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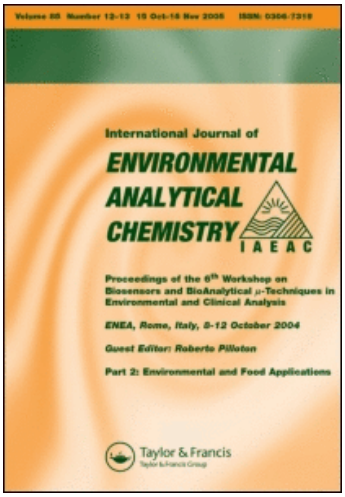

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ACCELERATED SOLVENT EXTRACTION OF HERBICIDES IN AGRICULTURAL SOIL SAMPLES

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This study was undertaken for comparing the standard Soxhlet procedure with an Accelerated Solvent Extraction (ASE), an extraction procedure already accepted by the US EPA that uses organic solvents at high temperatures and pressures. Soil samples contaminated by atrazine, terbuthylazine, alachlor and metolachlor herbicides and by their main transformation products were utilised for this comparison. The soil samples were selected on the basis of different textural characteristics and organic carbon contents. The obtained results showed good recovery efficiency of the ASE technique, reducing extraction times and organic solvent usage.

Keywords: Herbicides; soil; extraction; Soxhlet; Accelerated Solvent Extraction

INTRODUCTION

Extraction of solid samples using a great quantity of organic solvents is a common practice in environmental laboratory analysis; it represents a large source of waste and increases purchase and disposal costs. International trends have pushed the organic chemistry laboratories towards a reduction of solvent usage to safeguard both the environment and human health in working places. In the United States all federal laboratories have to reduce solvent usage by about 50–90%.

Scientists have proposed many methods to solve this problem. Supercritical fluid extraction (SFE) can reduce the amount of solvents because it uses carbon dioxide as the principal extraction fluid. However, carbon dioxide's non polar nature limited its use to few apolar micropollutant classes [1]. Microwave extrac-

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tion can also reduce solvent usage but in this case, after extraction, analysts must manually separate extracts from solid samples by filtering or centrifuging [2,3]. Automated Soxhlet systems reduce the per-sample solvent volume to 50–100 mL; however, this method is time-consuming and labour-intensive [4]. In Automated Soxhlet systems, the sample is immersed into boiling solvent so that the solvent temperature shortens the required extraction time. A further increase in temperature beyond the solvent boiling point is not possible since these systems operate at atmospheric pressure. A further increase in the temperature should enhance the extraction process; this can be obtained only by applying pressure which maintains the solvent in the liquid state. This is the theoretical basis for a new extraction technique introduced by B. Richter and his collaborators [5] called Accelerated Solvent Extraction (ASE). As the extraction efficiency is increased, the time required to perform the extraction and the amount of solvent used are reduced. The solvents are maintained in the liquid state by applying pressure that can reach 20 Mpa into the extraction cell. The extraction of compounds from solid matrix is greatly accelerated by high temperature (up to 200°C) so that the process takes place in about 15 minutes. The procedure uses approximately 15 mL of solvent for a sample of 10 g and it can be completely automated. ASE has appeared as Method 3545 as fully accepted in June 1997, in Update III of the US EPA SW-846 Methods for the extraction of pollutants from solid samples. The chemical compound classes that can be extracted from biological samples (animal tissues, vegetal materials), soils, sediments, sludges and solid wastes are: semivolatiles (BNAs) [6] which includes PAHs [7], organophosphorus pesticides [8], organochlorine pesticides [9], chlorinated herbicides [10] and polychlorinated biphenyls (PCBs) [11].

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In the present work we compared the soil extraction efficiency of widely used herbicides in agricultural soils using two methods: ASE and the traditional Soxhlet apparatus, the more commonly used method for solid sample extraction. The herbicides considered were: s-triazines (atrazine, terbuthylazine and its transformation product desethylterbuthylazine) and chloroacetanilides (alachlor and metolachlor).

EXPERIMENTAL

Soil sample preparation

Agricultural soil samples were collected at the Manerbio experimental site [12], located about 15 km South of Brescia, near the Mella river, a tributary of the Oglio river. The site pertains to the fundamental level of Po plain with a substra-

tum of sandy or gravel-sandy type. The underground profiles were investigated from surface level to a depth variable from 115 to 150 cm in the different sites.

The sampling procedure started with the opening of a pit of one meter square by a reverse-arm machinery. For the geo-pedological analyses the samples were collected in the middle of the different soil pedological horizons while for the pesticide determinations the samples were obtained by the insertion of a battery of special metal boxes (4 cm of height) in the open profile wall. The upper 30 cm sample was sampled by a metal shovel in the middle part of the thickness [12].

The main crop at the experimental site was maize, sometimes alternated with barley or other cereals. Triazine herbicides were applied every year for ten-fifteen years before the present investigation. The first sampling campaign was undertaken in May'94, one month after the herbicide application (Primalyam TZ: metolachlor 30%, terbuthylazine 15%). Three other pits were excavated in November'94, in June'95 and November'95, respectively 7, 14, and 19 months after the last herbicide application. After collection, all soil samples were frozen in sealed dark glass bottle and stored at –20°C.

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The geo-pedological characteristics of the different soil samples (pH in water, percent of organic carbon, texture classification, exchangeable cationic capacity, exchangeable acidity, total carbonate content) were determined in the different horizons according to Italian Standard Methodology [13].

For the determination of pesticide content a large sample of soil (400–500 g) was defrosted and dehydrated at air condition and laboratory temperature (20–22°C) for 96 hours. The determination of the water content of the air dried soil samples was done in laboratory by treating 10 g of soil in a stove at 105 °C for 24h. The dry sample was sieved with a 2 mm sieve. The skeleton (the amount of soil sample superior to 2 mm) was weighted and discarded.

For recovery experiments, 40 g soil samples were spiked with herbicide standards diluted in 20 mL of methanol; the resulting mixture was stirred for about 2 hours and the solvent was allowed to evaporate during the night. Three different levels of spiking were undertaken: 10, 5 and 2.5 ng/g for atrazine, terbuthylazine and DET; 20, 10 and 5 ng/g for alachlor and metolachlor.

ASE extraction apparatus

Extraction were performed on an ASE^{TM} 200 Accelerated Solvent Extraction system (Dionex Corp., CA, USA). The ASE system contains stainless steel sample cells with electronically controlled heaters and pumps that maintain the programmed parameters. IR sensors detect the fluid levels during extract collection. An automatic system shuts off the instrument upon sensor failure. When the actual pressure overcomes the programmed value due to the heat expansion of

the solvent, a valve is opened and a small quantity of solvent (0.1–0.2 mL/cycle of valve) is collected. It is compatible with a wide range of organic and aqueous-based solvents. Two carousels (one for samples and the other for collection vials) with 4 rinse positions allows the extraction of up to 24 samples. The system detects three sizes of cells (11, 22 and 33 mL) and solvent volumes are automatically adjusted as a function of the cell size. The operator can program different extraction conditions for each sample. The process follows these steps: sample cell loading; solvent introduction and pressurisation; sample cell heating; static extraction (temperature and pressure constant for a programmed time); transfer of extract to sealed vial with fresh solvent; nitrogen purge; loading of a new cell. All of the steps are automated. For operation, the system requires air at 400 kPa to 670 kPa and N_2 at 670 kPa to 1340 kPa.

Extraction methods

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The following extraction procedure with the Soxhlet apparatus was used. 50 g air-dried and sieved soil sample was extracted with pesticide-free grade methanol (150 mL) in a Soxhlet extraction apparatus for 8 hours. The extract was evaporated in Rotavapor with the water bath at 40–45°C in negative pressure condition . 1–2 mL of Milli Q water were added to the methanol-water obtained mixture in order to reach a 3 mL volume. The water mixture was then passed through a 3 mL Extrelut filled column (Merk, Germany). The function of the diatomaee flour powder contained in the Extrelut column was for removing the water content of the extract, while the pesticide content was eluted with 15 mL of ethylacetate. The ethylacetate extract was reduced to 0.25 mL volume and injected in a gas chromatograph [12].

The following ASE conditions were used for the soil sample extraction: 40 g soil sample, divided in two 20 g sub-samples, were loaded into two 22 mL cells fitted with a cellulose filter and stainless steel frit at the outlet; the inlet cap was finger-tightened and the cells loaded into the carousel; the extraction was performed with methanol as the extraction solvent; cell pressure and temperature were respectively 10 MPa and 125°C; after the combined heating and static extraction period (10 min), the static valve was opened and fresh solvent was introduced to flush the lines and cell, and the extract was collected in the vial. During this solvent flush step, 50–100% of the extraction cell volume was pumped into the cell. The pump valve was then closed and the purge valve was opened for 90 sec allowing pressurised nitrogen (0.8 Mpa) to force the solvent out of the extraction cell and into the collection vial. The entire procedure required less then 15 min/sample and used approximately 30 mL of solvent for 20 g soil sample. Different tests were carried on changing solvent (water or

methanol), temperature (100 and 125°C) and time of static step (5 and 15 ruin). At the end, the extracts of the two subsamples were jointed and concentrated as described for Soxhlet extracts.

GC analyses of soil samples

GC analyses of the herbicides and of the transformation products was performed on a Carlo Erba 5160 Mega Series gas chromatograph equipped with a nitrogen-phosphorus selective detector (NPD80-FL), with a fused capillary column (Sil 13 – Chrompack, 50 m x 0.25 mm I.D., film thickness 0.2–0.25 μm). The chromatographic condition were the following: carrier (helium) flow =1.5 mL/min; make up (nitrogen) flow = 30 mL/min; hydrogen flow = 3 mL/min, air flow = 250 mL/min; detector temperature = 300°C; oven programmed temperature : from 60 to 180°C at 7.5 °C/min, from 180 to 250 °C at 1.5°C/min, from 250 to 270°C at 5°C/min; injection in splitter/splitless with a program of temperature from 100 to 250°C and the spitter valve close for 60 sec. The peak identity of the compound was confirmed with a Ion Trap Detector (ITD 800- Finnigan MAT, USA) working in total ion current conditions.

The detection limits for the soil samples in this condition were: 0.1 ng/g for atrazine, terbutilazine and DET; 0.5 ng/g for alachlor and metolachlor. The pure chemicals for quantitative analyses were supplied by Alltech (USA).

RESULTS

Description of typical pedological profile as well as textural and physic-chemical data for each soil horizon are reported inTable I. The profiles were characterised on the basis of information provided by mean samples of each pedological horizon. All samples profiled exhibit well defined distribution of pedological fundamental horizons A/B/C. The soil is well evolved and typical of middle sandy plain. The superficial horizon (0–32 cm) is sandy loam, followed by a Bw horizon (32–50 cm) with a high content of clay. Below one meter of depth, the soil horizon is mainly sandy with low clay content. The organic carbon content is high only in the Ap upper horizon while it reduces with depth so as CEC.

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TABLE I Pedological characteristics of Manerbio soil samples

Horizon	*Ap 1*	*Ap2*	*Bw*	*BC*	*C1*	*C2*
Depth of sample (cm)	0–25	25–32	32–50	50–84	84–102	102–135
pH in H_2O	7.2	7.7	7.6	7.6	7.5	8.1
Organic carbon (%)	1.8	1.0	0.3	0.1	0.2	0.0
coarse sand (2–0, 1 mm)	37.1	27.0	30.8	38.0	74.2	83.4
fine sand (0,1–0.05 mm)	15.9	15.9	13.7	15.0	5.3	7.4
coarse silt (0.05–0.02 mm)	9.0	11.5	11.5	12.0	3.5	3.5
fine silt (0.02–0.002 mm)	19.5	23.5	16.5	12.5	4.0	3.5
clay (<0.002 mm)	18.5	22.0	27.5	22.5	13.0	2.8
Texture class	sandy loam	loam	clay loam	sandy clay loam	sandy loam	sand
CEC (meq/100g)	13.9	12.3	11.3	10.5	6.1	1.5
Exchangeable acidity (meq/100g)	0	0	0	0	0	0
Total carbonate content (%)	2	0.2	0	0	0	17.7

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In the ASE recovery and reproducibility experiments (Tables II and III) topsoil sample (0–25 cm) was chosen for soil extraction because soil samples collected from the upper horizons usually show the greatest analytical difficulty for extraction, concentration and clean-up (presence of humic acids, other organic pollutants, water). Therefore, the recovery results of topsoil samples are usually lower than those obtained analysing deeper soil samples while the replicate variability is generally higher. Topsoil chromatogram (Figure 1) shows the matrix complexity obtained with a soil sample spiked with the considered herbicides.

The recovery results are reported in Table II, using three different herbicide spiked amounts for ASE and only one, the intermediate, for Soxhlet extraction. Expected values represent the total herbicide content: environmental content (see Table III) plus spiked amount. ASE extraction was performed at 125°C. The best recovery efficiencies (ranging from 60 to 99%) were obtained for alachlor, metolachor and desethylterbuthylazine while the recovery for atrazine and terbuthylazine ranged from 47 to 83%. The results for these two herbicides show a great variability of the replicates as indicated by high relative standard deviation (RSD) values. Comparing ASE with Soxhlet results, ASE values relative to Soxhlet recovery are always equal or greater than 100% at the same spiked level for all the considered herbicides; therefore, both the extraction techniques showed similar results in terms of recovery.

Differences between expected and obtained concentrations for the considered compounds are shown in Figure 2; R^2 values approximate the unit value for alachlor, metolachor and desethylterbuthylazine while terbutilazine and atrazine points are not well correlated.

TABLE II Results of herbicide recovery experiments (ng/g) with superficial Manerbio soil (11/95)

	Terbuthylazine				*Desethylterbuthylazine*			
Extraction technique	*Mean n=3*	*RSD (%)*	*Expected value*	*Recovery (%)*	*Mean n=3*	*RSD (%)*	*Expected value*	*Recovery (%)*
ASE	3.3	39.4	6.7	**49**	4.1	10.9	5.3	**77**
ASE	7.0	32.4	9.9	**71**	7.1	6.1	8.5	**84**
ASE	8.7	35.6	15.9	**55**	11.0	10.5	14.7	**75**
Soxhlet	7.0	19.0	11.4	**62**	6.3	2.7	8.1	**78**
	Metolachlor				*Alachlor*			
Extraction technique	*Mean n=3*	*RSD (%)*	*Expected value*	*Recovery (%)*	*Mean n=3*	*RSD (%)*	*Expected value*	*Recovery (%)*
ASE	10.9	20.6	14.1	**77**	6.3	21.5	6.3	**99**
ASE	14.1	16.3	20.3	**70**	10.6	15.0	12.5	**85**
ASE	24.2	9.8	32.8	**74**	21.8	13.7	25	**87**
Soxhlet	12.8	16.8	17.6	**73**	9.3	17.2	12.5	**74**
	Atrazine							
Extraction technique	*Mean n-3*	*RSD (%)*	*Expected value*	*Recovery (%)*				
ASE	1.8	36.1	3.8	**47**				
ASE	5.8	37.9	7.0	**83**				
ASE	7.9	24.0	13.2	**60**				
Soxhlet	5.9	15.4	7.0	**84**				

In order to improve the reproducibility of the ASE procedure, other conditions were tested extracting superficial unspiked soil samples and varying temperature, static time and extraction solvent (Table III). ASE extraction at 125°C for 15 min shows RSD values significantly lower than the 5 min static time procedure and the observed replicate variability is better than the Soxhlet extraction.

The atrazine concentration in the superficial soil samples was too low and the presence of matrix interferences in the ASE extraction did not allow its quantification. Alachlor was absent in all the soil samples.

An experiment was also undertaken using water as extraction solvent; the water extracts were concentrated on a C18 column and eluted by ethylacetate [14]. Results show lower recoveries than with methanol as the extraction solvent. Further experimental tests might optimise the water extraction; this extraction technique might represent a procedure for studying the leaching of herbicides from soil samples.

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TABLE III Reproducibility evaluation (ng/g) of different extraction techniques with superficial Manerbio soil (11/95)

				Terbuthylazine							*Desethylterbuthylazine*						
				Extractions							*Extractions*						
Method	*Solvent*	*Temperature (°C)*	*Time(min)*	*I*	*II*	*III*	*IV*	*V*	*Mean*	*RDS %*	*I*	*II*	*III*	*IV*	*V*	*Mean*	*RDS %*
ASE	Methanol	125	15	6.0	6.2	5.9	6.1	5.6	**6.0**	4	2.0	1.8	1.7	2.1	2.1	**1.9**	9
ASE	Methanol	125	5	3.3	2.8	3.2	4.6	4.1	**3.6**	20	3.3	1.5	2.1	2.1	1.9	**2.2**	31
ASE	Methanol	100	5	2.2	2.4	3.3			**2.6**	22	1.1	2.1	1.3			**1.5**	35
ASE	Water	125	5	1.5	1.1	2.1			**1.6**	32	0.8	0.7	1.1			**0.9**	24
Soxhlet	Methanol	50–60	450	4.2	6.1	4.1	6.4	4.9	**5.1**	21	1.5	1.8	1.3	2.3	1.9	**1.8**	22
				Atrazine							*Metolachlor*						
				Extractions							*Extractions*						
Method	*Solvent*	*Temperature(°C)*	*Time(min)*	*I*	*II*	*III*	*IV*	*V*	*Mean*	*RDS %*	*I*	*II*	*III*	*IV*	*V*	*Mean*	*RDS%*
ASE	Methanol	125	15	nd	nd	nd	nd	nd			6.2	8.0	7.1	7.7	6.3	**7.1**	11
ASE	Methanol	125	5	nd	nd	nd	0.7	nd	**0.7**		10.2	5.2	8.8	8.8	5.9	**7.8**	27
ASE	Methanol	100	5	nd	nd	nd					3.8	6.5	3.8			**4.7**	33
ASE	Water	125	5	0.5	0.3	0.4			**0.4**	25	3.0	2.5	3.2			**2.9**	12
Soxhlet	Methanol	50–60	450	0.8	0.6	0.7	0.7	0.9	**0.7**	15	4.5	6.3	4.6	5.5	4.5	**5.1**	16

The herbicide content of soil samples collected in different campaigns and extracted with ASE procedure (5 min static temperature, 125°C) are reported in Table IV In the Manerbio soil profile, a maximum concentration in the top soil and a decreasing trend in the lower horizons was observed. Terbuthylazine and metolachlor reached the highest concentrations, 14 and 4 ng/g respectively, in the superficial horizon while in the deeper samples the contamination decreases to 0.2–0.3 ng/g level. The concentrations of atrazine may be considered as a memory effect from a previous use of this herbicide. The differences in the topsoil samples can possibly be attributed to variability in the sampling site.

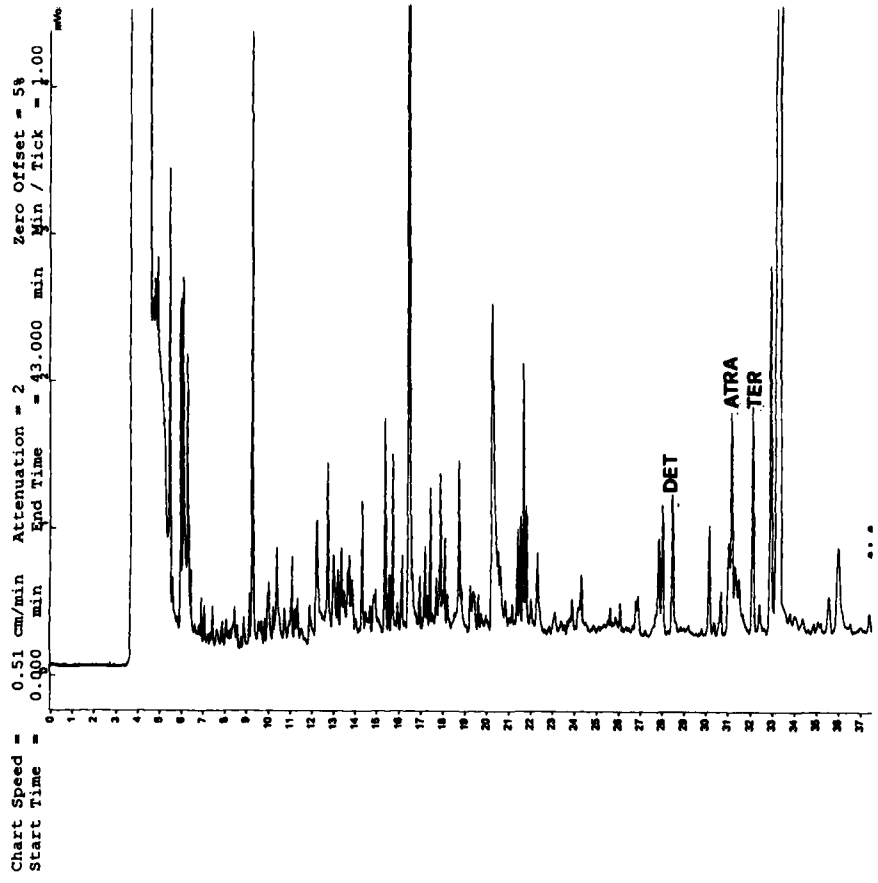

FIGURE 1 GC-NPD chromatogram of superficial soil sample spiked with herbicides

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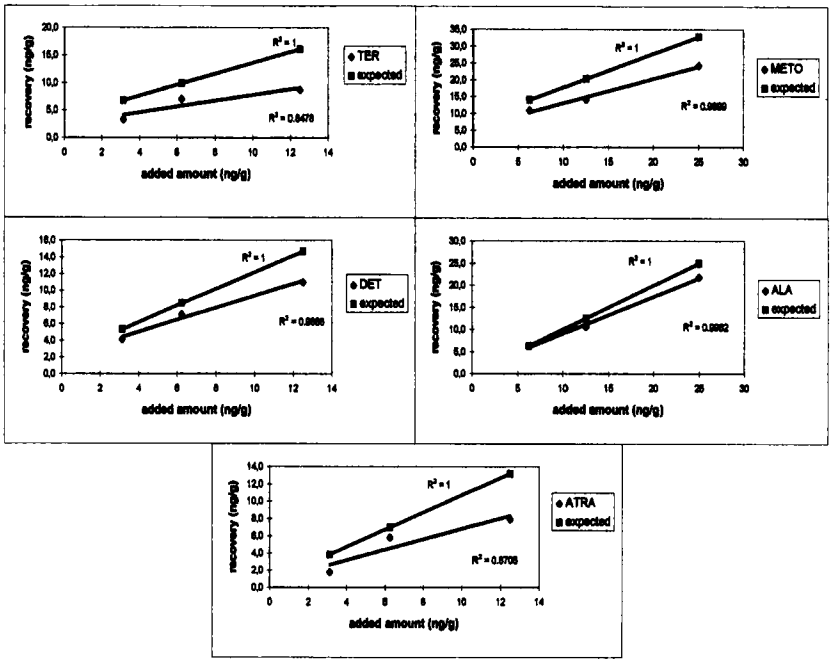

FIGURE 2 Regression curve of expected and obtained concentrations in spiking experiments with herbicides

TABLE IV Herbicide content (ng/g) of soil samples collected in different campaigns

Sampling date	*Depth (cm)*	*Terbuthylazine*	*Desethyl- terbuthylazine*	*Metolachlor*	*Atrazine*
May-94	5–22	3.9	2.9	13.8	nd
	22–33	0.4	0.7	1.4	nd
	34–38	0.3	1.5	0.0	nd
	42–46	0.0	0.4	0.0	nd
	50–54	0.0	0.3	0.0	0.5
	58–62	0.0	0.0	0.0	nd
	66–70	0,0	0.0	0.0	0.5
	74–78	0.0	0.2	0.0	0.5
	82–86	0.0	0.0	0.0	0.9
	90–94	0.0	0.0	0.0 0.0	nd
Nov-94	5–22	6.0	3.2	6.0	nd
	22–33	3.7	2.4	7.3	nd
Jun-95	5–22	5.3	2.9	7.5	1.2
	22–33	1.1	2.5	0.0	1
Nov-95	6–25	3.6	2.2	7.8	0.7

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DISCUSSION AND CONCLUSION

The recovery efficiency obtained in this study for herbicide extraction from soil samples is in agreement with the results of other investigations conducted for the recovery evaluation of semivolatile compounds (BNAs) [6], PAHs [7], organophosphorus pesticides [8], organochlorine pesticides [9], chlorinated herbicides[10] and polychlorinated biphenyls (PCBS)[11]. Therefore, the ASE technique showed the same recovery efficiency as the traditional standard Soxhlet extraction.

Previously used sample preparation and extraction methods for the herbicide determination included: soil drying, Soxhlet extraction for 7 h, followed by evaporation to small solvent volume, solid phase cleanup, evaporation and analysis. In contrast with this procedure, sample preparation for ASE is straight forward. The use of ASE for soil extraction can, in fact, dramatically reduces the time and solvent volumes normally associates with herbicide analyses. The automation capabilities of the instrument can also reduce analyst labour and time, increasing sample throughput. The ability of this method is most likely due to enhanced solubilization, which occurs at elevated temperatures and pressures.

In conclusion, the ASE technique was demonstrated to be a valid alternative extraction method for the analysis of herbicide contaminated soil samples.

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