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Analysis of phenoxyalkanoic acid herbicides and their phenolic conversion products in soil by microwave assisted solvent extraction and subsequent analysis of extracts by on-line solid-phase extraction–liquid chromatography

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Abstract

A multiresidue method for the determination of phenoxyalkanoic acid herbicides and their phenolic conversion products in soil was developed. The method was based on microwave-assisted solvent extraction (MASE) of soil samples by an aqueous methanolic mixture and subsequent analysis of extracts by automated solid-phase extraction followed by on-line high-performance liquid chromatography and diode array detection. MASE parameters (extraction temperature and time, composition of the extraction mixture and extraction volume) were optimized with respect to analyte recoveries. The method was validated with two types of soils containing 1.5 and 3.5% organic matter, respectively, both types containing fresh and aged residues of sought analytes. Under the selected analytical conditions when soils with fresh residues were analyzed all target analytes were recovered above 80% from the soil containing 1.5% organic matter, while limits of identification at the level of 20–40 ng/g were achieved. From the soil containing 3.5% organic matter the least polar phenolic analytes exhibited slightly reduced recoveries, while identification limits of 30–50 ng/g were achieved. Samples with aged residues exhibited reduced recoveries for some analytes, the reduction amounting up to 6–12% within 1 month of aging period depending on soil organic matter.

Keywords: Soil; Environmental analysis; Microwave-assisted solvent extraction; Extraction methods; Phenoxyalkanoic acids; Pesticides

1. Introduction

Phenoxyalkanoic acids are postemergence systemic herbicides with hormonic action used mainly in cereals for the control of broad-leaved weeds. They are considered as hazardous compounds and due to their high water solubility, especially of the dissociated forms, are amenable to transport to the water environment via leaching [1–4] and run-off processes [5,6] depending upon the sorption and degradation kinetics [7]. Sorption on soil components depends on a combination of parameters relevant to the chemical composition of minerals and the mechanical and textural characteristics of soils [2,8–15], while their degradation in soil is a result of both microbial action and photodegradation [16–19].

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Various toxic phenolic compounds are produced as intermediates in their degradation pathway [16,20–22].

Phenoxyalkanoic acids have been analyzed by both gas (GC) [23–33] and liquid (HPLC) [34–41] chromatographic methods. GC based methods require a pre-analysis derivatization step to increase the volatility of these weak acids and HPLC seems therefore to be more attractive for their analysis. Residues in soils have been traditionally extracted by solid–liquid shake-flask extraction techniques using aqueous alkaline media [16,34,42] or organic solvent–water mixtures [27,40,42] as extraction solvents. However, in recent years extraction methods based on instrumental techniques such as microwave-assisted solvent extraction (MASE) [43] and supercritical fluid extraction (SFE) [44–46] have been also reported. The high sample throughput and relatively small required extraction times obtained by MASE make this technique quite attractive.

The objective of this work was to develop an analytical method for the simultaneous determination of phenoxyalkanoic acid herbicides and their phenolic conversion products in soils at ng/g levels based on MASE of soil samples and subsequent analysis of extracts by solid-phase extraction (SPE) followed by on-line HPLC analysis with diode-array detection. Five phenoxyalkanoic acid herbicides [2,4-D (2-methyl-4-chlorophenoxy)acetic acid (MCPA), 2,4,5-T, dichlorprop and 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB)] and two phenolic conversion products (2,4-dichlorophenol and 2,4,5-trichlorophenol) were selected as model compounds.

2. Experimental

2.1. Reagents and solvents

Analytical standard materials for target compounds were purchased from Riedel-de Haën (Seelze-Hanover, Germany). Stock standard solutions for each compound separately were prepared in methanol at a concentration of 1 mg/ml and were stored under deep freeze (−24 °C). Mixed working standard solutions at concentrations of 50, 25, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 μg/ml were prepared every 2 months in methanol and stored also under deep freeze.

Potassium dihydrogenphosphate and potassium hydrogenphosphate (3 H_2O) were purchased from Riedel-de Haën and Merck (Darmstadt, Germany), respectively. Orthophosphoric acid was purchased from Carlo Erba (Milan, Italy).

Water used in HPLC mobile phase was laboratory-distilled and filtered through a 0.2 μm membrane filter (Schleicher and Schuell, Dassel, Germany). Methanol and acetonitrile were of HPLC grade and purchased from Merck.

2.2. SPE materials

Disposable Hysphere-GP (10×2 mm) SPE cartridges for the PROSPEKT system were purchased from Spark Holland (Emmen, Netherlands).

2.3. Soil matrix

The method was developed and conditions optimized by use of a sandy silt loam soil (33.7% silt, 12.8% clay and 49.7% sand) containing 1.5% organic matter and pH 7.4, which is a typical Mediterranean soil (soil A). The soil was air dried and sieved through a 2 mm sieve before use. The remaining moisture content in the air dried soil was 1.4% and thus all calculations were based on a dry mass basis of the air dried soil. In the absence of a soil rich in organic matter, the method was also validated with soil samples artificially enriched in organic matter (3.5%) by addition of the appropriate amount peat (soil B).

Fortified soil samples were prepared by addition of 1 ml portions of the appropriate calibration solutions (made in methanol) per 10 g of soil samples transferred into 200-ml glass vessels. These vessels were agitated for 2 h for better analyte distribution and solvent evaporation by use of an horizontal shaker. Samples were either processed afterwards (fresh residues) or after storage under cold conditions (refrigerated) for the designated periods (aged residues).

2.4. Instrumentation

(i) The MSP 1000 laboratory Microwave System

(CEM, Matthews, NC, USA) equipped with a 12 vessel carousel operated in the closed-vessel mode was used for the microwave-assisted solvent extraction step. PTFE-lined extraction vessels were used and during operation both temperature and pressure were monitored in a single vessel; a sensor monitoring the solvent leaks in the interior of the microwave oven was also in use.

(ii) The HPLC system consisted of the Model 222D pump and the Model 232C gradient controller (SSI, State College, PA, USA). For the injection of calibration solutions the Basic Marathon autosampler (Spark Holland) equipped with 20 μl injection loop was employed. Analytes were detected by the Model 996 diode-array detector (Waters, Milford, MA, USA) equipped with 10 μl flow sell. The acquired signal was recorded by a PC operated under the Millenium software (Waters). The solid-phase extraction of the aqueous soil extracts was performed on-line using the automated PROSPEKT system (Spark Holland).

2.5. Analytical procedure

Soil samples (10 g) were processed by MASE at 80 °C for 10 min in the presence of 50 ml of a mixture of 10 m*M* phosphate buffer (pH 7)–methanol (50:50, v/v). After the extraction soil extracts were cooled to room temperature and centrifuged at 5000 rev./min for 10 min. The supernatant was diluted up to 250 ml with distilled water (final methanol percentage at 10%). The extract was then filtered through a 0.2 μm membrane filter (Schleicher and Schuell) and acidified at pH 3 with orthophosphoric acid.

Aliquots of 20 ml of the soil extract were subsequently processed automatically on the Hysphere-GP (10×2 mm) SPE cartridges by the PROSPEKT system. Before sample loading the SPE cartridges were conditioned with 10 ml of methanol followed by 10 ml of HPLC-water (pH 3). The flow-rate during the conditioning and sample loading steps was 4 ml/min. After sample loading the SPE cartridges washed with 2 ml of HPLC water (pH 3) at a flow-rate of 2 ml/min and the retained solutes were subsequently eluted on-line onto the analytical column by the mobile phase flow in the backflush mode.

Table 1
HPLC gradient regime

Time (min)	Solvent A (%)	Flow (ml/min)
00	95	1.0
03	80	1.0
35	0	1.0
40	0	1.4
45	95	1.2
50	95	1.0

HPLC analysis was performed on a Nucleosil 100-5 C_{18}, 250×4.6 mm, 5 μm reversed-phase column (Macherey–Nagel, Düren, Germany) thermostated at 30 °C. The mobile phase was a binary gradient mixture of 5 m*M* phosphate buffer at pH 3 (Solvent A) and a mixture of acetonitrile–HPLC water (90:10) (Solvent B). The gradient composition and the flow-rate settings are shown at Table 1. The diode-array detector was operated in the range 190–400 nm with an acquisition rate of 2 spectra/s. Analytes were quantified via 9-point external standard calibration curves from 2 to 1000 ng injected amounts. Quantitative measurements were performed at 215 nm.

3. Results and discussion

3.1. Method optimization

The composition and the volume of the extraction solvent along with the extraction temperature and duration were optimized with respect to analyte recoveries using soil A and samples spiked at the level of 0.5 μg/g.

During preliminary experiments the parent compounds of phenoxyalkanoic acids were satisfactorily recovered from soils by 50 ml of a 10 m*M* phosphate buffer (pH 7) at 100 °C for 10 min extraction duration, however, under these conditions the recoveries of the phenolic conversion products were lower than 30%. The increase of pH of the extraction medium up to 10 did not affect the recoveries of the latter compounds. The use of an organic modifier in the extraction medium was therefore necessary to increase the recovery of these compounds. Acetone, methanol and acetonitrile were tested. Acetone re-

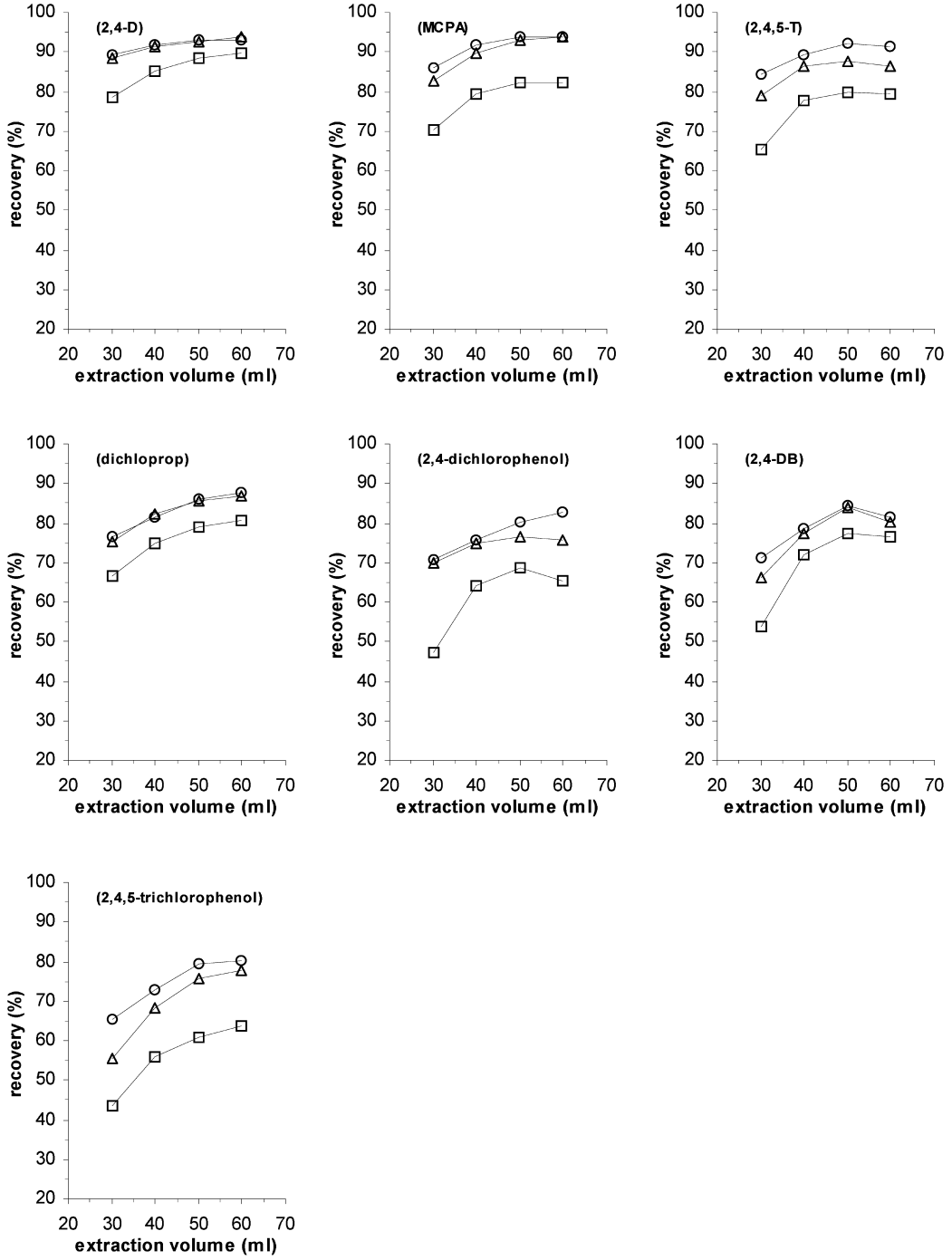

Fig. 1. Analyte recoveries vs. extraction volume when 40% (□), 50% (△) and 60% (○) of MeOH in a 10 m*M* phosphate buffer (pH 7) was used as extraction solvent. All other experimental conditions are given in the text.

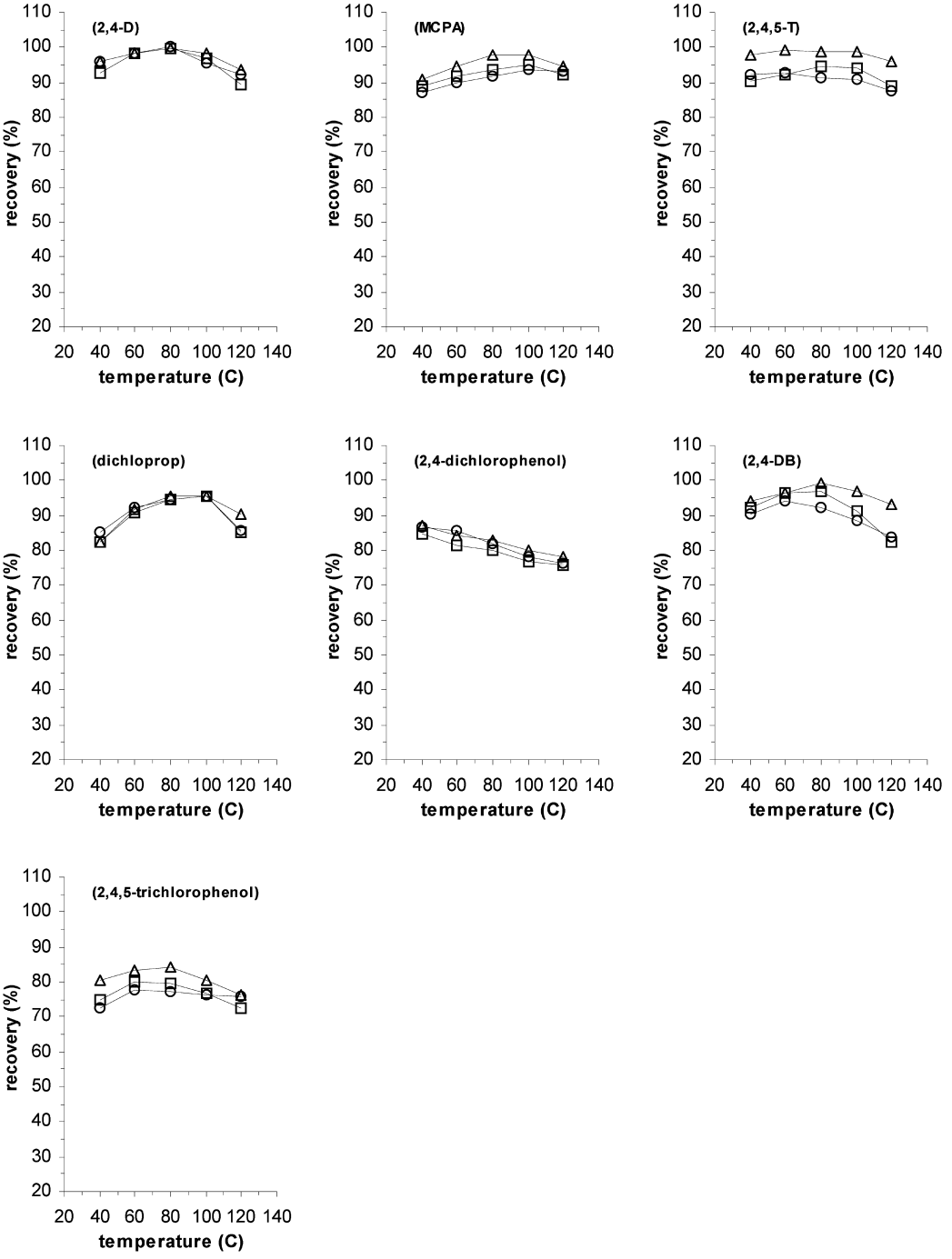

Fig. 2. Analyte recoveries vs. extraction temperature when the extraction time was set at 5 (□), 10 (△) and 15 min (○). All other experimental conditions are given in the text.

sulted to better recoveries than methanol, however the amount of coextractives was much higher, while acetonitrile was not so efficient. Methanol was therefore selected for further use.

Analyte recoveries were calculated against extraction volume at different methanol compositions, while the extraction temperature and duration were set at 120 °C and 15 min, respectively (Fig. 1). Analyte recoveries were increased when the volume of the extraction mixture was increased up to 50 ml. Further increase of the extraction volume resulted in no significant improvement on analyte recoveries, while in some cases the recovery of the analytes was decreased. The percentage of methanol necessary to achieve analyte recovery values better than 75% was 50%. Further increase of methanol percentage resulted in no significant improvement of analyte recoveries. Therefore 50 ml of a mixture of 10 m*M* phosphate buffer (pH 7)–methanol (50:50, v/v) was selected for further use.

Analyte recoveries were subsequently calculated against the extraction temperature at different extraction times (Fig. 2). Except 2,4-dichlorophenol analyte recoveries were increased slightly with the extraction temperature increased up to 80 °C, while further increase of extraction temperature resulted in smaller recovery values. The recovery of 2,4-dichlorophenol was decreased linearly by increasing the extraction temperature. Reduced extraction efficiencies at elevated temperatures might be due to evaporation losses especially for the relatively volatile phenolic compounds. Analyte recoveries increased when the extraction time was increased from 5 to 10 min, however further increase of the extraction time to 15 min provided slightly smaller values. Therefore 80 °C extraction temperature for 10 min duration was selected for further use.

3.2. Validation data

The method was validated by use of both soil A and soil B (Table 2). Detector response was linear through the whole calibration range (2–1000 ng) for all analytes; linear regression coefficients (r^2) were equal or better than 0.9999. All analytes were recovered above 80% at the level of 0.5 μg/g from soil A, while from soil B the least polar phenolic compounds exhibited slightly lower recoveries. Analyte identification limits (taken as the lower fortification levels where analytes could be detected at a signal-to-noise ratio above three and identified by their UV spectra) in addition to matrix interference peaks were also depended on the volume of the processed soil extract. By processing 20 ml of soil extract (corresponding to 0.8 g of soil) identification limits of 20–40 ng/g were achieved for the soil A and 30–50 ng/g for the soil B (Table 2). Better limits of detection could be achieved, however, by processing higher volumes of the soil extract. Method repeatability was very good with RSD values of

Table 2
Analyte[a] recoveries and limits of detection in two different soil types

No.	Analyte	t_R (min)	Soil A (1.5% organic matter)			Soil B (3.5% organic matter)		
			Recoveries (RSD%, $n=3$)		LOD (ng/g)	Recoveries (RSD%, $n=3$)		LOD (ng/g)
			50 ng/g	500 ng/g		50 ng/g	500 ng/g	
1	2,4-D	21.2	107 (3)	99 (1)	30	109 (7)	94 (2)	40
2	MCPA	21.9	98 (4)	98 (1)	40	105 (8)	92 (1)	50
3	2,4,5-T	23.5	100 (3)	99 (1)	20	92 (6)	90 (1)	40
4	Dichloprop	23.8	100 (2)	95 (1)	30	101 (8)	94 (2)	40
5	2,4-Dichlorophenol	24.7	90 (6)	89 (1)	30	81 (6)	73 (3)	40
6	2,4-DB	26.7	108 (7)	99 (2)	40	80 (8)	75 (4)	50
7	2,4,5-Trichlorophenol	27.9	89 (7)	84 (1)	20	74 (9)	63 (3)	30

[a] Analytes are ordered by increasing retention time.

the analyte recoveries being less than 10% ($n=3$) for both soil types. Method reproducibility was tested by comparing mean recovery values of solutes from freshly spiked samples analyzed at different occasions during 1 month period; the RSD values of mean analyte recoveries between different sample sets did not exceeded by 10%.

Chromatograms from the analysis of a control (unspiked) and a spiked sample from Soil A fortified at the level of 50 ng/g are shown in Fig. 3. The ''hill-type'' peak located in the center of the chromatograms corresponds to the coextracted humic substances. For the soil B this peak was much higher, however other matrix interference peaks were at about of the same height. Using more selective detection techniques (like mass spectrometry) interferences from the matrix could be largely reduced [34].

MASE efficiency was also compared with the traditional (very time consuming) solid–liquid flask-shaking extraction technique. Portions (10 g) of soil A spiked at the level of 0.5 μg/g were extracted

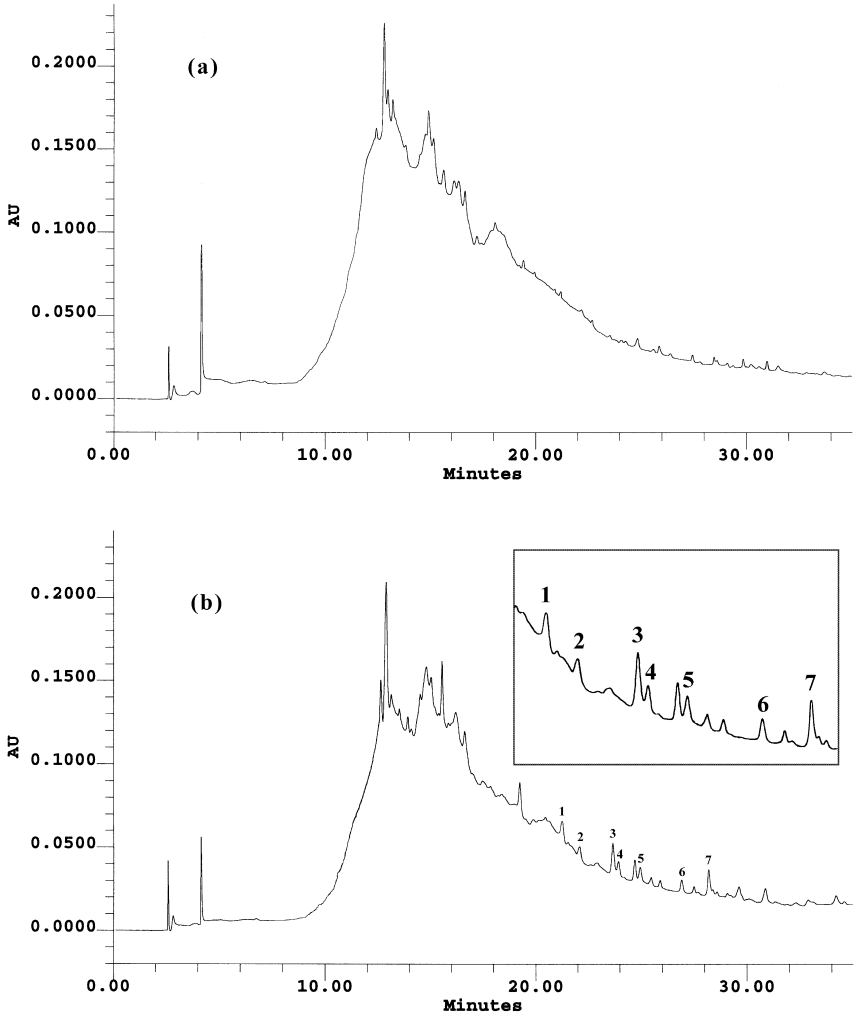

Fig. 3. Chromatograms from the analysis of a soil sample containing 1.5% organic matter unspiked (a) and spiked at the level of 50 ng/g (b). The inset figure is a magnified portion of the latter chromatogram. Analytes are numbered as in Table 2. The experimental conditions are given in the text.

with 50 ml of a mixture of 10 m*M* phosphate buffer (pH 7)–methanol (50:50, v/v). Samples were shaken into 200-ml screw capped glass vessels for 2 and 18 h (overnight), respectively, by use of a horizontal shaker. Equilibration period up to 18 h gave recoveries no better than 75% for the least polar phenolic analyte (Table 3).

In addition to freshly spiked soil samples the MASE technique was also validated with the analysis of aged residues. Samples from both soil types were spiked at the level of 0.5 μg/g and stored in the refrigerator (~4 °C). Samples were analyzed at weekly intervals up to 1 month (Fig. 4). From the soil A the most polar analytes (2,4-D, MCPA, 2,4,5-T and dichlorprop) were efficiently recovered after 1 month of storage. The extraction efficiency of the least polar 2,4-DB and the two phenolic metabolites 2,4-dichlorophenol and 2,4,5-trichlorophenol was reduced by 6–8%. From the soil B 2,4-D, MCPA and 2,4,5-T were extracted efficiently after 1 month, but the extraction efficiency of dichlorprop was reduced by 8%. For the least polar analytes the extraction efficiency of 2,4-dichlorophenol was reduced by 12%, while for 2,4-DB and 2,4,5-trichlorophenol by 6–7% (Fig. 4). Under refrigerated conditions it is expected that the soil microbial action to be inhibited and thus the reduction of the extraction efficiency might be attributed to either analyte-soil interactions resulting in a more stable incorporation of the analytes into the soil matrix and/or to evaporation losses, especially for the volatile phenolic conversion products. Despite the repellent effect of clay minerals (negatively charged) to negatively charged phenoxyalkanoic acids, these week acids are still sorbed on soil components and various mechanisms have been proposed [47]. Reduced extraction efficiency of aged phenoxyalkanoic acid residues has been reported also in the literature, especially for soils rich in organic matter [43].

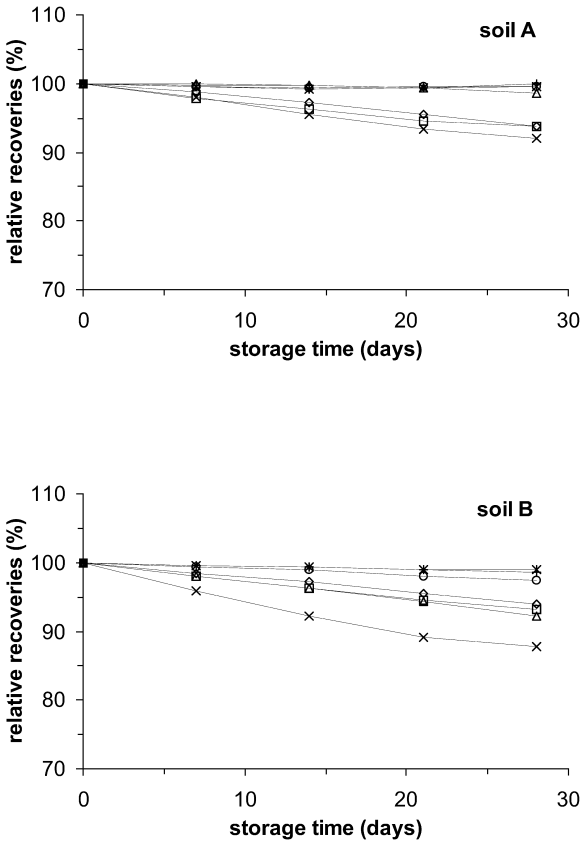

Fig. 4. Effect of soil storage time on the extraction efficiency of 2,4-D (○), MCPA (*), 2,4,5-T (+), dichloprop (△), 2,4-dichlorophenol (×), 2,4-DB (□) and 2,4,5-trichlorophenol (◊) from two soil types (A, B). Each data point represents an average of three replicates. Relative recoveries have been calculated by normalizing % of the values observed for freshly spiked soils. Soil characteristics and experimental conditions are given in the text.

Table 3
Analyte[a] recoveries from soil A spiked at the level of 0.5 μg/g and processed by solid–liquid flask-shaking technique for 18 h

Analyte	Recovery (RSD%; $n=3$)
2,4-D	99 (1)
MCPA	98 (3)
2,4,5-T	98 (1)
Dichloprop	99 (5)
2,4-Dichlorophenol	83 (1)
2,4-DB	92 (1)
2,4,5-Trichlorophenol	75 (2)

[a] Analytes are ordered by increasing retention time.

The proposed method was also applied for the analysis of field weathered soil samples. Soils were collected from a rice field where phenoxyalkanoics are also used for the control of broad-leaved weeds. Soil samples were collected 3 months after application, as rice fields are not accessible to soil sampling at earlier times due to the water cover. None of the sought analytes was detected in the soils analyzed.

4. Conclusions

MASE has been proven an effective technique for the analysis of phenoxyalkanoic acid herbicides in soils including also their major phenolic conversion products. It provides efficient analyte recoveries along with small extraction time and high sample throughput. Analysis of soil extracts by on-line SPE–HPLC is performed automatically in a single step and requires low operator involvement. The proposed method can be used effectively for the analysis of sought analytes in contaminated soil sites as well when present at trace levels (ng/g) in agricultural soils.

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