ELSEVIER

Journal of Chromatography A, 897 (2000) 279–293

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Continuous microwave-assisted extraction, solvent changeover and preconcentration of monophenols in agricultural soils ☆

M.A. Crespín, M. Gallego, M. Valcárcel*

Analytical Chemistry Division, Campus de Rabanales, University of Córdoba, E-14071 Córdoba, Spain

Received 31 January 2000; accepted 28 June 2000

Abstract

An automatic extraction, preconcentration and clean-up module for the extraction of phenolic compounds from soils was developed; the separation and quantitation of each phenol is accomplished by GC–MS. The sorption–desorption of thirteen phenols on soils containing variable amounts of organic carbon (0.05–3.4%) and clay minerals (2–43%) at pH 5.7–8.6 was investigated. For this purpose, uncontaminated soils were spiked with 5 or 20 μg of each phenol per g of soil; the soils were then stored at 4°C for at least 3 months prior to analysis in order to simulate analyte–matrix interactions other than material losses and environmental degradation in actual contaminated soils. The organic carbon content in acid and alkaline soils affects the sorption of chlorophenols but not that of alkylphenols. On the other hand, alkylphenols are preferentially sorbed by neutral soils, the process being influenced by the clay mineral content. Based on the results, alkylphenols interact more strongly with agricultural soils than do chlorophenols; also, both types of compound are less strongly sorbed by loamy sand soils owing to their increased sand contents.

Keywords: Soil; Environmental analysis; Clays; pH effects; Microwave-assisted extraction; Extraction methods; Phenols; Chlorophenols; Alkylphenols

1. Introduction

Phenolic compounds are industrially employed as intermediates and in the production of dyes, plastics and pharmaceuticals. Consequently, most phenols in soil come from industrial waste sources; some, however originate from degradation of natural sources (e.g., lignine and humic acids) or pesticides. These substances are hazardous to human health, so much so that some are on the lists of priority pollutants of the European Union and de US Environmental Protection Agency [1].

The interaction between phenols and soils can be affected by various factors such as variations in clay mineral and organic matter contents; in addition, microbial activity can transform phenols into other products that persist in soil in the form of either free or bound residues [2]. Binding of phenols to soil organic matter inhibits their mineralization and may therefore constitute a delayed hazard if the phenols are subsequently released. Binding can thus play a central role in neutralizing phenolic compounds provided their subsequent release is somehow limited. The likelihood of phenolic compounds being

☆Presented at the 9th Symposium on Handling of Environmental and Biological Samples, Porto, 10–13 October 1999.

*Corresponding author. Tel.: +34-957-218-616; fax: +34-957-218-616.

E-mail address: qa1meobj@uco.es (M. Valcárcel).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00784-6

released from soils varies according to whether the analytes are incorporated by adsorption (after only a few minutes of interaction) or by sequestration (which takes weeks or even months) [3]. The former phenomenon is involved at the early stages of sorption, where H-bonding and van der Waals forces prevail. On the other hand, sequestration involves sorption at remote microsites within the soil matrix. When the total organic carbon in sediments exceeds 0.1%, this is considered to be the dominant factor in the partitioning of hydrophobic organic compounds such as phenols; however, the composition of the organic matter (especially the amount and type of humic acids present) can affect the sorption of compounds onto the sediment [4]. Clay minerals play a prominent role in the sorption of phenols by soils as a result of its association with organic carbon, which not necessarily covers inorganic surface-rathes, it may promote chemically-induced oxidative coupling owing to the nearness of the required reactive soil constituents [5]. Soil pH affects the sorption of organic compounds such phenols in two ways, namely: by altering the surface charges of the clay minerals [6] and the sorbent capacity of the organic matter [7], and by ionizing the hydroxyl group in the phenols, thereby changing the ease with which it can be sorbed by organic matter and clay mineral surfaces [3].

Analyses for organic environmental pollutants require complex procedures involving several steps such as extraction, clean-up and preconcentration prior to quantitation. Extraction is often performed by refluxing organic solvents, but new techniques such as supercritical fluid extraction, accelerated solvent extraction and microwave-assisted extraction (MAE) have lately become available for this purpose. The MAE technique, for example, has been used to extract pesticides [8–11], polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) [12,13], and phenols [9,13–18]. It was also used to extract 14 phenols spiked to soil suspensions at 115°C for 20 min, with recoveries of 55–80% for alkylphenols and chlorophenols, but low recoveries (10–20%) for nitrophenols owing to their degradation [14]. Phenols and cresols were also extracted by MAE from spiked soil matrices by adding variable amounts of activated charcoal in order to strengthen analyte-matrix interactions [16]; the results showed MAE to be a highly powerful technique for extracting cresols from soils containing up to 10% of adsorptive charcoal in their matrices. A comparative study of MAE and Soxhlet extraction for five phenols in soils was recently conducting using special experimental designs [18]. MAE was found to provide the better results; both techniques, however, gave recoveries lower than 50% for 2-methylphenol.

A thorough literature scan revealed the lack of systematic studies about the effect of different types of soil (variable pH, organic carbon and clay minerals contents) on the sorption of phenols. The purpose of this work was thus to use an automatic extraction, preconcentration and clean-up system including a microwave oven for the extraction of phenolic compounds from various types of soils with a view to assessing the influence of soil organic matter, clay minerals and pH on the sorption/desorption of phenols. The soils used were spiked 3 months before analysis in order to allow any analyte-matrix interactions to occur over the weathering period to an extent similar to that in actual contaminated soil of similar properties [16]. Thirteen phenolic compounds and seven different types of soils (including acid and alkaline ones) were used for this purpose.

2. Experimental

2.1. Standards and solvents

Phenol, 4-methylphenol (4-MP), 2,5-dimethylphenol (2,5-DMP), 3,4-dimethylphenol (3,4-DMP), 2-*tert*.-butylphenol (2-TBP), 4-chloro-3-methylphenol (4-C-3-MP), 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 3,4-dichlorophenol (3,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), pentachlorophenol (PCP) and 3-nitrophenol (3-NP), and methyl nonanoate (internal standard) were obtained from Aldrich (Madrid, Spain) and used as received. Ethyl acetate, ethanol, acetone and *n*-hexane, all in HPLC grade, were purchased from Romil (Loughborough, UK). XAD-2 styrene–divinylbenzene, 50–100 μm, was supplied by Serva (Heidelberg, Germany). Stock standard solutions containing a 10 mg/ml concentration of each phenol were prepared in 99.9%

acetone and stored in glass-stoppered bottles at 4°C. Appropriate volumes of the stocks were diluted with acetone or ethyl acetate to prepare more dilute solutions containing phenols at the microgram per millilitre level.

2.2. Instruments and apparatus

The concentration of monophenols in the soils were determined with a chromatograph/mass spectrometer (HRGC 8000/MD800, Fisons, Madrid, Spain). GC separations were performed with a 30 m TRB-5 fused-silica capillary column of 0.25 mm I.D. packed with a 5% phenyl-methylpolisiloxane stationary phase of 0.25 μm film thickness from Teknokroma (Barcelona, Spain). Helium carrier gas (6.0 grade, Air Liquide, Seville, Spain) was passed at a rate of 1 ml/min with the aid of an electronic pressure control. The GC injection port, GC–MS interface and MS source temperatures were maintained at 250, 250 and 200°C, respectively. The column temperature was initially set at 60°C for 3 min and then raised at 6°C/min to 85°C, where it was held for 6 min; a subsequent ramp to 160°C at 10°C/min and another ramp to 250°C (held for 3 min) at 12°C/min were programmed. The optimal GC–MS conditions were established by using a mixture containing 10 μg/ml of each phenol and the internal standard (methyl nonanoate) in ethyl acetate; the injected volume was 1 μl. Mass spectra were obtained in the electron impact ionization (EI) mode at 70 eV. In the full-scan mode, the scanned mass range was m/z 50–450 and spectral data were acquired at a rate of 1.8 scans/s. The time for solvent delay was set to 3 min and sample injections were done in the split mode (split ratio 1:20).

The extraction system comprised a household microwave oven equipped with a magnetron of 2450 MHz with a nominal maximum power of 800 W as marketed. A piece of PTFE tubing of 0.5 mm I.D. for sample aspiration was inserted into the microwave oven through the vent holes in order to avoid drilling its walls. The continuous flow manifold consisted of two peristaltic Gilson Minipuls-3 pumps fitted with poly(vinyl chloride) and Solvaflex pumping tubes for aqueous and organic solutions, respectively; two Rheodyne 5041 injection valves; two laboratory-made PTFE filters, one of them packed with glass wool (4 cm×2 mm I.D.) to remove the largest solid particles and the other furnished with a paper disk (3.8 cm^2 filtration area) to filter fine particles; a custom-made phase separator furnished with a Fluoropore membrane (1 μm, pore size, Millipore, Spain) described elsewhere for liquid–liquid extraction [19]; and a PTFE solid-phase extraction column (3 cm×3 mm I.D.) containing 50 mg of XAD-2 sorbent.

2.3. Physical characterization of the soils

Agricultural soil samples from the A horizon were air-dried at room temperature for 1 week, ground, sifted to a particle size less than 2 mm and treated with a view to their classification. Soil pH was determined with a glass electrode in a 1:2.5 (g of soil/ml of distilled water) mixture; the slurry was magnetically stirred for 10 min and allowed to separate before the supernatant pH was measured. The total organic carbon content was determined by photometry, using sulfuric potassium dichromate as reagent [20]. The clay content in each soil sample was determined densitometrically [21]; the sedimentation rate allowed the distribution of soil compounds by particle size to be determined. Based on the results, the soils were classified in terms of their clay, silt and sand contents [22].

2.4. Preparation of spiked soils

In order to study the influence of soil class on the sorption of phenols, seven representative agricultural soil samples encompassing wide ranges of pH, clay and organic matter contents were selected. Uncontaminated soils (blank samples) were previously analysed and then spiked with the phenols following reported recommendations [16]. For this purpose, the soil samples were air-dried at room temperature for 1 week and sifted to a particle size below 2 mm. Two 100 g aliquots of each soil were individually spiked with 50 ml of acetone containing 0.5 or 2 mg of 13 phenols in order to study the influence of the pollutant concentrations on the sorption process. After spiking, the samples were allowed to air-dry (ca. 10 h) with shaking every 30 min at the beginning and 1 h at the end. The samples were then stored in amber glass-stoppered bottles at 4°C for at least 3

months before their first extraction. The phenols were assumed to be uniformly distributed in the 100 g of sample; also, because the soils still retained residual moisture throughout the storage period, any analyte-matrix interactions were assumed to have occurred over the weathering period and to an extent similar to that in actual contaminated soil of similar properties. In fact, the extraction results for analytes spiked in this manner did not vary significantly from those for native analytes. The spiked samples remained stable at 4°C for at least 8 months after their first extraction but were rendered unusable after opening the bottles more than 20 times (probably through analyte evaporation).

2.5. Analytical methods

The soil samples (0.5–1 g) were weighed inside 100 ml PTFE bottles to which 30 ml of extractant (*n*-hexane–acetone, 95:5) was added. The bottle cap was drilled for insertion of the aspiration tube of the sample channel and the bottle was then placed in the microwave oven, in front of the magnetron; the extraction was performed at a power of 500 W for 10 min. After extraction, pump 1 was started as depicted in Fig. 1, 18 ml of the organic extract (sampling time, 10 min; flow-rate 1.8 ml/min) was aspirated and cooled by immersion in an ice beaker and then filtered to prevent clogging of the membrane of the phase separator by the colloid fraction of the soil. The filtered organic solution was mixed with 0.1 *M* NaOH; extraction of the phenols into the alkaline solution developed in a continuous manner along a knotted tube of 500 cm at the end of which the phenols were separated by means of a membrane phase separator (PS). The preconcentration of phenols on the XAD-2 sorbent required the prior adjustment of the pH to 1 with a 0.5 *M* HNO_3 stream. Phenols were retained on the sorbent column and the sample matrix was sent to waste. Residual aqueous solution in the column and connections was dried by passing an N_2 stream for 5 min. Elution was accomplished by injecting 125 μl of eluent (ethyl acetate containing 10 μg/ml of internal standard) into the N_2 stream. The eluate was collected in glass vials containing anhydrous sodium sulphate (desiccator) and 1 μl aliquots were used for GC–MS analysis. The following experiment required flushing and conditioning of the sorbent column with 1 ml of ethanol and 1 ml of Milli-Q water. Occasionally, the filters were flushed with a 10^{-2}*M* HNO_3 stream.

3. Results and discussion

3.1. Optimization of the extraction conditions

The optimal conditions for the extraction of

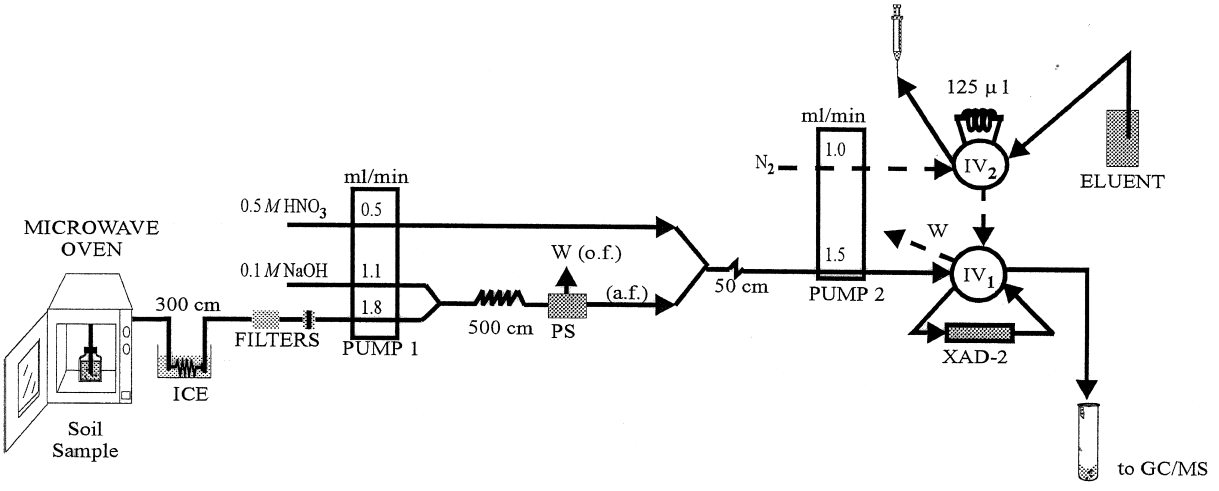

Fig. 1. Experimental design for the continuous extraction, sorption/clean-up and elution of phenolic compounds in soil samples. PS=membrane phase separator; o.f. and a.f.=organic and aqueous phase; W=waste; eluent=ethyl acetate; GC–MS=gas chromatograph–mass spectrometry system.

phenols from soils were established by using a sample of the sandy clay loam textural class as test soil. This soil, of pH 8.4, had intermediate organic carbon (1.4%) and clay mineral contents (22%). The test soil (containing no phenols) was spiked with 5 μg of each phenol per g or soil as described in the Section 2.4 and stored for 3 months.

The efficiency with which pollutants can be extracted from soil using microwave energy depends on solvent polarity, microwave power and extraction time. Therefore, the effects of these variables were the first to be examined, using a continuous system similar to that of Fig. 1. For this purpose, 1 g of spiked soil was extracted with 30 ml of solvent containing between 0 and 10% of acetone in *n*-hexane at a variable microwave power for 10 min. The most relevant results are listed in Table 1; the recovery was calculated by assuming 100% to organic standards containing the same amount spiked to the soils (5 μg/g) that were directly injected into the gas chromatograph. The extraction yields for alkylphenols were all very low, probably because they interact more strongly with the soil samples than do the other phenols. The highest recoveries were obtained using 1–5% acetone at full microwave power; however, under these conditions and at a microwave power of 750 W, the extractant was evaporated, so the recoveries obtained were unreliable. The extraction efficiencies for chlorophenols and 3-nitrophenol were more markedly affected by the organic solvent polarity than that by the microwave power, so the best results were obtained for 5 and 10% of acetone in *n*-hexane. Also, the recovery of pentachlorophenol was lower than those of the other chlorophenols as consequence of its degradation by the microwaves [14]. At a high microwave power (e.g., 750 W), and above 1% acetone concentrations, higher recoveries were obtained for chlorophenols and 3-nitrophenol as a result of the solvent being evaporated owing to the increased polarity of the extractant and of the microwave power giving rise to large quantitation errors. In order to avoid evaporation of the organic solvent and the potential decomposition of the phenols, a solution containing 5% acetone in *n*-hexane was used as extractant and the microwave power was set at 500 W. Under these conditions, and with this soil type, alkylphenols were recovered by less than 25% and the other phenols (pentachlorophenol excepted) by more than 50%. As the extraction efficiency was also dependent on the extraction time, this study was completed by keeping the sample in the oven for variable times (1, 5, 10 or 15 min). The recovery of the phenols increased with increasing extraction

Table 1
Percent recoveries of phenols from soils obtained by using MAE at variable power and proportions of acetone in *n*-hexane as extractant

	250 W				500 W				750 W			
	0%	1%	5%	10%	0%	1%	5%	10%	0%	1%	5%	10%
Phenol	20	36	48	24	26	50	57	31	41	55	70	28
Alkylphenols												
4-Methylphenol	9	17	18	8	10	20	23	9	16	26	30	12
2,5-Dimethylphenol	4	6	8	7	5	8	10	8	7	8	11	8
3,4-Dimethylphenol	4	8	11	9	5	9	12	10	8	12	18	13
2-*tert*.-Butylphenol	17	16	13	10	23	20	17	11	16	14	14	8
Chlorophenols												
4-Chloro-3-methylphenol	17	42	58	60	19	49	65	67	27	50	80	79
2-Chlorophenol	45	55	67	65	60	70	75	68	73	79	85	80
4-Chlorophenol	15	45	66	66	20	51	78	70	25	62	79	88
2,4-Dichlorophenol	41	57	70	72	56	66	75	76	60	73	100	107
3,4-Dichlorophenol	10	39	63	65	15	45	73	75	17	57	102	116
2,4,6-Trichlorophenol	23	41	55	73	30	52	70	71	45	54	75	77
Pentachlorophenol	3	6	20	35	7	12	32	39	6	9	26	28
3-Nitrophenol	1	20	40	34	1	23	50	42	2	35	70	61

Table 2
Chemical and flow conditions for the extraction and preconcentration/clean-up of phenols in soils

Extraction	Optimum range	Selected value
Microwave		
Power, *W*	250–500	500
% Acetone in *n*-hexane	1–5	5
Time, min	10	10
Liquid–liquid		
Sampling time, min	10	10
Organic flow-rate, ml/min	1.8	1.8
NaOH conc., *M*	0.1	0.1
NaOH flow-rate, ml/min	0.9–1.1	1.1
Extraction coil length, cm	400–600	500
Solid-phase		
HNO_3 conc., *M*	0.5	0.5
Amount of sorbent (XAD-2), mg	45–60	50
Eluent (ethyl acetate) volume, μl	125	125
HNO_3 flow-rate, ml/min	0.5	0.5
N_2 flow-rate, ml/min	0.5–1.5	1.0

time; beyond 10 min, however, overpressure caused the PTFE bottle to break. Table 2 lists the selected values of the variables for the microwave-assisted solid–liquid extraction of the phenols.

In order to boost the sensitivity and selectivity of the proposed method, we used a preconcentration/clean-up system used elsewhere for phenols in water samples [23]. In this solid-phase extraction system, however, the phenols were retained on an XAD-2 sorbent column in an aqueous medium at pH 1 rather than in *n*-hexane. As a result, the organic extract from the microwave oven required liquid–liquid extraction with an aqueous solution prior to insertion into the sorbent column. For solvent changeover [Fig. 1] the organic stream, which might contain solid particles, was filtered and then extracted with the aqueous solution. As the phenols are uncharged at low pH values (their pK_a values are generally higher than 8), alkaline solutions at variable pH were tested. Above pH 12, the recoveries of all phenols remained constant, so a 0.1 *M* NaOH solution was used for medium changeover. The alkaline solution from the membrane separator was mixed with a stream of 0.5 *M* HNO_3 in the merging-zones mode to adjust the sample pH to 1 before preconcentration in the sorbent column. The optimal values of the chemical and physico-chemical variables are given in Table 2. The effects of the flow variables influencing the extraction, preconcentration and elution processes (viz., flow-rates, extraction coil length and eluent volume) were also investigated. Their optimum ranges and selected values are also listed in Table 2.

3.2. Chromatographic behaviour

Phenols were separated underivatized by GC, and their retention was examined by using two chromatographic columns of different polarity. For this purpose, a sandy clay loam soil spiked with 1 ml of standard solutions containing the phenols studied in amounts between 30 ng and 20 μg in acetone per g of soil was used as blank; after the acetone was evaporated (ca. 10 min), each soil sample aliquot was treated inside the microwave oven and then aspirated into the flow system of Fig. 1. The short time elapsed between spiking of the soil with the phenols and its analysis minimized analyte-matrix interactions, so the phenol recoveries were quantitatively reliable enough to construct calibration graphs. Sensitivity was higher with the TRB-5 column (the more polar) than with the TRB-1 column. The performance and reliability of the proposed method was assessed by determining the sensitivity (slope of the calibration

Table 3
Sensitivity and precision of the proposed chromatographic method

Compound	m/z[a]	Sensitivity (10^{-4})	Detection limit (ng/g)	RSD (%)
Phenol	65,66,***94***	2.7	15	6.8
Alkylphenols				
4-Methylphenol	77,**107**,*108*	5.1	10	5.4
2,5-Dimethylphenol	77,107,***122***	4.5	20	5.7
3,4-Dimethylphenol	77,**107**,*122*	5.4	20	6.0
2-*tert*.-Butylphenol	107,**135**,*150*	1.2	25	7.1
Chlorophenols				
4-Chloro-3-methylphenol	77,**107**,*142*	4.1	20	6.7
2-Chlorophenol	64,***128***,130	5.3	20	4.5
4-Chlorophenol	65,***128***,130	4.0	10	7.9
2,4-Dichlorophenol	98,***162***,164	3.3	10	4.7
3,4-Dichlorophenol	99,***162***,164	2.8	10	7.0
2,4,6-Trichlorophenol	132,***196***,198	2.0	20	3.9
Pentachlorophenol	165,230,***266***	1.0	25	6.7
3-Nitrophenol	65,93,***139***	1.9	50	6.2

[a] m/z values in italics are M^+ values; those in bold face are for base peaks and those underlined are quantitation values.

graph), analyte detectability, linearity range and relative standard deviation. The results, and the m/z values used for identification/quantitation, are listed in Table 3. The equations for the standard curves (correlation coefficients ranged from 0.996 to 0.999) were obtained by plotting the analyte-to-internal standard peak area ratio against the phenol concentration. The linear range was similar for all phenols (30–20000 ng/g) except PCP and 2-TBP (60–20000 ng/g), and 3-NP (120–20000 ng/g). Detection limits (based on a signal-to-noise ratio of 3) and relative standard deviations (obtained by measuring 11 samples containing 1 μg/g of each phenol) are also listed in Table 3.

3.3. Soil characterization in terms of sorption phenols

Phenolic compounds can reach soil through various, well-defined processes leading to their gradual disappearance or immobilization. The sorption of phenols by soil is affected by (1) the properties of the phenols themselves, (2) the surface character of the sorbents (humic acids and clay minerals included) and (3) the soil pH. Various Mediterranean soils (ca. 20) were characterized according to textural class, pH and organic carbon content in order to encompass a wide range of soil classes. The procedure used to determine each parameter is described in the Section 2.5. The textural class (sand, silt and clay mineral contents) were determined by using a triangular diagram [22]. Soils with clay minerals between 2 and 43%, organic carbon contents between 0.05 and 3.4%, and an acid, neutral or alkaline character were studied in the sorption experiments. The most organic soil (3.4%) was collected from a holm oak/pine forest, 0–10 cm below ground surface. Table 4 summarizes the physico-chemical and mineralogical properties of the seven soils selected. Two uncontaminated sub-samples of each soil type were spiked at two concentration levels (5 and 20 μg/g) with the thirteen phenols studied in order to examine the influence of their concentrations on their sorption by the soils; a sorption-equilibration period of 3 months was observed after spiking in order to simulate native soils. Aliquots of 0.5–1 g of each soil were analysed using the proposed method. Bottles were capped between samplings to avoid volatilization and contamination. After opening 15–20 times, bottles were discarded as they exhibited losses of several phenols. Therefore, no periodic sorption experiments during storage were carried out owing to the risk of analyte evaporation, so only the

Table 4
Soil characteristics

Soil	Textural class	Particle size analysis			% Organic carbon	pH
		% Sand	% Silt	% Clay		
1	Sandy loam	65	21	14	0.7	5.7
2	Sandy loam	55	31	14	3.4	5.9
3	Sandy loam	67	23	10	0.9	8.6
4	Loam	43	45	12	1.6	8.0
5	Sandy clay loam	53	21	26	0.3	7.1
6	Loamy sand	86	12	2	0.05	7.6
7	Clay	22	35	43	0.6	8.3

overall sorption of phenols in different agricultural soils after 3 months of storage was evaluated.

The colloid fraction of soil plays a central role in adsorption–desorption phenomena. In Mediterranean agricultural soils, this fraction consists primarily of clay minerals, accompanied by small amounts of organic matter, oxides and hydroxides. In addition, soil pH influences the surface charge of the colloid. As can be seen from Table 5, the recoveries of phenols from all the soils studied were higher when they were spiked with 20 μg/g than with 5 μg/g; thus, the average extraction efficiency for alkylphenols was 65 and 34% with 20 and 5 μg/g, respectively, and that for chlorophenols 87 and 67% with 20 and 5 μg/g respectively. This was attributed to the presence of a small number of high-affinity sites leading to a non-linear sorption behaviour. The experimental results (see Table 5) can be described in terms of a kinetic model composed of two separate compartments; one (for 5 μg/g) represents sorption to high affinity sites (linear sorption) and the other (for 20 μg/g) the non-linear sorption behaviour. A kinetic study of pentachlorophenol sorption at different concentrations in a soil previously revealed that the extent of sorption–desorption is concentration-dependent [24].

3.4. Effect of organic carbon on sorption

The effect of organic matter on phenol sorption was studied by using two acid soils (containing 14% clay minerals) and two alkaline ones (containing 11±1% clay minerals); their organic carbon contents were 0.7–3.4%. The specific soils used for this purpose were 1, 2, 3, and 4 in Table 4.

Fig. 2 shows the variation of the recoveries of alkylphenols and chlorophenols with increase in the amount of organic carbon in the soils (spiked with each phenolic compound at two different concentrations, viz., 5 and 20 μg/g soil). In all soils studied (1, 2, 3 and 4) the initial contaminant concentration was found to influence the desorption rate. Thus, at the higher phenol concentration (20 μg/g), the influence of the organic carbon content was less marked than that at the lower one (5 μg/g). In the acid soils (Fig. 2A), phenol recoveries increased (minimum adsorption) with increase in the amount of organic carbon, the effect being more marked for chlorophenols than for alkylphenols. This can be ascribed to organic substances such as humic acids adsorbed on the mineral surface being active and fully accessible for phenol sorption at low carbon contents; however, as the organic carbon increases, these organic substances adopt interfacial configurations that reduce phenol sorption [6]. The alkaline soils (Fig. 2B) provided the opposite results: phenol recoveries decreased with increasing organic carbon content. In this type of soil, organic matter is negatively charged so the hydroxyl group in alkylphenols (pK_a 10–11) is undissociated, the positive charge on the ring prevails owing to the electron-releasing character of the methyl group and electrostatic interactions take place between the organic matter and alkylphenols. The presence of chloro or nitro groups in the phenols endows them with an electron-withdrawing character; on the other hand, the organic matter can act as an electron-donor via its aromatic structural units. Strong binding is thus possible between the two, the effect being more pronounced at high organic carbon contents [25]. As

Table 5
Percent recovery of phenols spiked to agricultural soils 3 months before analysis[a]

Compound	Soil 1		Soil 2		Soil 3		Soil 4		Soil 5		Soil 6		Soil 7	
	5 μg/g[b]	20 μg/g[b]	5 μg/g[b]	20 μg/g[b]	5 μg/g[b]	20 μg/g[b]	5 μg/g[b]	20 μg/g[b]	5 μg/g[b]	20 μg/g[b]	5 μg/g[b]	20 μg/g[b]	5 μg/g[b]	20 μg/g[b]
Phenol	54.3±4.1	83.0±5.4	58.0±4.3	85.0±5.4	54.0±2.2	80.1±5.4	47.0±3.8	77.0±5.4	60.0±4.4	87.3±5.4	70.4±4.6	87.8±5.4	65.8±4.5	88.0±5.3
Alkylphenols														
4-Methylphenol	39.0±2.4	93.7±4.8	40.0±2.5	94.0±4.7	35.8±2.4	84.8±4.6	26.0±1.8	82.0±4.6	5.0±0.4	40.0±2.5	67.0±4.0	92.2±4.9	40.0±2.5	80.0±4.6
2,5-Dimethylphenol	22.2±1.6	80.0±5.3	25.0±1.6	82.0±4.1	14.0±0.9	63.0±3.4	10.0±0.7	61.0±3.4	3.0±0.2	11.0±0.7	61.8±3.7	81.0±4.3	19.2±1.2	68.0±3.9
3,4-Dimethylphenol	26.8±1.7	72.0±3.6	28.1±1.8	73.0±3.6	15.7±1.0	61.0±3.3	11.0±0.8	59.0±3.3	4.0±0.3	12.0±0.7	77.3±4.6	88.0±4.7	20.0±1.2	65.0±3.7
2-*tert*.-Butylphenol	30.0±2.2	68.0±4.8	31.0±2.2	69.0±4.9	22.5±1.7	59.5±4.4	15.0±1.2	58.0±4.4	4.0±0.3	12.0±0.9	65.6±4.8	80.0±5.6	28.0±2.1	65.0±4.5
Chlorophenols														
4-Chloro-3-methylphenol	63.0±5.0	98.0±7.0	68.0±5.3	100.0±7.0	77.1±5.8	102.2±7.2	69.0±5.4	99.0±7.0	45.0±3.6	69.0±5.4	90.0±6.5	102.0±7.2	84.0±6.2	99.0±7.1
2-Chlorophenol	48.4±2.8	91.0±5.1	73.0±3.5	96.1±4.3	80.4±4.0	95.2±3.9	68.0±4.4	92.0±3.8	70.0±3.1	89.8±3.7	85.1±4.4	90.0±4.5	79.3±3.6	97.1±4.4
4-Chlorophenol	52.4±4.6	80.4±5.7	82.2±6.4	94.0±6.6	88.4±6.6	102.3±7.2	70.0±5.5	99.0±7.0	82.0±6.4	90.4±6.5	95.2±6.8	103.0±7.3	81.3±6.0	98.0±7.0
2,4-Dichlorophenol	49.0±3.2	92.1±5.2	71.2±3.5	95.0±4.2	82.0±4.9	92.5±5.0	70.0±3.5	90.0±3.8	81.0±4.2	91.2±4.5	87.0±4.3	96.0±4.6	80.0±3.8	91.7±3.9
3,4-Dichlorophenol	46.7±3.7	78.1±5.8	74.1±5.6	84.6±6.2	78.0±5.8	83.3±6.2	67.0±5.3	81.0±6.0	69.3±5.5	81.8±6.0	85.0±6.2	97.0±6.8	79.0±6.0	92.5±6.5
2,4,6-Trichlorophenol	44.6±2.5	90.0±4.0	69.6±3.5	97.4±4.0	80.0±3.8	102.3±4.3	65.0±3.0	98.0±4.1	75.4±3.6	99.0±4.4	88.0±4.0	99.3±4.3	79.6±3.8	97.0±4.4
Pentachlorophenol	36.0±3.0	43.0±1.9	39.0±3.0	45.0±3.4	33.1±2.6	42.1±3.2	28.0±2.2	40.0±2.8	35.0±2.7	54.8±3.8	55.0±4.0	66.3±4.7	35.0±2.7	40.1±2.8
3-Nitrophenol	42.0±3.2	87.1±5.5	63.0±4.1	93.4±5.6	58.4±4.5	91.1±5.5	42.0±3.3	86.7±5.6	52.0±3.6	80.7±5.0	67.0±4.6	89.4±5.6	59.0±5.0	89.0±5.7

[a] Percent recovery±standard deviation (n=5).
[b] Concentration spiked.

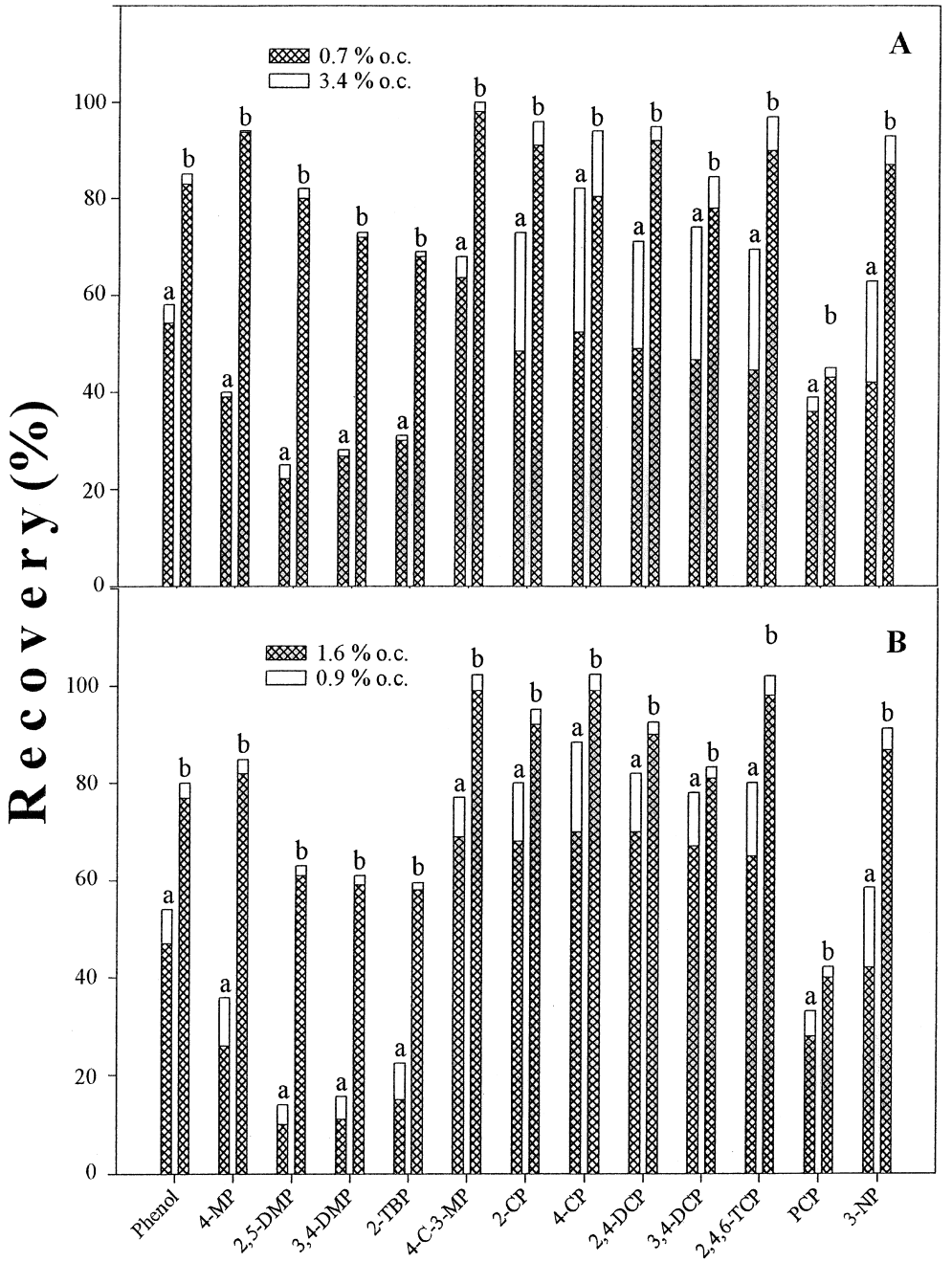

Fig. 2. Recovery of phenols as a function of the organic carbon (o.c.) content in acid soils 1 and 2 (A) and alkaline soils 3 and 4 (B). For soils spiked with 5 (a) and 20 μg/g (b) of phenols, respectively. See Table 4.

can be seen from Fig. 2, 4-C-3-MP in acid and alkaline soils behaves more like methylphenols than like chlorophenols because the effect of its methyl group prevails over that of the chlorine atom. The behaviour of 3-NP in this respect is similar to that of chlorophenols.

3.5. Effect of the clay minerals on sorption

All clay contains hydrous aluminium silicates that have sheet-like structures; accordingly, clays can be classified as two-layer and three-layer clays. Clay minerals can acquire a net negative charge by ion replacement (Si and Al ions are replaced by other metal ions of similar size but lesser charge). On the other hand, the charge of hydroxyl groups in the aluminium octahedral layer in clay minerals is related to soil pH. Therefore, the clay mineral fraction in Mediterranean soils plays a prominent role in the sorption of organic/inorganic compounds owing to its ion-exchange capacity and to its ability to incorporate molecules of these compounds into its structure.

Taking into account that only two acid soils were studied and they contained the same amount of clay minerals, both were discarded for this experiment; thus, only alkaline soils, with variable amounts of clay minerals, were used. Soil 4 was also discarded as it contained an amount of clay minerals similar to that of soil 3 in addition to abundant organic carbon. Consequently, only soils 3, 5, 6 and 7 (Table 4) with average pH values of 7.9 ± 0.7 and organic carbon contents of 0.5 ± 0.4, were selected. The highest average extraction efficiency (minimum adsorption in the soil) was obtained for soil 6 (68 and 88% for alkylphenols and chlorophenols, respectively, with PCP omitted for calculations) which had the lowest organic carbon and clay minerals contents so it probably contained the phenols preferentially in a deposited state. As shown in Fig. 3A, the recoveries of alkylphenols decreased with increasing clay mineral content up to 26%; surprisingly, the recoveries from soil 7 (43% clay minerals) were similar to those from soil 3 (with only 10% clay minerals). This can be explained as follows: clay minerals affect the sorption of alkylphenols in soils to an extent dependent on the types of clay minerals (illite, montmorillonite, kaolinite, etc.) they contain and their contents; in fact, some types of clay minerals such as kaolinite have a low ion-exchange capacity and are poorly adsorbent; thus, soil 7 may contain clay minerals differing markedly from those in the other soils [26]. Therefore, this study might be completed with X-ray diffraction experiments intended to determine the types of clay minerals present in the selected soils. The adsorption of alkylphenols arises from their ability to stabilize positive charges on the ring by virtue of the electron-releasing nature of alkyl groups, thereby facilitating their interaction with the negative charges in the clay. Chlorophenols (Fig. 3B), exhibited no linear dependence on the clay mineral content because, at the pH of the soils studied, these compounds may be partly ionized and hence unable to interact with the clays, which will be negatively charged. As in the previous experiment 4-C-3-MP and 3-NP behaved similarly to alkylphenols and chlorophenols, respectively.

3.6. Effect of soil pH on sorption

The pH of soil can affect the sorption of phenols because the surface charges of clay minerals vary significantly with it [6]. In addition, organic matter (especially humic acids) behaves rather differently depending on the soil pH, which alters its sorbent capacity: humic acids contain acidic functions such as carboxylic (pK_a 2–6) and phenolic groups (pK_a 8–12).

The influence of soil pH on the sorption of phenols was studied by using soils 1, 3, 4 and 5, with organic carbon contents of 0.3–1.6% and clay minerals contents of 10–26%. Soils with extreme values (2 and 7) were discarded because they contained the largest amounts of organic carbon (3.4%) and clay minerals (43%), respectively; also, soil 6 was excluded because it contained the smallest amounts of organic carbon and clay minerals. Taking into account the little dependence of alkylphenol recoveries on the amount of organic carbon, only the effect of soil pH on the clay mineral content is examined in this section. With chlorophenols as their recoveries were more markedly affected by organic carbon content than by clay mineral content, soil pH was primarily related to the organic carbon content. The effect of soil pH on the sorption of phenols is illustrated in Fig. 4; for alkylphenols (Fig. 4A), recoveries decrease from an acid to a neutral pH because alkylphenols are neutral at pH 7.1 and clay increases in negative charge with increasing pH. In the alkaline region, recoveries increase linearly with increasing soil pH by effect of alkylphenols being negatively charged and hence less prone to interact with the clay minerals. If the data for soil 7 (43% of

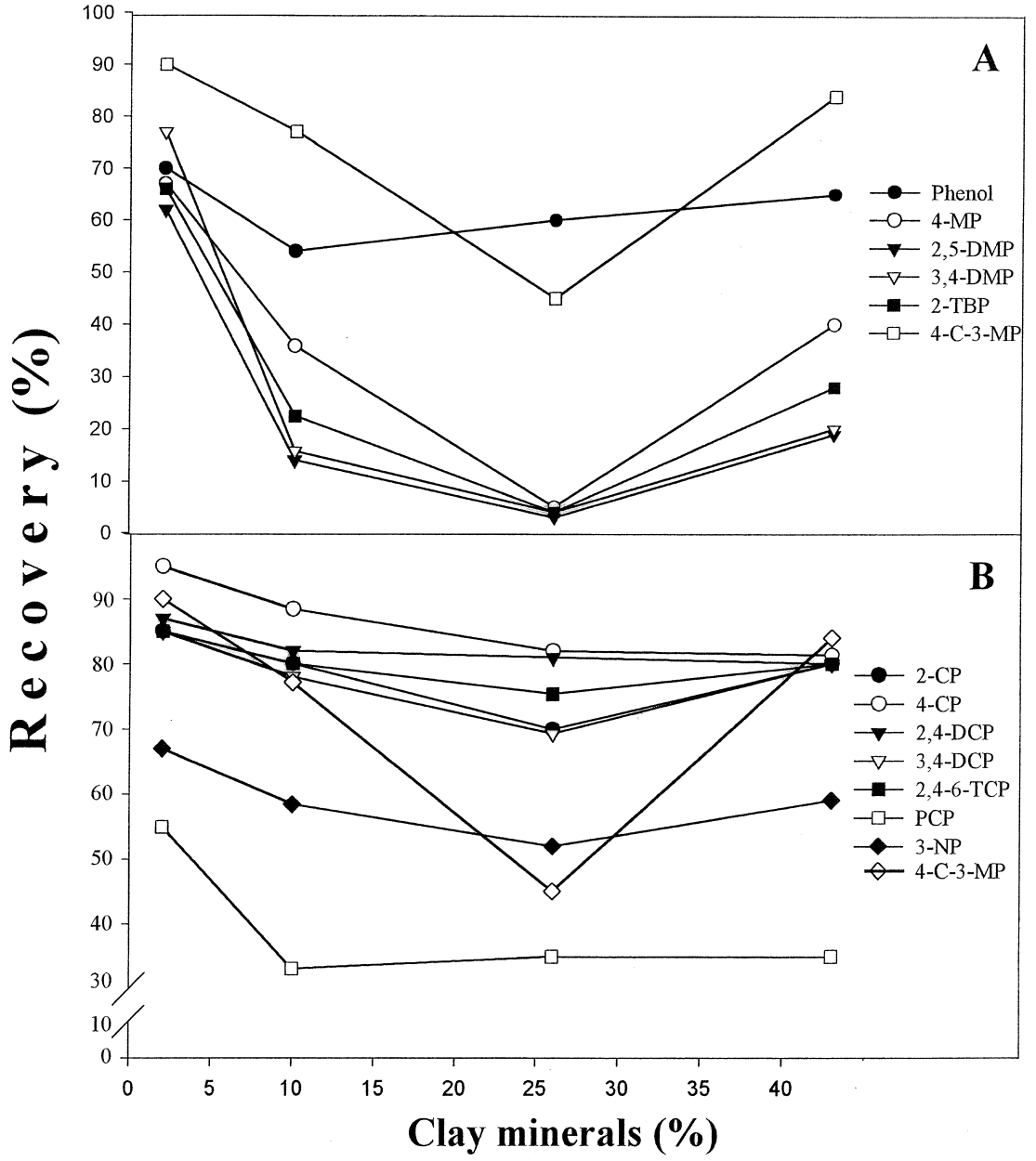

Fig. 3. Influence of the clay mineral content on the recovery of alkylphenols (A) and chlorophenols and 3-nitrophenol (B) from soils 3, 5, 6 and 7.

clay minerals and pH 8.3) are included in the Figure, points deviate from linearity, possibly because of the clay type contained in this soil, which confirms the results of the previous section. In summary, soil pH is critical to the adsorption of alkylphenols because it affects the sorbent properties of clays and the degree of ionization of alkylphenols; these effects are especially marked at neutral pH values (recoveries of ca. 5%). With chlorophenols (Fig. 4B), recoveries increase from acid to neutral pHs but change little in the alkaline region. By exception, the soil of pH 8.0 exhibits markedly decreased recoveries owing to its

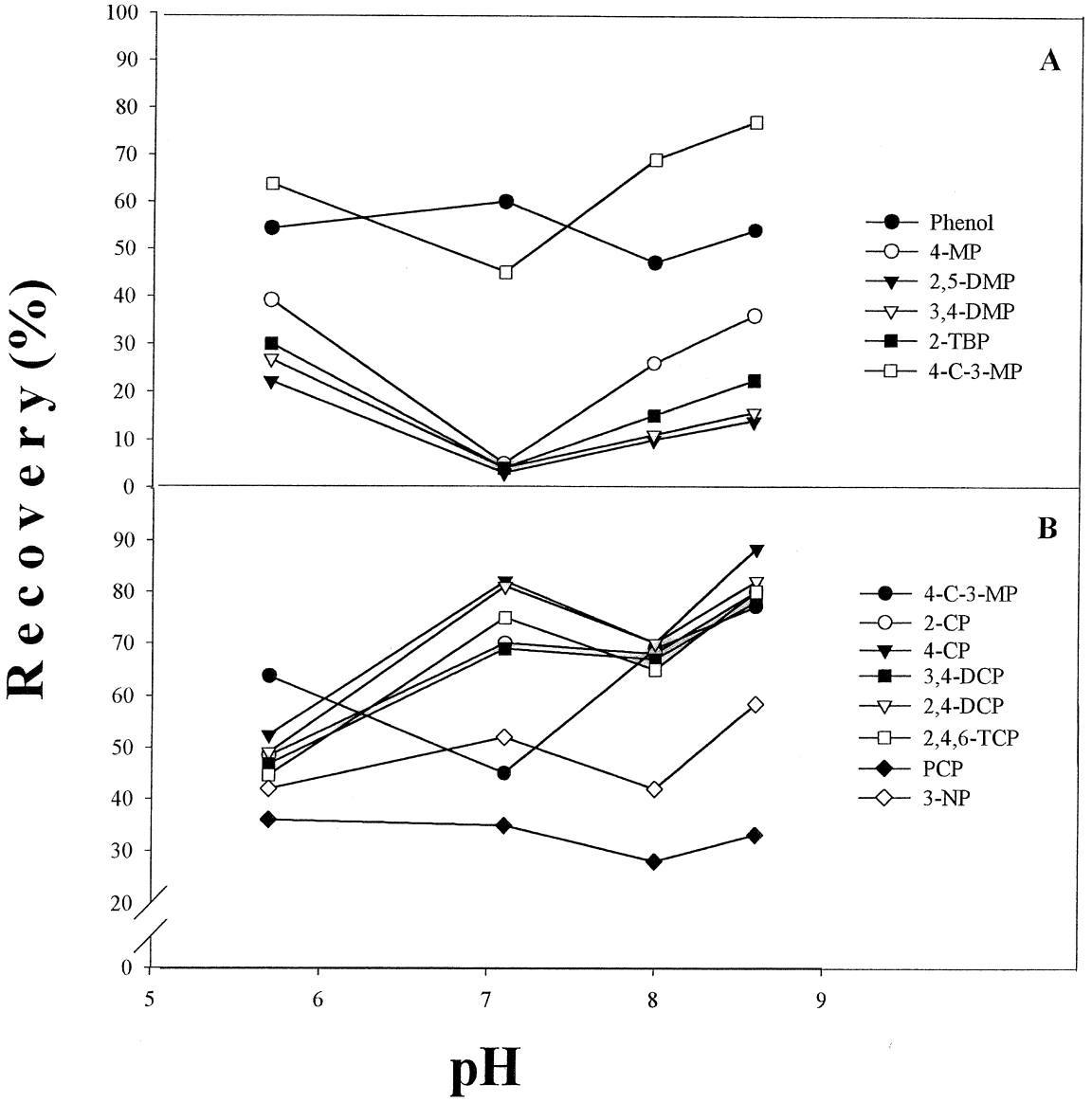

Fig. 4. Effect of soil pH on the recovery of alkylphenols (A) and chlorophenols and 3-nitrophenol (B) from soils 1, 3, 4 and 5 (see Table 4).

greater content in organic carbon (1.6%), the effect of which prevails over that of pH. The increased sorption by acid soils can be explained as follows: phenolic groups in humic acids are in molecular form while carboxylic groups are partially ionized; under these conditions, chlorophenols (in a neutral state) are more readily adsorbed onto organic mater surfaces. At lower pH values, humic acids are more hydrophobic as a whole and favour the sorption of chlorophenols. On the other hand, the hydrophilic character of humic acids decreases the sorption of chlorophenols by alkaline soils [7].

3.7. Comparison of the recoveries of phenols from different soils

In order to relate the sorption of phenols to soil properties, a bar graph showing the average sorption values for the phenols studied (with phenol, 3-NP and PCP omitted from the calculations) in the

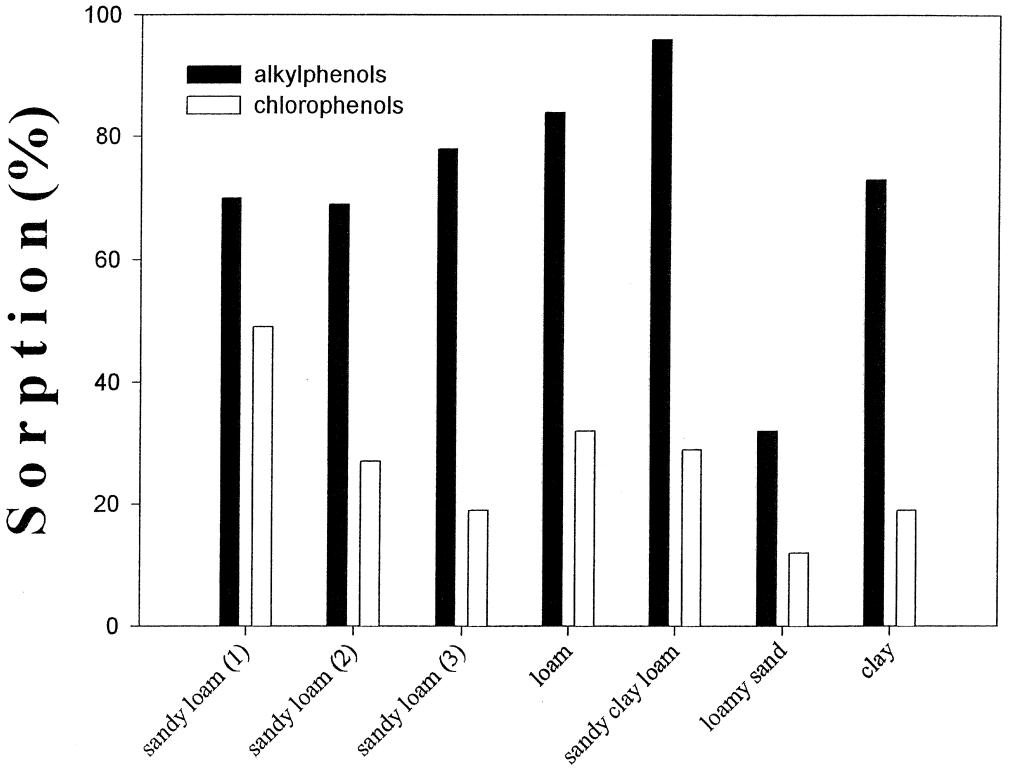

Fig. 5. Average sorption of phenolic compounds from the different soil classes tested. For details, see Table 4.

different soils was constructed. As can be seen from Fig. 5, alkylphenols interacted more strongly with all the soils tested except the loamy sand soil owing to its high sand content (86%) and its negligible clay and organic carbon contents. On the other hand, chlorophenols were much less strongly adsorbed; their strongest interaction was that with the sandy loam soil, with an acid pH (5.7) and a low organic carbon content (0.7%). These findings can be extrapolated to agricultural chlorinated pesticides that are hydrolysed to chlorophenols (e.g., 2,4-dichlorophenoxyacetic acid or 4-chloro-2-methylphenoxyacetic acid); the persistence of these pesticides in soils is related to their hydrolysis; the more marked this is, the higher is the proportion of chlorophenols, which are scarcely adsorbed by the soil matrix and are thus easily leached by irrigation or rain water, eventually becoming aquifer, river and lake pollutants. However, the soils used in this work were isolated from their native environment for 3 months so the analytes were incorporated into them by sequestration; one can therefore expect the persistence of phenols in environmentally exposed soils to be much lower.

Acknowledgements

This work was supported by grant PB-95-0977 from Spanish DIGICyT. The authors also acknowledge the assistance of Dr. J. L. González in characterizing the soils studied.

References

[1] EPA Method 604, Phenols in Federal Register, Friday October 26, 1984, Environmental Protection Agency, Part VIII, 40 CFR Part 136'', p 58.
[2] J.M. Sarkar, R.L. Malcolm, J. Bollag, Soil. Sci. Soc. Am. J. 52 (1988) 688.
[3] J. Dec, J. Bollag, Soil Sci. 162 (1997) 858.

[4] F. Kopinke, J. Pörschmann, U. Stottmeister, Environ. Sci. Technol. 29 (1995) 941.
[5] W.D. Burgos, J.T. Novak, D.F. Berry, Environ. Sci. Technol. 30 (1996) 1205.
[6] K. Takimoto, K. Ito, T. Mukai, M. Okada, Environ. Sci. Technol. 32 (1998) 3907.
[7] J.L. Gundersen, W.G. Macintyre, R.C. Hale, Environ. Sci. Technol. 31 (1997) 188.
[8] F.I. Onuska, K.A. Terry, Chromatographia 36 (1993) 191.
[9] V. López-Avila, R. Young, R. Kim, W.F. Beckert, J. Chromatogr. Sci. 33 (1995) 481.
[10] S.J. Stout, A.R. daCunha, D.G. Allardice, Anal. Chem. 68 (1996) 653.
[11] G. Xiong, B. Tang, X. He, M. Zhao, Z. Zhang, Z. Zhang, Talanta 48 (1999) 333.
[12] V. López-Avila, J. Benedicto, C. Charan, R. Young, W.F. Beckert, Environ. Sci. Technol. 29 (1995) 2709.
[13] V. López-Avila, R. Young, J. Benedicto, P. Ho, R. Kim, W.F. Beckert, Anal. Chem. 67 (1995) 2096.
[14] V. López-Avila, R. Young, W.F. Beckert, Anal. Chem. 66 (1994) 1097.
[15] M.P. Llompart, R.A. Lorenzo, R. Cela, J.R.J. Paré, Analyst 122 (1997) 133.
[16] M.P. Llompart, R.A. Lorenzo, R. Cela, K. Li, J.M.R. Bélanger, J.R.J. Paré, J. Chromatogr. A 774 (1997) 243.
[17] M.P. Llompart, R.A. Lorenzo, R. Cela, J.R.J. Paré, J.M.R. Belanger, K. Li, J. Chromatogr. A 757 (1997) 153.
[18] A. Egizábal, O. Zuloaga, N. Etxebarría, L.A. Fernández, J.M. Madariaga, Analyst 123 (1998) 1679.
[19] M. Gallego, M. Silva, M. Valcárcel, Anal. Chem. 58 (1986) 2265.
[20] A.L. Page, R.H. Miller, D.R. Keeney, Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed., Soil Science Society of America, Madison, WI, 1989.
[21] C. Benítez, E. Bellido, J.L. González, M. Medina, Biores. Technol. 63 (1998) 147.
[22] Organización de las Naciones Unidas para la Agricultura y la Alimentación, Guía para la Descripción de Perfiles de Suelo, Rome, 2nd ed., 1977.
[23] M.A. Crespín, S. Cárdenas, M. Gallego, M. Valcárcel, Rapid Commun. Mass Spectrom 12 (1998) 198.
[24] J.P. Divincenzo, D.L. Sparks, Environ. Sci. Technol. 31 (1997) 977.
[25] J. Pörschmann, U. Stottmeister, Chromatographia 36 (1993) 207.
[26] R.W. McCabe, in: D.W. Bruce, D. O'Hare (Eds.), Inorganic Materials, 2nd ed, Wiley, Chichester, 1996, p. 313.