	ASE	proc B	ASE	proc B	ASE
2-CP[a]	23	17	3	1	310	209	136	105	4	2
4-CP	126	58	12	8	1217	716	622	237	16	9
2,4-DCP	1528	1017	143	69	11751	9504	6334	5139	115	47
4-C-3-MP[a]	10	4	nd[b]	nd	1009	480	178	pd[c]	19	8
2,4,6-TCP	288	235	24	10	2987	3050	1180	1210	31	11
PCP	254	187	14	nd	2615	pd	1611	1432	nd	nd

[a] $^{13}C_6$-labeled 4-CP was used as the internal standard. [b] nd = not detected. [c] pd = peak disturbance by overlapping peak.

fore, the influence of increasing the exposure time on the extraction efficiency in SPME was investigated here.

Using water extracts (ASE procedure A), we found that increasing the exposure time from 20 to 50 min resulted in only slightly improved extraction yields by factors of about 1.5 and 1.1 for the mono-, di-, and trichlorinated phenols and for 2,3,4,6-TeCP and PCP, respectively. Similar studies were also done with the acetonitrile-modified ASE extracts, with the exposure time being varied in the range 20−80 min at 20 min intervals. With an exposure time of 60 min, unexpectedly high extraction yields were observed (Figure 6). The peak areas increased by up to 5 times compared with the values obtained at 20 min. As shown in the next paragraph, this behavior was reproducible. We assume that this increase in the extraction rate is caused by swelling of the polyacrylate fiber in the acetonitrile-containing solution with the resulting alteration of the distribution equilibrium of the analytes between fiber and aqueous solution. The decrease in extraction yields at higher exposure times cannot yet be explained.

The TIC chromatograms of chlorophenols using the optimized ASE-SPME procedure B (upper chromatogram) and the initial ASE-SPME conditions (lower chromatogram) are compared in Figure 1.

Evaluation of the ASE-SPME Procedures. The optimized ASE-SPME procedures A and B were evaluated with respect to precision and limits of detection. To calculate the reproducibility, six WS3 soil samples each spiked at a concentration level of 100 μg kg^{-1} of chlorophenol were extracted by applying both ASE procedures. Subsequently, the resulting extracts were analyzed using SPME exposure times of 30 (procedure A) and 60 min (procedure B). The results are given in Table 1. For procedure A, the relative standard deviations (RSD) are in the range from 6.7% (2-CP) to 16.9% (2,3,4-TCP) for the combination of both techniques. Slightly higher RSD values ranging from 9.3% (2-CP) to 20.3% (PCP) were obtained for procedure B.

To estimate the detection limits (LOD), samples of the lowly polluted wetland soil WS3 spiked at concentration levels of 100, 10, and 1 μg kg^{-1} were extracted and analyzed using procedures A and B. The LOD values (see Table 1) were calculated by applying the 3σ criterion.

As shown in Table 1, detection limits in the low-ppb range were achieved using both procedures, with the LOD values resulting from procedure B being lower (1.1−6.7 μg kg^{-1}). Santos et al.[15] described in a recent paper the determination of chlorophenols in soils using SFE combined with liquid chromatography−electrochemical detection. LOD values in the range from 3 (2-CP) to 150 μg kg^{-1} (PCP) were reported.

Soil/Water Partitioning. To determine the soil/water partitioning of the chlorophenols, spiked WS3 soil samples (100 μg kg^{-1} of each compound) were extracted using ASE procedures A and B. To determine the total concentrations of analytes in DOM-loaded aqueous samples, using an internal calibration of SPME by means of deuterated or isotope-labeled surrogates is recommended.[16,17] Therefore, the appropriate amount of a $^{13}C_6$-labeled chlorophenol standard containing 4-CP, 2,4-DCP, 2,4,6-TCP, and PCP was added to the ASE extracts prior to SPME analysis. Chlorophenol extraction yields were related to the signal ratios at the specific ion traces, with the signal intensity of the labeled surrogate being set to 100% (Table 2). These partitioning studies demonstrated that ∼32−72% of each analyte could be extracted by using subcritical water at 125 °C (ASE procedure A). Using water/acetonitrile (95:5, v/v) as the extraction medium, the recoveries are still improved. As reported,[14] the SPME step extracts ∼2−32% of the total quantity of each chlorophenol from the extractant water.

Analysis of Real-Soil Samples. The ASE-SPME procedure B described here was applied to the determination of chlorophenols in five soil samples taken from contaminated areas near Bitterfeld. To compare the results with those of a reference method, the soil samples were analyzed according to U.S. EPA method 3545 (see also ref 7) by applying a "classical" ASE procedure with organic solvents (acetone/*n*-hexane, 1:1, v/v) and subsequent GC/MS determination of the chlorophenols. The other ASE parameters were as follows: temperature, 100 °C; pressure, 15 MPa; static extraction time, 5 min.

For quantification, $^{13}C_6$-labeled internal standards (4-CP, 2,4-DCP, 2,4,6-TCP, and PCP) were added to the soil samples prior to the ASE procedure. To simulate in particular the binding of analytes to the soil matrix,[5] the internal standards were added (spiking) as described above (Experimental Section, Sample Preparation). Thus, 10 g of each soil sample was spiked with an appropriate amount of the labeled standards and stored for 24 h prior to ASE extraction. In the case of the highly loaded soils (K1 and K2), only 1 g of the sample was spiked, mixed with sea sand (1:9, w/w), and then extracted. The results of chlorophenol analysis are given in Table 3. The highest contamination with concentrations up to 12 mg kg^{-1} for each chlorophenol was found for soil K1, followed by K2 and WS1.

As shown in Table 3, most of the values resulting from the water extraction (procedure B) are significantly higher than those resulting from ASE with organic solvents (ASE). These results confirm that water under subcritical conditions extracts the chlorophenols more effectively from the soil matrix than the

organic solvents used. At higher analyte contaminations, the differences between the methods are less. We assume that, in these cases, the solvency of water for the sum of the extractable organics was exceeded.

Furthermore, it could be observed that the described ASE-SPME procedure is more selective than the extraction with organic solvents. Thus, the chromatograms of the organic extracts are disturbed by a large number of interfering peaks. The high selectivity of the described ASE-SPME procedure for the determination of chlorophenols in soils is in our opinion the result of the selective subcritical water extraction in combination with the SPME using the polyacrylate fiber.

In the present study, we used air-dried soil samples only, although there is a risk of analyte losses during the drying process, especially for the more volatile chlorophenols.

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