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Short communication

Fast determination of phenols in contaminated soils

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Abstract

An extraction method for the determination of phenols in contaminated soils, based on the application of solid-phase microextraction (SPME) coupled with GC–flame ionization detection analysis, was developed and tested. This method was developed using a natural soil spiked with phenol to a concentration level typical of an acute contamination event that can occur in an industrial site. The effects of the extraction parameters (pH, extraction time and salt concentration) on the extraction efficiency were studied and the method was then applied to determine the pollutant concentration at the beginning and during the biological treatment of a soil, contaminated with phenol and 3-chlorophenol, respectively. The SPME results were validated by comparison with those obtained with an US Environmental Protection Agency certified extraction method. The SPME method was also successfully applied to the determination of the adsorption behavior of 3-chlorophenol on a natural clay soil and was shown to be suitable for different matrices and phenolic compounds. Application of SPME technique results in a sharp reduction of the extraction times with negligible solvent consumption.

Keywords: Soil; Solid-phase microextraction; Phenols

1. Introduction

Determination of phenols from contaminated soil is required to evaluate the extent of pollution and to apply the best soil remediation technology.

Quantitative analysis of phenols can be carried out by chromatographic techniques, either liquid (HPLC) or gas phase (GC), following extraction of the target analytes from the soil matrix. Up to now, the extraction step has been performed following the official methods issued by national and international environmental protection agencies, such as the US Environmental Protection Agency (EPA) 3500B and International Organization for Standardization (ISO) TC 190/SC3/WG6 methods, which require the use of relatively large volumes of organic solvents, with great concerns for their negative environmental effects and for their disposal. The analyte transfer from the soil matrix to the organic solvent is usually accelerated by different means such as ultrasonic treatment or microwaves [1], use of a supercritical solvent [2,3], accelerated solvent extraction (ASE) [4] or increase of the contact time between soil and

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solvent with a semi-continuous extraction method [5,6]. Nevertheless, all above procedures result to be time consuming, requiring up to 24 h for the analysis of a single soil sample. Therefore, the development of an extraction method of phenols from contaminated soils which could guarantee fast and reliable results with a lower solvent consumption, is strongly required.

A possible answer to this need comes from a quite new extraction method, solid-phase microextraction (SPME), that has been developed and widely applied in the last decade for the extraction of organic compounds from liquid [7] and gas matrices [8,9] and allows for a great reduction of the extraction times, with negligible solvent consumption [10]. The SPME technique was applied to the determination of phenols from aqueous matrices [11–13] using a polar stationary phase (polyacrylate) and a Carbowax-divinylbenzene (CW–DVB) fiber [13], with several data on method development and optimization. Recently, the SPME technique was also used for the determination of phenols from soil in conjugation with ASE [14], and directly from landfill leachate and soil [15]. Finally, direct headspace SPME determination of phenol in soils has been reported [16], where an in situ derivatization of the sample was performed.

The purpose of this work is to assess the feasibility of the SPME technique and to demonstrate the significance of its results for fast determination of phenolic compounds in different soil matrices. Phenol and 3-chlorophenol were chosen as representative of the phenolic compounds class; a soil sample from an industrial site (soil 1) and a natural clay soil sample collected from a cave (soil 2), both spiked with phenol or 3-chlorophenol, were used as matrices. The selected concentration level for spiking was that typical of acute pollution events that may occur in industrial sites [17]. The effect of the main extraction parameters, such as contact time, pH value, salt concentration, was studied along with the interference of the soil matrix on the extraction efficiency. Besides, monitoring of a biological method for phenol and 3-chlorophenol degradation in soil 1 and the determination of the adsorption isotherm of 3-chlorophenol on soil 2 were considered for application and validation of the SPME method.

2. Experimental

2.1. Reagents

Phenol crystals (99.5% pure), 3-chlorophenol (99% pure), acetone, hexane and 2-propanol (pesticide grade) methanol and ethanol (HPLC grade), hydrochloric acid (37%), sodium and potassium chlorides were all purchased from Carlo Erba (Milan, Italy). 2-Bromophenol (purity ≥98%), used as internal standard, was purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Preparation of soil samples

2.2.1. Soil 1

The soil selected for the composting study was collected from an industrial site located near Rome. Preliminary extraction tests indicated that phenols concentration was below the detection limit. The soil was air dried and passed through a 2-mm sieve. The particle size distribution of the soil fraction below 2 mm was the following: 21% wt. below 2 μm, 12% between 2 and 6 μm, 15% (6–20 μm), 12% (20–60 μm), 13% (60–200 μm), 13% (200–600 μm), 14% above 600 μm [19]. The moisture content of the sieved soil was 15% (wt. %), whereas the soil pH measured in a 0.1 *M* KCl solution was 6.5 [19]. The total organic carbon (TOC), measured with a Strohlein CMAT 550 LI instrument, as difference between total carbon and inorganic carbon, was negligible.

A 9-kg soil sample was spiked with 300 ml of a 10 g/l phenol solution in distilled water, in order to obtain a phenol concentration of approximately 1 g per kg of wet soil. Another 9 kg soil sample was spiked with 300 ml of a 5 g/l 3-chlorophenol solution in distilled water corresponding to about 0.5 g/kg of wet soil. Contamination was performed by sparging the phenol and 3-chlorophenol solutions on the two sub-samples, respectively and then by mixing each one to achieve a homogeneous pollutant distribution.

The contaminated soils were then treated with a microbiological composting method [18]. In order to determine the clean-up efficiency, three different operating conditions were investigated in three sepa-

rate reactors: spiked soil with no added chemicals or nutrients (reactor A), spiked soil with nutrient salts (reactor B) and spiked soil with nutrient salts and wastewater treatment dewatered sludge as the inoculum (reactor C). Soil sampling was made twice a day by collecting about 40 g of soil from each laboratory-scale reactor after mixing the composted soil.

2.2.2. Soil 2

The soil used for the adsorption study was constituted by 70% clay and 30% sand. It was a natural soil collected from a cave in Rome. Preliminary extraction tests indicated that phenols concentration was below the detection limit. The soil was dried at 105°C and then autoclaved (P=1 atm; T=120°C; time=15 min; 1 atm=101 325 Pa) in order to avoid interference due to microbial activity. The soil pH measured in a 0.1 *M* KCl solution was 7 [19].

The adsorption study was carried out by isotherm determination of 3-chlorophenol at 25°C. Each compound was tested by filling eight glass bottles with 20 g of prepared soil and 100 ml of polluted solution at different concentrations. A 10-ml volume of soil suspension was collected from each stirred bottle after 30 min of mixing, corresponding to the time required to achieve the equilibrium concentration of the pollutant, previously determined. Each collected suspension was then centrifuged, to obtain a soil and a liquid sample for phenol determination.

2.3. Extraction and analytical methods

Determination of phenol and 3-chlorophenol in the spiked soil was obtained by the SPME technique coupled to gas chromatography–flame ionization detection (GC–FID). The SPME extractions were done using a manual SPME device purchased from Supelco (Bellefonte, PA, USA). An 85 μm polyacrylate fiber was used, since it is recognized to have the highest sensitivity for polar compounds as phenols [11,13]. A 2-g sample of the soil spiked with phenol or a 0.2-g sample of the soil spiked with 3-chlorophenol, was added to 31 ml of distilled water, previously poured into a 35-ml extraction glass. Hydrochloric acid was added to the soil suspension to keep pH 1. The SPME fiber was immersed in the soil suspension, that was kept in agitation by means of a magnetic stirrer, for a fixed extraction time (60 min), at room temperature and with always the same stirring rate. Choice of proper value for the extraction parameters, such as pH, extraction time, salt concentration are discussed in the following section. Quantitative determination of phenol and 3-chlorophenol in the spiked soil was obtained applying the internal standard method, adding a known quantity of a standard compound (2-bromophenol).

US EPA method 3540 was also applied to the extraction of phenol or 3-chlorophenol from the spiked soil, using again the internal standard method. The liquid extract or SPME content, obtained with the above described extraction procedures, were then analyzed by means of GC–FID, using an Autosystem XL gas chromatograph (Perkin-Elmer, Norwalk, CT, USA), equipped with a split–splitless injector and managed by a proprietary TurboChrom software. The analysis were carried out on a 30 m×0.25 μm I.D. BP5 capillary column with 5% phenyl polysiloxane (SGE, Ringwood, Australia) operating isothermally (60°C) for 4 min and then programmed at 25°C/min to 250°C. Both injector and detector temperature were kept at 250°C.

The splitless mode was maintained for 4 min, when a liquid extract obtained from the EPA method was analyzed, and for a time corresponding to the desorption time, when the SPME fiber content was to be determined. The carrier gas was nitrogen with a column head pressure of 1 bar.

3. Results and discussion

3.1. Method development

The effects of the different extraction parameters on the extraction efficiency were studied before starting the application of SPME to the determination of phenols concentration in contaminated soils. Based upon previous results [11,14] it was decided to focus on the following parameters: extraction time, pH of the extraction environment, salt concentration in the extraction environment.

The effect of the extraction time on the extraction

efficiency was determined for a sample of soil 1, spiked with phenol as described in the Experimental section, adding 2-bromophenol, as internal standard, to the aqueous phase. As shown in Fig. 1, the equilibrium conditions were not attained, even if extraction times up to 2 h were used. The GC peak areas of both phenol and 2-bromophenol were observed to increase at higher extraction times. The SPME absorption profiles, calculated using the theoretical approach proposed by Ai [20], are also shown in Fig. 1.

The equation used to determine the SPME absorption profile is as follows:

$$A = A_0[1 - \exp(-at)] \quad (1)$$

where A is the GC peak area at time t, A_0 is the GC peak area under equilibrium conditions and a is a parameter that gives a measure of how fast the absorption equilibrium can be reached in the SPME process. It is worthwhile to point out that the calculated time profiles for both phenol and 2-bromophenol were obtained with the same value of this parameter. The equilibration time, defined as the time required to absorb on the SPME fiber at least 95% of the equilibrium amount, was also estimated by Eq. (1), resulting in 170 min. Nevertheless, as it can be argued from Fig. 1, the extraction time did not have any relevant effect on the ratio between the GC peak areas of phenol and 2-bromophenol.

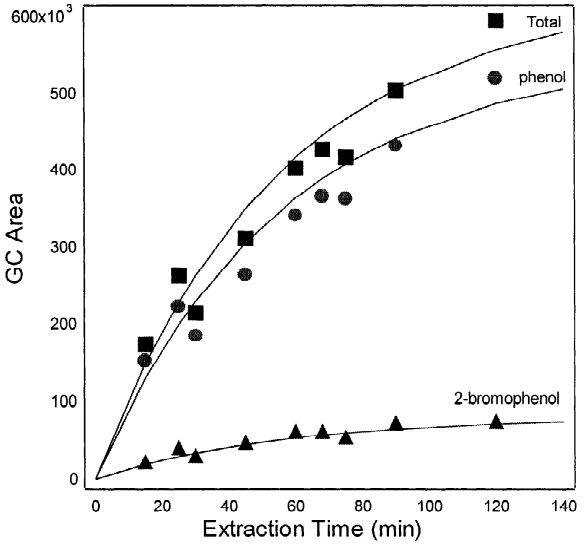

Fig. 1. Effect of extraction time on extraction efficiency from a soil sample: phenol (●), 2-bromophenol (▲), total GC peak area (■), and model predictions (continuous lines), versus extraction time; model parameters (Eq. (1)): $a = 0.018\ \text{min}^{-1}$, $A_{oPh} = 5.5 \cdot 10^5$, $A_{o2\text{-}br} = 8.0 \cdot 10^4$.

Similar results, not reported for sake of conciseness, were obtained by performing SPME extraction on an aqueous phenol solution, indicating that the missed achievement of equilibrium can be attributed to the slow analyte transfer from the liquid to the SPME fiber rather than to slow desorption from the soil sample.

Therefore, it is possible to conclude that phenol may be determined also by SPME technique under non-equilibrium conditions, provided that an internal standard method is applied.

The effect of salt on extraction efficiency was then investigated with regard to the extraction of phenol from a soil matrix. Four extraction conditions were tested: extraction in pure distilled water, in a 150 and a 300 g/l NaCl solution, and in a saturated NaCl solution. When NaCl was added to the extraction solution, the quantity of extracted phenol was observed to increase dramatically, up to threefold. This value is lower with respect to those previously reported for phenol [11,14] which ranged from 5 to 5.5. This difference can be in part attributed to a slower achievement of equilibrium between matrix and SPME fiber when salt is added, that was confirmed by the experimental absorption time profile for phenol and by that predicted by Eq. (1), not reported here for sake of conciseness. Apart from these considerations, the obtained results clearly indicate that even when a soil matrix is present, extraction in a possibly saturated salt solution leads to an increased phenol absorption on the fiber allowing to increase the method sensibility.

Finally, the effect of pH was tested by comparing the extraction efficiency from a sample of soil 1 in pure distilled water and in a pH 1 HCl solution. The results clearly indicated that the pH value did not appreciably affect the extraction efficiency of both phenol and the internal standard 2-bromophenol, confirming previous observations [11,12].

3.2. Optimal extraction conditions

Since the development of a general procedure for

phenols determination in highly polluted soil was pursued, it was considered to select safe and robust extraction conditions, that could be extended to the whole class of phenolic compounds. For this reason the optimal extraction conditions used in this work are somewhat different from those discussed so far. Namely, the extraction time was set to 60 min, in order to extract approximately 50% of the equilibrium amount (see Fig. 1). Moreover, pH 1 was used, since with this condition also phenolic compounds with low pK_a can be determined correctly. Besides, all extractions were performed without salt, being the scope of this work limited to phenols determination in highly polluted soils, where method sensitivity is not a key issue. Finally, extractions were made at room temperature, as the effect of temperature on extraction efficiency was negligible, as far as the ratio between phenol and internal standard is concerned.

3.3. Applications and validation of SPME method

The quantitative determination of phenol and 3-chlorophenol concentration in the spiked soils by SPME was performed using the internal standard method, as described above. Calibration curves for both analytes were built, allowing to determine the ratio between the weights of phenol (3-chlorophenol) and 2-bromophenol as a function of the ratio between the chromatographic peak areas of phenol (3-chlorophenol) and 2-bromophenol, measured by GC–FID. It is worthwhile to point out that each calibration curve, obtained by fitting 10 experimental data points, remains linear (correlation coefficient greater than 0.98) between 200 ng/ml and 30 μg/ml phenol concentrations and between 20 ng/ml and 3 μg/ml 3-chlorophenol concentrations, which largely include the range of interest.

Degradation of phenol and 3-chlorophenol with the microbiological composting method described above was monitored measuring the pollutant concentration in the spiked soil with the SPME method. The results of phenol and 3-chlorophenol degradation processes were compared under selected conditions with those provided by the application of the EPA 3540 standard extraction method (Table 1).

Table 1
Comparison between extraction performances of EPA method 3540 and solid-phase microextraction applied to phenol and 3-chlorophenol determination in contaminated soil

Performances	SPME	EPA method 3540
Phenol concentration (mg/kg dry soil)		
Sample 1[a]	795±18.9	800±10.3
Sample 2[b]	423±8.5	456±6.4
Sample 3[c]	212±3.1	214±3.0
3-Chlorophenol concentration (mg/kg dry soil)		
Sample 4[d]	553±27.1	592±16.0
Sample 5[e]	188.6±2.63	187.3±3.01
Sample 6[f]	5.32±1.43	8.02±0.62
Advantages	Fast Small soil samples No solvent required	Low cost apparatus
Disadvantages	Moderate cost, since one fiber can be used for up to 100 samples	Time-expensive Large soil samples Large solvent volumes

[a] Soil 1 contaminated with phenol with no nutrients or inoculum added.
[b] Same soil of sample 1 after 12 days of composting treatment.
[c] Same soil of sample 1 after 14 days of composting treatment.
[d] Soil 1 contaminated with 3-chlorophenol and added with inoculum.
[e] Same soil of sample 4 after 47 days of composting treatment.
[f] Same soil of sample 4 after 102 days of composting treatment.

Table 2
Equilibrium concentrations of 3-chlorophenol in the liquid phase and adsorbed on soil 2, compared to the initial concentration in the liquid polluted solution (all data obtained with SPME)

Equilibrium concentration liquid phase (mg/l)	Equilibrium concentration soil 2 matrix (mg/kg)	Total concentration[a] (mg/l)	Initial concentration liquid phase (mg/l)	% Error[b]
34.1	39.8	42.1	50	15.8
181.3	170.5	215.4	200	7.7
254.5	217.4	298.0	300	0.66
347.2	280.0	410.6	400	2.65
469.6	379.1	545.4	500	9.0
512.6	455.4	597.2	600	0.46
686.5	542.7	795.1	800	0.61

[a] Total concentration is the sum of 3-chlorophenol equilibrium concentrations in the liquid solution and adsorbed on the soil sample, the latter normalized in terms of equivalent liquid concentration.
[b] % Error between initial liquid concentration and total equilibrium concentration.

This comparison is relevant to the extraction and analysis of phenol from three soil samples with no nutrients or inoculum added, and of 3-chlorophenol from three inoculated soil samples, characterized by a different degree of phenol degradation. For both analytes, concentrations determined with SPME are comparable with those obtained applying the EPA 3540 extraction method.

Finally, the possibility to extend the SPME method also to different soil types was investigated. The SPME method was applied to describe the adsorptive behavior of 3-chlorophenol on a sample of soil 2, as described in the Experimental section. Validation of SPME was obtained by verifying mass balance of 3-chlorophenol. Namely, the initial concentration of 3-chlorophenol mixture was compared with the sum of the equilibrium concentrations of the liquid solution and of the soil sample, the latter being normalized in terms of equivalent liquid concentration. The comparison between these data for different initial concentrations is shown in Table 2. The observed agreement is remarkably good at higher initial concentrations, but still acceptable at the lowest investigated concentrations, even if larger errors were observed. Besides, the collected data allowed us to verify that the adsorption isotherm of 3-chlorophenol on soil 2 is clearly linear in the investigated concentration range.

4. Conclusions

The SPME method was successfully applied to the determination of phenol and 3-chlorophenol in contaminated soils and to monitor their degradation behavior during microbiological treatment; the results were comparable with those obtained applying the EPA standard extraction methods to the same soil samples. The SPME was also used to determine 3-chlorophenol concentration in a natural clay soil, whose adsorptive behavior was also characterized. Thus, it seems possible to extend the SPME method also to other soil matrices. Finally, it is worthwhile to point out that these results were obtained without reaching equilibrium between liquid and SPME stationary phase. This opens new perspectives for the SPME extraction method, since a sharp reduction of the extraction time can be obtained, provided that an internal standard method is used for quantitative analysis. Of course, the validity of SPME method has to be considered limited to spiked soil; for its definitive validation, investigation on a really contaminated soil is necessary.

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