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Multiresidue herbicide analysis in soil samples by means of extraction in small columns and gas chromatography with nitrogen–phosphorus and mass spectrometric detection

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Abstract

A rapid multiresidue method has been developed for the analysis of 21 nitrogen containing herbicides in soil. The compounds included widely used herbicides belonging to triazines, thiocarbamates, uracils, nitroanilines, chloroacetamides and oxadiazon, a non-classified heterocycle. The method is based in the extraction by sonication of soil samples placed in small columns using a low volume of ethyl acetate. Residues were determined by gas chromatography with nitrogen–phosphorus detection. Two capillary columns, HP-1 and HP-1701, were compared and the best resolution was obtained with the HP-1 column, which was then used in the residue determination. Recovery through the method was studied in the range 0.2 to 1 $\mu g\, g^{-1}$ and average recoveries varied from 89% to 109% with a relative standard deviation between 2% and 10%. The detection limit of the method ranged from 0.001 to 0.02 $\mu g\, g^{-1}$ for the different herbicides. Confirmation of residue identity was performed by GC–MS in the selected-ion monitoring mode.

Keywords: Soil; Environmental analysis; Pesticides

1. Introduction

Herbicides are the main group of pesticides used in current agriculture, being soil applied in many cases or transported to soil after application.

A large variety of methods have been used in the determination of herbicides in soil. Analysis of herbicide residues is commonly carried out by gas chromatography (GC) with nitrogen–phosphorus detection (NPD) or electron-capture detection (ECD) and, in some cases, coupled with mass spectrometry (GC–MS) [1,2] being high-performance liquid chromatography (HPLC) [3] an alternative technique.

The extraction of residues from soil is currently accomplished by conventional methods using mechanical shaking or Soxhlet extraction with different organic solvents, alone or in mixtures with water [4–10]. Solid-phase extraction (SPE) has been widely used for the determination of pesticides in water and has also been proposed as a rapid technique for the extraction of pesticides from soil [11]. Other technologies like supercritical fluid extraction (SFE) [12–14] and microwave-assisted extraction [15–17] have been employed in the last years with successful results. Nevertheless, the routine use of SFE in sample preparation is considered to be expensive and, in addition, a better understanding of the matrix effects is necessary before this technique can be used as a standard extraction method. On the other hand, the use of microwave-assisted extraction has some manual steps during the experimental procedure and

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requires special microwave systems designed for organic analysis, due to safety reasons.

The extraction of herbicide residues by conventional methods has, however, several disadvantages. These methods often use great amounts of toxic organic solvents and are time consuming procedures that need a lot of laboratory space as well as glassware. Miniaturization of these systems is then a recent trend, with the aim of reducing solvent requirements and increasing productivity. The purpose of this work is to develop a rapid analytical multiresidue method for the determination of nitrogen containing herbicides in soil using a low volume of organic solvent in the sample extraction. Herbicide levels are subsequently determined by capillary GC with NPD detection and residue identity is confirmed by GC–MS in the selected ion monitoring mode. The compounds included in this study are important herbicides belonging to triazines (atrazine, terbumeton, terbuthylazine, prometryn, metribuzin, terbutryn and cyanazine), thiocarbamates (EPTC, molinate, thiobencarb and triallate), uracils (bromacil and lenacil), nitroanilines (ethalfluralin, trifluralin, dinitramine and butralin), chloroacetamides (propachlor, alachlor and metolachlor) and a non-classified heterocycle (oxadiazon). The proposed method is applied to the analysis of various spiked and treated soil samples from agricultural fields located in different areas of Spain.

2. Experimental

2.1. Chemicals

Herbicide standards were obtained from commercial sources: terbutryn, prometryn, terbumeton, terbuthylazine, atrazine and metolachlor from Ciba Geigy (Switzerland), alachlor, propachlor and triallate from Monsanto (USA), ethalfluralin and trifluralin from Eli Lilly (USA), lenacil and metribuzin from Bayer (Germany), molinate and EPTC from Zeneca Agrochemicals (UK), cyanazine from Shell (UK), oxadiazon, butralin and dinitramine from Condor (Middlesex, UK), bromacil from DuPont (USA) and thiobencarb from Kumai (Japan). All solvents were of analytical reagent grade (Panreac, Spain). Anhydrous sodium sulfate was purchased from Merck (Germany). Polypropylene columns (20 ml) with polyethylene frits of 20 μm pore size (Varian, USA) at the end and Whatman No.1 filter paper circles of 2 cm diameter (Whatman, UK) located over the frits were used in the extraction step.

2.2. Standards

A stock solution of the studied compounds was prepared, containing 1 mg ml^{-1} of each herbicide in ethyl acetate. Herbicide solutions used for fortification of soil samples were prepared by dilution of appropriate volumes of this stock solution.

2.3. Equipment

2.3.1. GC–NPD

A Hewlett–Packard 5890 Series II gas chromatograph equipped with a nitrogen–phosphorus detector and an automatic injector was used (Hewlett–Packard, Hoofddorp, Netherlands). Two fused-silica capillary columns were employed, HP-1 (crosslinked dimethyl siloxane) and HP-1701 (cyanopropylphenyl methyl siloxane), 30 m×0.25 mm I.D., 0.25 μm film thickness, supplied by Andaluza de Instrumentación (Spain). The carrier gas was helium at a flow-rate of 1 ml min^{-1}. Injection port and detector temperatures were maintained at 270°C. The oven temperature was kept at 80°C for 1 min and then programmed at 5°C min^{-1} to 140°C, held for 10 min and programmed at 5°C min^{-1} to 250°C, held 15 min. A 2 μl volume was injected splitless, with the valve closed for 1 min, in a double-taper glass liner with a nominal volume of 800 μl.

2.3.2. GC–ion-trap detection (ITD)

A Perkin–Elmer 8500 gas chromatograph equipped with a Finnigan ion-trap detector (ITD) (Perkin–Elmer, Norwalk, CT, USA), operated in the electron impact mode, was used. A fused-silica capillary column, BP-1 crosslinked dimethyl siloxane from SGE, Australia (12 m×0.22 mm I.D.), 0.25 μm film thickness, was employed with helium as carrier gas at 10 p.s.i. with gave a flow-rate of 0.9 ml min^{-1} (1 p.s.i.=6894.76 Pa). Temperature settings were: injector, 270°C, detector, 250°C. The oven temperature was maintained at 80°C for 1 min

and then programmed at 5°C min^{-1} to 140°C, held for 10 min and programmed at 5°C min^{-1} to 250°C, held 15 min. Samples were injected in splitless mode with the split valve closed for 1 min, in a wide bore glass liner with glass wool packing and a nominal volume of 900 μl.

Mass spectrometric acquisition parameters: The transfer line temperature was 250°C; mass range 40–350 daltons; scan-rate 0.5 s $scan^{-1}$, 3-μscan; radio frequency and voltage, 1.1 MHz and 0–7.5 kV; automatic gain control from 78 μs to 25 μs; solvent delay 3 min.

2.3.3. Extraction equipment

An ultrasonic water bath (Raypa, Spain) was used in the extraction procedure. The generator of this ultrasonic bath has an output of 150 W and a frequency of 35 kHz.

A 12-port vacuum manifold (Scharlau, Spain) was employed for the filtration of the extracting solvent.

2.4. Soil samples

The main physico-chemical properties (organic matter, pH and texture) of the eight soils used in this study are given in Table 1. Soil samples were collected from the plough layer (0–10 cm) of fields growing different crops in several areas of Spain. These samples were sieved to pass a 2 mm sieve and stored at −18°C until analyzed.

2.5. Procedure

A 5 g amount of sieved soil was placed in a polypropylene column. In the recovery experiments, samples were weighed in a weighing funnel (6 ml), fortified with 0.5 ml of a mixture of the different herbicides to give final concentrations in the range of 0.2–1 μg g^{-1} and transferred to plastic columns 10 min after to allow solvent evaporation. Soil samples were extracted with 4 ml of ethyl acetate for 15 min in an ultrasonic water bath at room temperature. The water level in the ultrasonic bath was adjusted to equal the extraction solvent level inside the columns. Columns were supported upright in a tube rack and closed with screw-type valves. After extraction, the columns were placed on the multiport vacuum manifold where the solvent was filtered and collected in graduated tubes. Soil samples were extracted again by sonication with another 4 ml of ethyl acetate (15 min). The extracting solvent was filtered and soil samples washed with additional solvent (2 ml). The total extract collected in 10 ml graduate tubes was concentrated in gentle stream of air to an appropriate volume for GC analysis (2–5 ml). A small amount of anhydrous sodium sulfate was added to dry the concentrated extract.

Table 1
Characteristics of selected soils

Field	Organic matter (%)	pH	Sand	Silt	Clay
A	0.79	8.64	30.7	36.2	33.1
B	1.69	8.0	29.7	38.4	31.90
C	0.9	5.4	59.8	24.8	15.3
D	0.5	5.7	85.9	9.5	4.6
E	1.02	4.98	25.91	43.22	30.87
F	1.50	4.45	29.63	56.47	13.90
G	0.97	7.7	44.36	37.42	18.22
H	1.75	6.7	64.81	23.65	11.54

2.6. Quantitation

The concentration of these compounds was determined by comparing the ratios of the peak areas in the sample with those found for mixtures of herbicides of known concentration.

3. Results and discussion

3.1. GC–NPD analysis

The analysis by GC–NPD of a soil sample, prior and after fortification with the 21 herbicides studied, is depicted in Fig. 1. Two columns with different polarity, a non-polar HP-1 and a medium polarity HP-1701, were used for multiresidue herbicide analysis. Table 2 shows the retention times obtained using these capillary columns. The best resolution was obtained with the HP-1 column, which provided an acceptable separation of all herbicides. Retention times obtained for the different herbicides were very precise, with a variation ranging, within a working day, from 0.009% to 0.06%, being the variation highest for the first eluting peaks. It can also be

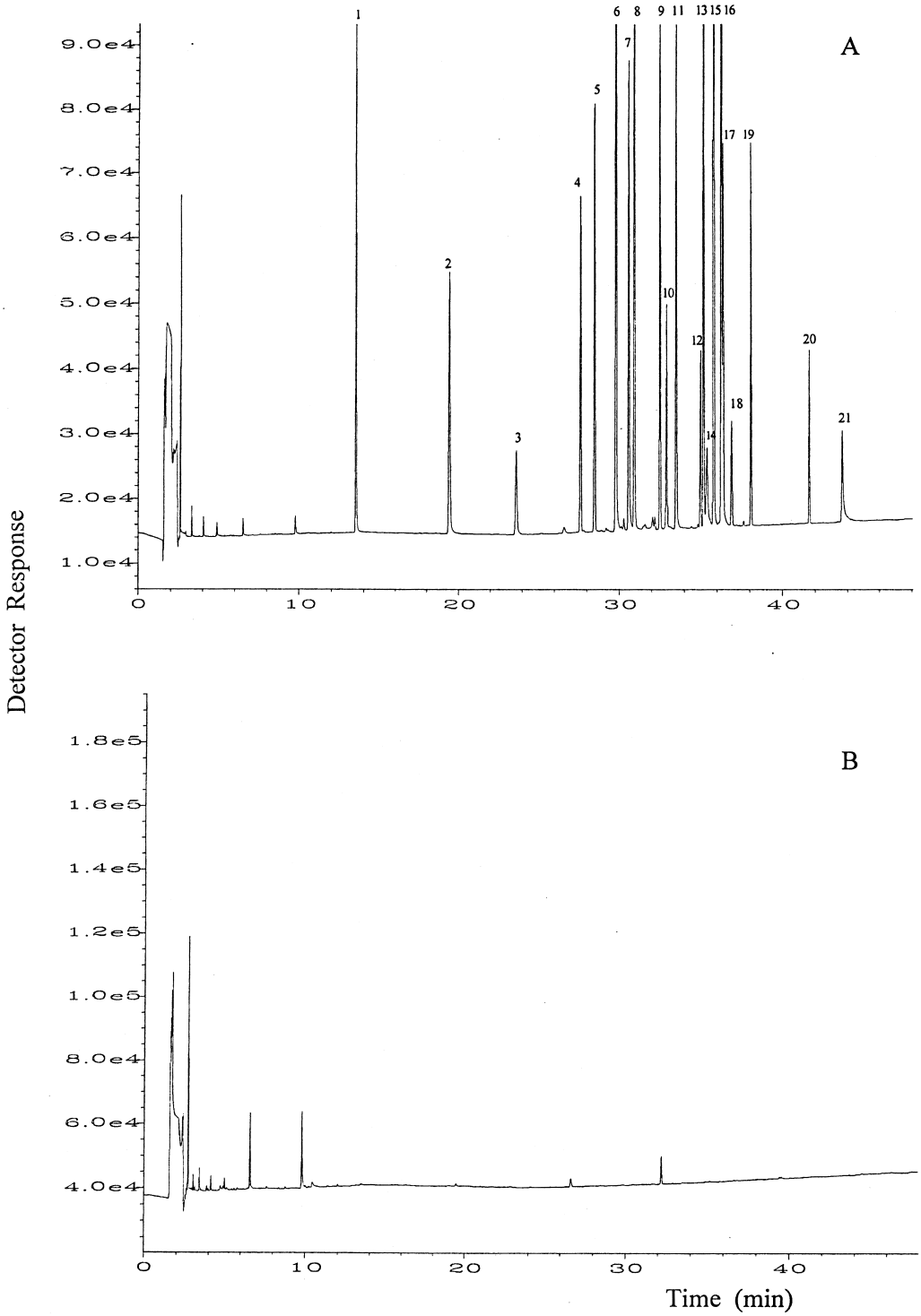

Fig. 1. GC–NPD chromatograms of soil extracts. (A) Soil fortified with herbicides at 0.5 $\mu g\, g^{-1}$. (B) Soil sample prior to fortification. Column HP-1. For peak numbers see Table 2.

Table 2
Retention times and detection limits of selected herbicides

No.	Compound	t_r(min)		
		HP-1[a]	HP-1701[b]	Limit of detection
1	EPTC	13.41	15.62	0.003
2	Molinate	19.23	25.73	0.003
3	Propachlor	23.37	32.78	0.007
4	Ethalfluralin	27.44	32.18	0.005
5	Trifluralin	28.31	32,78	0.003
6	Atrazine	29.58	35.89	0.002
7	Terbumeton	30.38	33.26	0.003
8	Terbuthylazin	30.73	36.81	0.002
9	Dinitramine	32.36	37.19	0.003
10	Triallate	32.76	35.66	0.007
11	Prometryn	33.33	38.80	0.003
12	Alachlor	34.84	38.98	0.01
13	Metribuzin	35.02	39.69	0.002
14	Bromacil	35.15	41.46	0.02
15	Terbutryn	35.63	39.69	0.001
16	Cyanazine	36.09	45.13	0.002
17	Thiobencarb	36.23	41.26	0.005
18	Metolachlor	36.75	42.07	0.01
19	Butralin	37.95	40.29	0.005
20	Oxadiazon	41.52	45.54	0.01
21	Lenacil	43.51	53.99	0.02

[a]Fused-silica capillary column coated with dimethyl siloxane.
[b] Fused-silica capillary column coated with cyanopropylphenyl methyl siloxane.

observed in Fig. 1 the few and un-important co-extractive interferences obtained in the blank soil extract, what allowed the determination of herbicides without errors. The HP-1701 showed a higher column bleeding that may decrease the sensitivity in some cases and, moreover, this column did not allow a good resolution of all peaks with the temperature programs assayed. Nevertheless, the HP-1701 shows a different selectivity for some compounds, in comparison with column HP-1, which is made evident by the different elution order of various herbicides and these differences in retention times can be useful for the confirmation of residues.

3.2. Recovery

Soil samples were spiked with 0.2, 0.5 and 1 $\mu g\,g^{-1}$ of the studied herbicides and analyzed by GC–NPD following the procedure described above. The average recoveries obtained were always higher than 88% with standard deviations equal or lower than 10% (Table 3). These results are in agreement with those obtained in previous studies using conventional methods [5,8,9,18].

3.3. Detection limit and linearity

Fig. 2 shows a representative chromatogram of a soil sample fortified with 0.01 $mg\,g^{-1}$ of each compound. The limit of detection of the GC–NPD method ranged from 0.001 to 0.02 $mg\,g^{-1}$ for the 21 herbicides studied (Table 2), considering a signal-to-noise ratio equal or higher than 3.

The detector response was linear in the assayed range. The linearity of the method was tested by analysing triplicate solutions over the range 0.01–1 $\mu g\,ml^{-1}$ of the studied herbicides.

3.4. GC–ITD

The confirmation of herbicide residues was accomplished by GC–ITD under the conditions described above. The retention times of the herbicides and the main ions found in their mass spectra are summarized in Table 4. The structural assignments for these ions have been reported previously [8,9,19]. Herbicides were quantitated by selecting the base peak of their mass spectra, after the acquisition of the total ion chromatogram of the sample. Injection of blank extracts showed no coextracted interferences at the herbicide retention times. A BP-1 non-polar column, which was shorter than that used in GC–NPD analysis, was used in the GC–ITD determination. Although overlaping of some peaks occurred with this column, herbicide residues can be quantitated due to their different main ions. All the herbicides studied can be identified by their mass spectra, in the NBS library, at levels near 1 ng per compound. The detection limit was about 0.01 $\mu g\,g^{-1}$ for each compound, except for the uracil herbicides, bromacil and lenacil, which was near 0.02 $\mu g\,g^{-1}$. This limit of detection is considered acceptable for its use in the confirmation of herbicide residues in soil.

3.5. Real samples

Soil samples were collected in various areas of Spain from commercial fields growing different

Table 3
Recovery of herbicides added to soil samples[a]

Herbicide	Recovery (mean±S.D., %)		
	1 μg g^{-1}	0.5 μg g^{-1}	0.2 μg g^{-1}
EPTC	95±3	97±4	98±8
Molinate	98±4	94±3	93±6
Propachlor	94±6	93±5	93±7
Ethalfluralin	97±5	95±4	90±6
Trifluralin	96±4	92±5	91±6
Atrazine	100±4	97±5	99±7
Terbumeton	91±4	89±4	102±6
Terbuthylazine	100±4	94±3	96±6
Dinitramine	101±4	90±3	90±8
Triallate	98±4	103±5	106±8
Prometryn	103±4	90±5	100±6
Alachlor	100±5	96±7	91±9
Metribuzin	94±9	104±5	102±8
Bromacil	102±2	95±6	89±5
Terbutryn	89±5	90±8	109±10
Cyanazine	108±8	94±4	96±4
Thiobencarb	91±4	92±5	94±4
Metolachlor	95±7	93±5	96±6
Butralin	97±4	95±4	94±6
Oxadiazon	99±4	100±8	96±6
Lenacil	98±7	96±6	90±8

[a]Results based on five replicates using soil samples taken from field H.

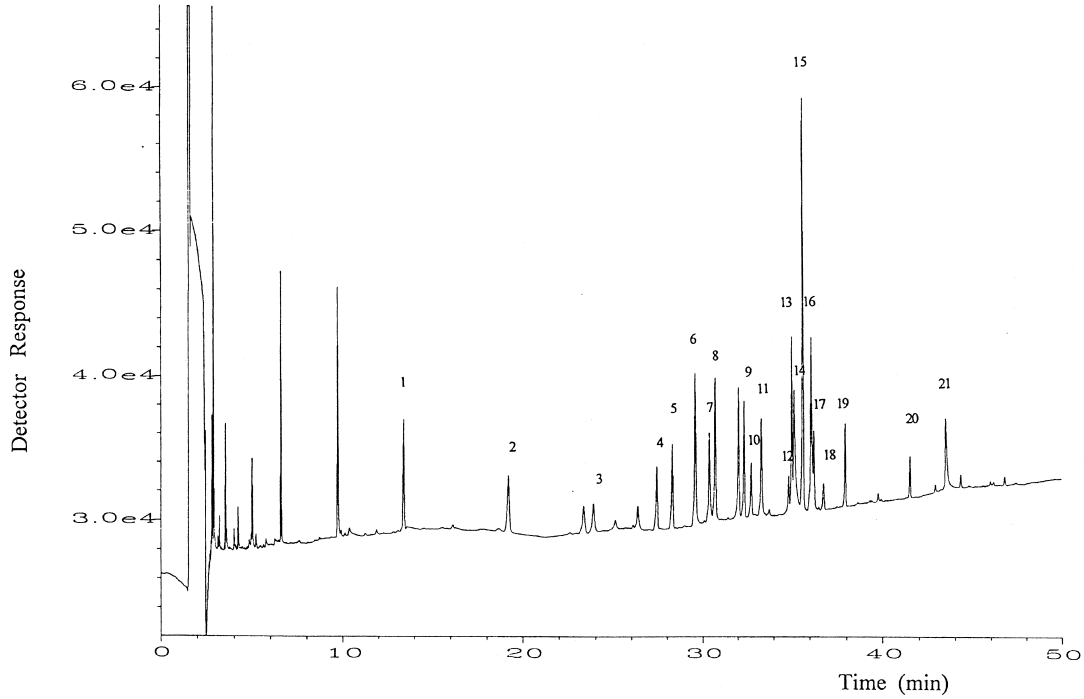

Fig. 2. GC–NPD chromatogram of a soil extract fortified with herbicides at 0.01 μg g^{-1}, except for uracils that were spiked at 0.02 μg g^{-1}. Column HP-1. The numbered peaks refer to the herbicides listed in Table 2.

Table 4
Main ions found in the mass spectra and retention times of herbicides

No.	Compound	Main ions (m/z[a])	t_r(min)
1	EPTC	128–132–**190**	6.49
2	Molinate	**126**–158–187	10.19
3	Propachlor	**120**–176–212	12.04
4	Ethalfluralin	**276**–316–333	14.04
5	Trifluralin	**264**–306–336	14.39
6	Atrazine	173–**200**–215	15.59
7	Terbumeton	**169**–210–225	16.10
8	Terbuthylazine	173–**214**–230	16.54
9	Dinitramine	261–**305**–322	18.49
10	Triallate	86–143–**268**	18.54
11	Prometryn	184–226–**241**	20.34
12	Alachlor	**160**–188–238	22.44
13	Metribuzin	144–**198**–215	24.59
14	Bromacil	149–188–**205**	25.34
15	Terbutryn	170–**226**–241	25.34
16	Cyanazine	212–**225**–241	25.81
17	Thiobencarb	**100**–125–257	26.14
18	Metolachlor	131–**162**–238	27.08
19	Butralin	250–**266**–295	28.19
20	Oxadiazon	**175**–258–344	33.09
21	Lenacil	136–**153**–235	36.39

[a]Base peak in bold.

crops (corn, tomato and wheat). Soil was sampled at different times after treatment and the concentration of herbicides found in these samples are given in Table 5. The chromatograms obtained for three representative soil samples are depicted in Fig. 3. Analysis of real samples showed the validity of the method used, which allowed the determination and identification of herbicides present in the samples.

Table 5
Levels of herbicides found in treated soils

Soil	Compound	Level ($\mu g\ g^{-1}$)±S.D.
A[1]	Atrazine	0.05±0.006
B[1]	Atrazine	0.07±0.009
C[2]	Ethalfluralin	0.05±0.007
D[2]	Ethalfluralin	0.04±0.001
E[3]	Terbutryn	0.02±0.004
F[3]	Terbutryn	0.02±0.004
G[4]	Terbuthylazine	0.55±0.06

1: Corn fields sampled after harvest. 2: Tomato fields sampled at harvest. 3: Wheat fields sampled at harvest. 4:Experimental plot sampled one month after treatment.

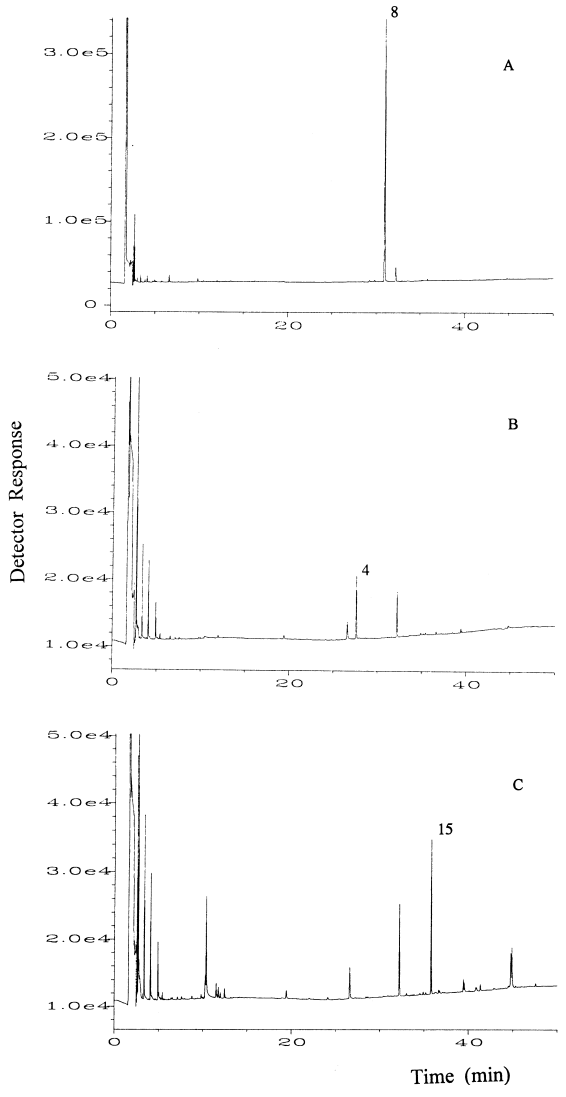

Fig. 3. GC–NPD chromatograms of treated soil samples: (A) 8=terbuthylazine, 0.5 $\mu g\ g^{-1}$, (B) 4=ethalfluralin, 0.06 $\mu g\ g^{-1}$, (C) 15=terbutryn, 0.03 $\mu g\ g^{-1}$. Column HP-1.

4. Conclusions

The results of this study show that the proposed procedure is simple and economic, requiring only small volumes of solvent and then decreasing the hazard for both human health and the environment.

This method has proved to be a rapid and sensitive procedure for the simultaneous determination of

nitrogen containing herbicides in soil. The number of herbicides determined using this method was 21, but since several different chemical classes of herbicides were represented, other compounds of the same classes could likely by simultaneously analyzed with only minor modifications of the method.

Results obtained in the analysis of treated samples confirmed the efficiency and reliability of this method for the multiresidue analysis of herbicides in soil.

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