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DETERMINATION OF HERBICIDES RESIDUES IN SOIL BY SMALL SCALE EXTRACTION

Luciana POLESE*

Eliana Freire Gaspar de Carvalho DORES**

Elaine Fátima Galatti JARDIM*

Sandro NAVICKIENE*

Maria Lúcia RIBEIRO*

ABSTRACT: Herbicides such as trifluralin, simazine, atrazine, metribuzin and metolachlor are used in Brazilian agriculture. The efficiency of a small scale method for determination of these herbicides and two degradation products (deisopropylatrazine and deethylatrazine) in soil samples was evaluated. The compounds were extracted from soil samples (5 g) with 20 ml of ethyl acetate in a mechanical shaker for 50 min. Following the extraction, the supernatant was dried through anhydrous sodium sulphate, concentrated and analysed by high resolution gas chromatography (HRGC) with thermionic specific detection (TSD). Mean recoveries obtained from soil samples fortified at three different levels ranged from 81 to 115% with relative standard deviation (RSD) values varying from 1.2 to 12.7%. The method detection limits ranged from 0.01 to 0.06 mg kg⁻¹. The methodology was applied using soil samples from farms located near the town of Araraquara, in the State of São Paulo, Brazil.

KEYWORDS: Soil; herbicides residues; gas chromatography.

Introduction

As a result of agricultural practices, the soils are being continually polluted by pesticides. Brazil plays an important role in the world agrochemical market. Agrochemicals are used for weed and pest control of different crops, mainly in fruits, vegetables, soybean, cotton and sugar cane^{7,3}.

Since the Brazilian pesticide market is focused on herbicides in comparison with insecticides and fungicides⁷ and the agricultural use of herbicides trends to increase³ it is important to estimate the soil contamination by herbicides.

Methods for the determination of herbicides in soils have been described in the literature. Different organic extractive solvents^{1,5,6,8,9,12,13} have been proposed, as well as, various extraction procedures such as liquid-liquid^{1,9,12,13}, Soxhlet^{5,13}, solid phase extraction^{8,12} and supercritical fluid extraction^{9,12} have been tested. Florisil cartridges^{1,5,12} and gel permeation chromatography⁵ have been commonly used as purification techniques. Analyses have been carried out by liquid chromatography with diode array detector^{5,9} and mainly by packed or capillary gas chromatography with electron-capture and nitrogen-phosphorus detectors^{1,6,8,12,13}.

In general, the efficiency of the procedures is evaluated by means of recovery analysis^{5,6,12,13}, comparison with other procedures^{10,13} or application to field samples which have been treated with the herbicides^{12,13}.

The aim of this study is to evaluate the applicability of a small scale method for simultaneous determination of five herbicides (trifluralin, simazine, atrazine, metribuzin and metolachlor) and two metabolites (deisopropylatrazine and deethylatrazine) in soil samples. The establishment of the experimental conditions followed a described methodology¹⁰ developed for the determination of atrazine in soil.

Materials and methods

Apparatus

A Varian 3300 (Varian Associates, Sunnyvale, CA, USA) gas liquid chromatograph equipped with a split-splitless injector, a thermionic specific detector (TSD) and a Varian 4290 integrator were used. A fused silica capillary column (30 m x 0.25 mm I.D.; J&W Scientific, Folsom, CA, USA) with DB-5 (5% phenylmethylpolysiloxane) liquid phase (film thickness 0.25 mm) was employed. Injections were performed in splitless mode. The column temperature programme was as follows: initial temperature 140°C (held for 1 min), increased at 2°C min⁻¹ to 240°C (held for 1 min), increased at 10°C min⁻¹ to 260 °C and held at the final temperature for 10 min. The injector and detector temperatures were 240°C and 300°C, respectively. Nitrogen (99.999% pure) was used as carrier gas, at 0.7 ml min⁻¹. Detector gases: air 189 ml min⁻¹, hydrogen 1.5 ml min⁻¹ and nitrogen 31 ml min⁻¹.

Reagents and standards

Ethyl acetate (Mallinckrodt Baker Inc., Paris, Kentucky) was nanograde. Anhydrous sodium sulfate (Mallinckrodt) was of analytical grade. Reference standards of deisopropylatrazine, deethylatrazine, trifluralin, simazine, atrazine, metribuzin and metolachlor were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Standard solutions were made up in ethyl acetate and stored at -18°C.

Soil samples

Soil samples were collected in two farms located near the town of Araraquara, in the State of São Paulo, Southeast, Brazil; on January, 1999 and June, 1999, respectively. The samples were taken in sugar cane fields. Soil samples were collected in five points from the first farm at two depths (0-25 cm; 25-50 cm). Twelve samples from the second farm were taken at depth of 0-25 cm. The soils were sampled, packed in paper boxes and brought to the laboratory.

Sample preparation and fortification

300 g of each soil samples was air-dried, passed through a 2 mm sieve, homogenized and stored in a closed vessel. Fortified soil samples were prepared by adding 1.0 ml of each standard solution to 5.0 g of soil. The standard solution was added to the soil sample drop by drop while verifying that the solution covered the soil particles completely. After fortification the samples were kept at room temperature for 1 h to allow evaporation of the solvent.

Extraction procedure

An analytical sample of 5.0 g was weighed in a 25 ml screw-capped flask; 20 ml of ethyl acetate was added and the flask was shaken in a mechanical shaker (Thermolyne, Dubuque, Iowa, USA) for 50 min. The soil was allowed to settle and the supernatant was poured to another flask containing 1 g of anhydrous sodium sulfate. 10.0 ml of the organic layer was transferred to a round-bottom flask and concentrated to approximately 0.5 ml using a rotary evaporator. The final extract was reconstituted to an adequate volume (1.0 ml) with ethyl acetate.

Gas chromatographic analysis

Suitable aliquots (1 ml) of soil extracts and standard solutions were injected into a gas chromatograph. The percentages of recoveries were calculated by comparing the average chromatographic peak heights of the standard, fortified samples and unfortified samples. Quantification of herbicides in soil samples was performed using a three point linear calibration curve plotting peak heights versus concentrations.

Results and discussion

The establishment of the experimental conditions was based on a method developed by Steinwandter¹⁰ for the determination of atrazine in soil. In this method, after adding water and ethyl acetate, atrazine was extracted from two contaminated soil samples by shaking on a mechanical shaker for 16 h.

In the present study, five herbicides (trifluralin, simazine, atrazine, metribuzin, metolachlor) and two metabolites (deisopropylatrazine, deethylatrazine) were extracted from soil with ethyl acetate by shaking on a mechanical shaker for 50 min. The herbicides were selected among those released by the Brazilian legislation for field application⁴. The efficiency of the extraction procedure was evaluated by means of recovery analyses with soil samples fortified at three different levels. As shown in [Table 1](#), the mean recoveries of four replicates of fortified soil samples ranged from 81% to 115%. The reproducibility of the method was demonstrated by the relative standard deviation (RSD) values that varied from 1.2% to 12.7%. The detection limits, determined by the described procedure¹¹, ranged from 0.01 mg kg⁻¹ to 0.06 mg kg⁻¹ for the studied compounds. Gas

chromatograms concerning an unfortified soil sample, a fortified soil sample and a standard solution are shown in [Figure 1](#). The total running time under the described conditions was approximately 27 min. The soil sample (sandy clay, pH 4.4, organic matter 0.7 %) used in the recovery analysis was free of the selected compounds.

Table 1 - Recoveries of selected compounds from fortified soil samples at different levels of concentration

Compounds	Addition (mg kg ⁻¹)	Recovery (%)		RSD (n=4, %)	Detection limit (mg kg ⁻¹)
		Range	Mean		
Deisopropylatrazine	0.1	73-97	84	12.0	0.03
	1.0	77-84	81	3.6	
	2.0	80-87	83	3.6	
Deethylatrazine	0.1	88-106	95	8.4	0.02
	1.0	93-119	103	11.0	
	2.0	85-100	92	6.8	
Trifluralin	0.2	94-119	109	10.5	0.06
	2.0	94-113	103	8.0	
	4.0	109-118	115	3.6	
Simazine	0.1	88-104	93	8.0	0.02
	1.0	96-109	102	5.8	
	2.0	92-96	95	2.0	
Atrazine	0.1	99-113	105	5.5	0.02
	1.0	98-104	102	3.0	
	2.0	107-110	108	1.2	
Metribuzin	0.1	80-109	98	12.7	0.04
	1.0	104-113	109	3.8	
	2.0	111-114	113	1.3	
Metolachlor	0.2	102-107	104	2.1	0.01
	2.0	90-102	95	5.3	
	4.0	110-116	112	2.6	

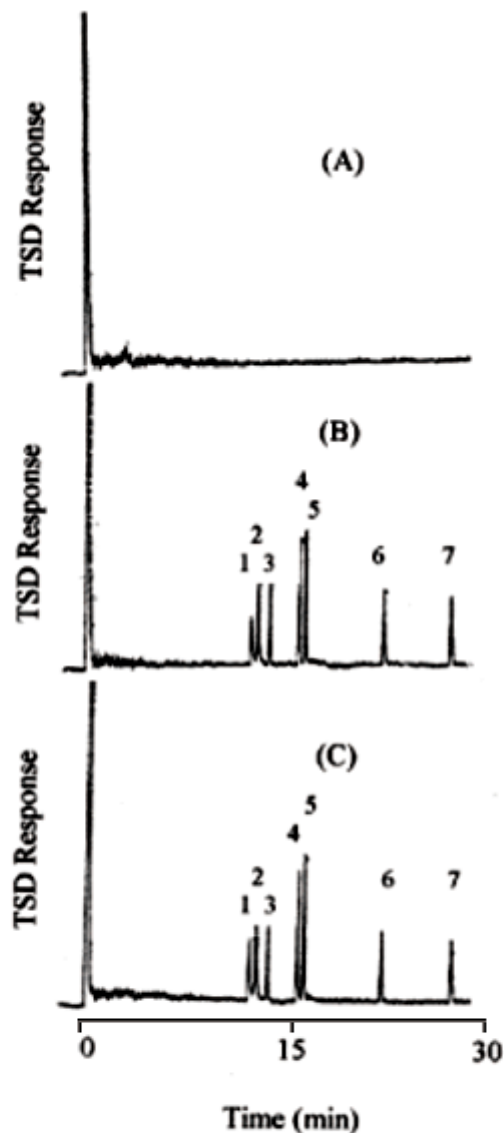


FIGURE 1 - Gas chromatograms of (A) soil sample; (B) fortified soil sample: (1) deisopropylatrazine (0.1 mg kg^{-1}); (2) deethylatrazine (0.1 mg kg^{-1}); (3) trifluralin (0.2 mg kg^{-1}); (4) simazine (0.1 mg kg^{-1}); (5) atrazine (0.1 mg kg^{-1}); (6) metribuzin (0.1 mg kg^{-1}); (7) metolachlor (0.2 mg kg^{-1}); (C) standard solution

After optimization of the experimental conditions, the presented method was applied to twenty two soil samples collected in sugar cane fields from two farms located near the town of Araraquara, in the State of São Paulo, Brazil. Some soil properties are presented in [Table 2](#). All of the soil samples had a pH between 4.1 and 5.8 and organic matter contents between 0.7 % and 2.6%. The soil samples were analysed in duplicate. Calibration graphs were constructed by the external standard method. A good linearity was achieved in the range $0.08\text{-}4.5 \text{ mg kg}^{-1}$, with correlation coefficients between 0.9998 and 1.0000. Clean up steps were unnecessary because no interfering peaks were observed in the chromatograms of the soil samples. Since the extractibility of the pesticides from soils decreases with time ², analyses were performed with different extraction times (50, 120 and 180 min). No residues of the studied compounds were detected under the selected conditions in the soil samples, which were collected in sugar cane fields. Further investigations are necessary in order

to verify the ability of the proposed method to extract aged residues from soil.

Table 2 - Characteristics of soil samples

Property	Range
Phosphorus (ppm)	3-54
Potassium (mEq / 100 cm ³)	0.02-0.4
Calcium (mEq / 100 cm ³)	0.8-6.4
Magnesium (mEq / 100 cm ³)	0.2-2.3
pH	4.1-5.8
Cation exchange capacity (mEq / 100 cm ³)	3.0-10.1
Organic matter (%)	0.7-2.6

Conclusion

The efficiency of a small scale method for determination of herbicides residues in soil was demonstrated by means of a recovery study with samples fortified at different levels of concentration, determination of the detection and quantification limits. The procedure did not require specific optimizations for the analysis of twenty two soil samples and the results indicated that the described method could be applied in monitoring studies to estimate the contamination of soils by herbicides.

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POLESE, L., DORES, E.F.G.C., JARDIM, E.F.G., NAVICKIENE, S., RIBEIRO, M.L. Determinação de resíduos de herbicidas em solo por extração em pequena escala. *Ecl. Quím. (São Paulo)*, v.27, p. , 2002.

RESUMO: *Herbicidas tais como trifluralina, simazina, atrazina, metribuzina e metolaclor são usados na agricultura brasileira. A eficiência de um método em pequena escala para a determinação destes herbicidas e dois produtos de degradação em amostras de solo foi avaliada. Os compostos foram extraídos das amostras de solo (5 g) com 20 ml de acetato de etila em agitador mecânico por 50 min. Após a extração, o sobrenadante foi filtrado em sulfato de sódio anidro, concentrado e analisado por cromatografia a gás de alta resolução com detector termiônico específico. Valores médios de recuperação obtidos de amostras de solo fortificadas em*

três níveis diferentes variaram de 81 a 115 % com valores de desvio padrão relativo entre 1,2 e 12,7 %. Os limites de detecção do método variaram de 0,01 a 0,06 mg kg⁻¹. A metodologia foi aplicada a amostras de solo de fazendas localizadas próximas à cidade de Araraquara, estado de São Paulo, Brasil.

PALAVRAS-CHAVE: Solo; resíduos de herbicidas; cromatografia a gás.

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* Departamento de Química Orgânica – Instituto de Química – UNESP – 14801-970 – Araraquara – SP Brasil.

** Universidade Federal do Mato Grosso – 78060-900 – Cuiabá – MS – Brasil.