

Determination of pesticides and their degradation products in soil: critical review and comparison of methods

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Pesticides are applied widely to protect plants from disease, weeds and insect damage, and usually come into contact with soil, where they undergo a variety of transformations that provide a complex pattern of metabolites. This article reviews the most relevant analytical methods for determining pesticides and their transformation products in soils. We address some recent advances in sampling and sample-preparation technologies for soil analysis. We discuss and critically evaluate procedures, such as liquid extraction methods (pressurized liquid extraction or microwave-assisted extraction) and solid-phase based methods (headspace solid-phase microextraction, solid-phase microextraction or matrix-solid-phase dispersion). Analysis of pesticides is generally carried out by gas chromatography (GC) or liquid chromatography (LC) coupled to different detectors, especially to mass spectrometers (MSs). However, alternative and/or complementary methods, using capillary electrophoresis (CE), biosensors and bioassays have emerged recently. We also consider the advantages and the disadvantages of the various methodologies.

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1. Introduction

Pesticides in soils continue to be studied more than any other environmental contaminant, because they are used widely to control pests that affect agricultural crops and pests in the home, yards, and gardens. There is also increasing interest in their transformation products (TPs), because they can be present at higher levels in soil than the parent pesticide itself.

Generally, pesticide TPs could show lower toxicity to biota than the parent compounds [1]. However, toxicological evaluation of pesticide TPs is also an emerging issue and the few scientific works that have been reported show insufficient results to establish a generality

[2]. In some instances, TPs are more toxic, so they represent a greater risk to the environment than the parent molecules [1,3–14].

Differences in the environmental behavior of many TPs, compared to the parents, include an increase of their mobility in soil; even when a TP is less toxic than its parent, it may still have the potential to produce an adverse impact on the environment [2,7,13,14]. As a result, there is a need to consider TPs during the environmental risk-assessment process.

In Europe, the EU Directive 91/414/EEC and its subsequent amendments establish that, before placing a new pesticide on the market, environmental data must be provided for all amounts of metabolites, and degradation and reaction products, which account for more than 10% of the amount of the active substance [15].

The fate of pesticides in soil is controlled by chemical, biological and physical dynamics of this matrix [16]. These processes can be grouped into those that affect persistence, including chemical and microbial degradation, and those that affect mobility, involving sorption, plant uptake, volatilization, wind erosion, run-off and leaching (Fig. 1).

Pesticides are degraded by chemical and microbiological processes. Chemical degradation occurs through reactions such as photolysis, hydrolysis, oxidation and reduction [3,17]. Biological degradation takes place when soil microorganisms consume or break down pesticides [1,4,18]. These microorganisms are

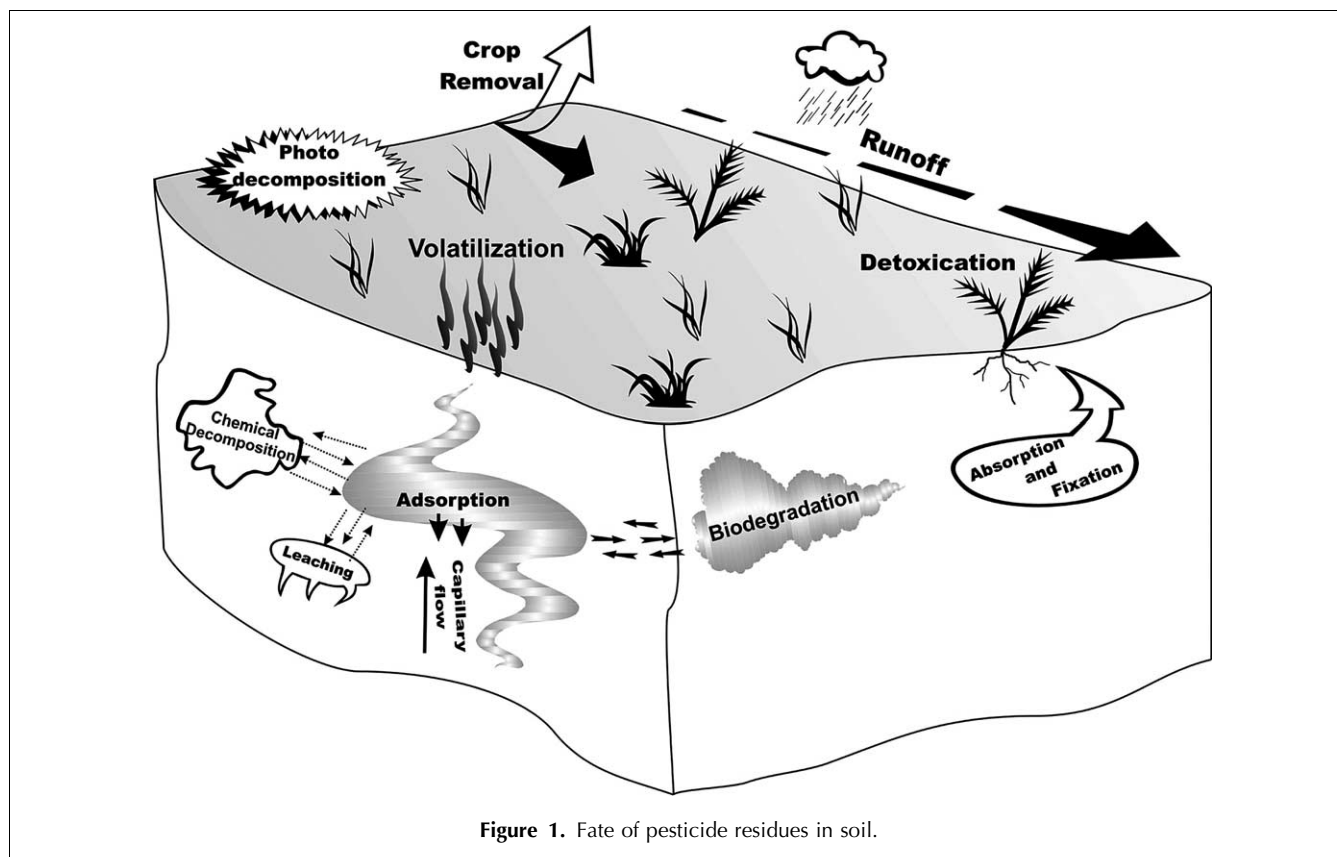


Figure 1. Fate of pesticide residues in soil.

mainly distributed in the top centimeters of the surface layer of the soil, where the organic matter acts as food supply [8]. The extent of degradation ranges from formation of metabolites (TPs) to decomposition in inorganic products [4,16,19].

In addition, some pesticides are chiral molecules. In that way, environmental studies have historically neglected to determine the adverse effects associated with particular enantiomers, including persistence in various environmental media. The racemic signature remains unchanged by physico-chemical removal mechanisms. However, microbial degradation and biological metabolism may be enantio-selective, and result in different effects and fates in the environment [20,21].

Whether they are destroyed over a period of few days by soil microorganisms or whether they are accumulated steadily from year to year, the fate of pesticides in soils varies greatly, depending on the type of soil, the climate and the agricultural practices used [22–24].

The development and the application of methodologies for determining pesticides and their TPs in soil is a challenging task, as a result of some of the inherent general properties of such type of samples:

- (a) The concentration of relevant analytes in soil samples can be extremely low. As a result, the corresponding analytical methods must provide

extremely high sensitivities, which are adequate for detection and quantification of these species at such levels.

- (b) There is a great variety of pesticides, covering a wide range of polarities, from the highly apolar to the water soluble; the latter are considered the most hazardous compounds, especially the dissociated forms, which can be transported to the water environment via leaching or run-off processes.
- (c) The strong binding of the analytes to soil; that requires special extraction techniques that play a crucial role in the subsequent analysis of these compounds.
- (d) The degradation products formed can constitute an extremely complex blend of substances (e.g., Lerch et al. [25] reported that 28 different metabolites of trifluralin were formed in soils, and, because standards were available for only trifluralin and six of its metabolites, the other TPs can only be identified from MS/MS data, but not quantified). This pointed out another problem: the lack of analytical standards.

As a result, chemical characterization of pesticides and TPs in soil demands state-of-the-art techniques for sampling and sample preparation, analyte separation, detection and quantification. We aim in this article to

provide a critical review of existing methods for analyzing pesticides and their TPs in soils.

2. Pesticides and TPs determined in soil samples

A large number of TPs from a wide range of pesticides have been documented [15,26,27]. Table 1 identifies those that have been determined in soils in the last five years as well as the main characteristics that condition their fate and the hazard for the environment and human beings. It is interesting to note that not many of all possible pesticide TPs have been monitored in soil, showing that there is a pressing need for more studies in this field.

Persistence and movement of these pesticides and their TPs are determined by some parameters, such as water solubility, soil-sorption constant (K_{oc}), the octanol/water partition coefficient (K_{ow}), and half-life in soil (DT_{50}) [28]. A pesticide is able to contaminate groundwater (leaching) if its sorption coefficient is low, its half-life long, and its water solubility high [15]. This is quite frequent because pesticides are increasingly polar, hydrosoluble, and thermolabile to diminish their toxicity and to facilitate their disappearance from the environment; at the same time, they must persist long enough to enable acceptable pest control [29]. Pesticides and TPs (Table 1) could be grouped into:

- (a) Hydrophobic, persistent, and bioaccumulable pesticides that are strongly bound to soil. Pesticides that exhibit such behavior include the organochlorine DDT, endosulfan, endrin, heptachlor, lindane and their TPs. Most of them are now banned in agriculture but their residues are still present [1,5,18].
- (b) Polar pesticides are represented mainly by herbicides [7–11,14,22,24,30–36] but also include carbamates [19,37–39], fungicides [4,21,40,41] and some organophosphorus insecticide TPs [3,6,34]. They can be moved from soil by runoff and leaching, thereby constituting a problem for the supply of drinking water to the population. The most researched pesticide TPs in soil are, undoubtedly, those from herbicides [8,10,11,13,14,24,29–31]. Several metabolic pathways have been suggested, involving transformation through hydrolysis, methylation, and ring cleavage that produce several toxic phenolic compounds [31].

All these pesticides and their TPs are retained by soils to different degrees, depending on the interactions between soil and pesticide properties. Most influential soil characteristic is the organic matter content [5,31,36,41,42]. The larger the organic matter content, the greater is the adsorption of pesticides and TPs

[7,9,13,20,37,43]. The capacity of the soil to hold positively charged ions in an exchangeable form is important with paraquat and other pesticides that are positively charged. This is why they are not in Table 1, even though they are among the most widely used herbicide groups. Strong mineral acid is required for extracting these chemicals, without any analytical improvement or study reported in recent years [44]. Soil pH is also of some importance. Adsorption increases with decreasing soil pH for ionizable pesticides (e.g., 2,4-D, 2,4,5-T, picloram, and atrazine).

3. Analytical procedures

Table 2 gives a summary of analytical methods used for the quantification of pesticides and their TPs in soil. The steps involved are matrix preparation, extraction, clean-up, fractionation and determination.

3.1. Matrix preparation and quality control

Soil samples, collected in the field, are commonly air-dried, grounded and sieved through a mesh with a grain size of 2 mm. One basic requirement is to assess how much analyte has been removed from soil by the selected extraction technique. However, the common practice of adding a known amount of the pesticide and/or TP to the soil, usually in an organic solvent, prior to extraction does not solve this problem [31,41,42]. This type of spiked sample will provide the accuracy and the precision of the analytical steps, but sometimes it is useless for measuring the efficiency of the extraction. It is essential that the contaminant is bound to the matrix in a configuration similar to that in the environment.

With the passage of time, unextractable or “bound” residues are often formed in substantial quantities after the application of pesticides to soil. Bound residues are considered, such as those soil residues of radioactive (generally ^{14}C)-labeled pesticides that remain in soil after exhaustive extraction with polar and non-polar solvents [4,17,19,24,45]. They can be distinguished in reaction products of the original pesticides and/or their metabolites with humic matter, and metabolites or molecular parts incorporated into humic matter.

“Aged soils” are soil samples spiked with pesticides and TPs and left at room temperature for periods of time that vary between 3 days and 2 years to evaluate the capacity of the pesticides and/or TPs to form “bound” residues in soil [5,7,9,13,20,31,36,37,41–43]. Evidently, these “aged soils” are far from comparable, and it needs to be taken into account that artificially spiked materials are not representative of real samples. Because of this, the idea of reference soils and derived reference materials (for analytical purposes) has been highlighted in the context of horizontal standardization of methods. However, there is a lack of natural matrix certified ma-

Table 1. TPs of different pesticides studied in soils in the last five years

Pesticide	K_{oc} (ml/gOc)	$T_{1/2}$ (days)	TPs	Characteristics	Ref.
<i>Organochlorines</i>					
γ -HCH	1100	400	1,2,3,5-Tetrachlorobenzene α -HCH	More toxic, persistent, bioaccumulable	[1–5]
DDT	635,000	3000	DDE, DDD	Toxic, persistent, bioaccumulable	[1,5]
Endosulfan	12,400	50	Endosulfan sulfate	Persistent	[5,18]
Endrin	8500–45,000		Endrin aldehyde	Persistent	[5]
Heptachlor	30,200	300	Heptachlor epoxide	Persistent, bioaccumulable	[5]
Pentachloronitrobenzene	41,000	120–300	Pentachloronitrobenzene Pentachlorothioanisole	Losses by volatilization, absorption to organic matter	[40]
<i>Organophosphorus</i>					
Diazinon	1000	40	2-Isopropyl-6-methyl-4-pyrimidinol	Hydrolysis product	[3]
Malathion	1800	1	Malaoxon	Oxidation product, more toxic	[3]
Glyphosate	24,000	47	Aminomethylphosphonic acid	More toxic	[6]
<i>Carbamates</i>					
Benomyl	1900	67	<i>N,N</i> -dibutylurea	Toxic, little persistent	[4]
Carbofuran	14–160	30–117	3-Hydroxycarbofuran 3-Ketocarbofuran	Hydrolysis products, toxic, little persistent	[19]
Diethofencarb	430	1–6	Nitroderivative at position 6 of the 3,4-diethoxyphenyl ring	TPs enhanced in dry conditions	[17]
<i>Triazine herbicides</i>					
Metribuzin	95	30–60	Deaminometribuzin Diketometribuzin Deaminodiketometribuzin	More toxic, microbial degradation	[7]
Simazine	130	60	Monodeethylsimazine	More toxic	[8]
Terbutylazine	500	30–90	Deethylterbutylazine	More toxic	[8]
Atrazine	100	60	Deethylatrazine Deisopropylatrazine Deethyldeisopropylatrazine	More toxic	[9–11]
Atraton	–	30	Deisopropylatraton	–	[11]
<i>Phenoxyalkanoic acid herbicides</i>					
2,4-D	45	34–333	2,4-Dichlorophenol	More toxic	[12]
MCPA	50–60	7–41	4-Chloro-2-methylphenol	More toxic	[12–14]
<i>Acidic herbicides</i>					
Bifenox	10,000	7–14	Bifenox acid	Chemical and microbial degradation	[12]
Clodinafop-propargyl	–	–	Clodinafop acid	–	[12]
<i>Chloroacetanilide herbicides</i>					
Metolachlor	90	26	2-Ethyl-6-methylaniline Metholachlor ethane sulfonic acid	Detected more often in groundwater	[28,31]
Alachlor	170	15	2,6-Diethylaniline 2-Chloro-2',6'-diethylacetanilide 2-Hydroxy-2',6'-diethylacetanilide	Detected more often in groundwater Formed twice faster than TPs of metolachlor	[28,31]
Acetochlor	74–428	8–12	Alachlor ethane sulfonic acid Acetochlor, oxanilic acid Acetochlor ethane sulfonic acid	Detected more often in groundwater	[30]
<i>Dinitroanilide herbicides</i>					
Trifluralin	8000–10,000	57–126	28 different metabolites	Strong binding to soil Less toxic	[25]
<i>Urea pesticides</i>					
Isoproturon	66	15–40	4,4-diisoprylazobenzene	Less toxic	[24]

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Table 1 (continued)

Pesticide	K_{oc} (ml/gOc)	$T_{1/2}$ (days)	TPs	Characteristics	Ref.
Phenylamide fungicide Methalaxyl (chiral compound)	50	7–170	Acid metabolite	Toxic product	[21]
Chloronitrobenzene fungicide Pentachloronitrobenzene	50	120–300	Pentachloroaniline, Pentachlorothioanisole	Toxic metabolites	[40]

terials containing relevant pesticides in soil, because these materials are difficult and expensive to characterize, store and maintain [5]. They are restricted to persistent organochlorine insecticides and some acidic herbicides.

3.2. Extraction

Extraction aims to remove as much as possible of the analyte from the matrix, so it is crucial to optimize the extraction parameters. The best extraction conditions for pesticides and TPs are established more and more by applying chemometrics (e.g., the orthogonal array [37] or a two-level full factorial designs [38]), which offer the advantage of saving time and improving the correlation of the different parameters.

3.2.1. Liquid–solid extraction. Current methodology frequently involves liquid–solid extraction (LSE) for the analysis of pesticides in soils. A number of disadvantages have been noticed with LSE methods; they are laborious, time-consuming, expensive, and subject to problems arising from the evaporation of large volumes of solvent, and the disposal of toxic or inflammable solvents. Table 3 outlines the advantages and disadvantages of LSE procedures in extracting pesticides and TPs from soils.

Soxhlet is one of the most frequently used techniques because it has been adopted in many standardized analytical methodologies for determining pesticides in soils [32]. However, this technique uses drastic conditions that have often broken the structural integrity of thermolabile pesticides and TPs. The long heating periods in the Soxhlet flask degrade *N*-methylcarbamates, sulfonyl urea, and chlorophenoxy acid herbicides.

In addition, Soxhlet is restricted to using strong organic solvents, which are not able to solubilize humate matter, so it fails to extract such chemicals from soil. The extent of this failure depends on the age of the soil and the content of organic matter in the soil. The prerequisite of an extractant should therefore be that it can dissolve the humate clots, which contains a more or less abundant fraction of xenobiotic. A common procedure involves saturating the soil with water to its capacity

(5% or so) to release adsorbed residues. It should be also noted that the more polar a pesticide is the better extraction is achieved using a higher percentage of water as extractant.

Because of this, pesticide residues in soil have been traditionally extracted by flask-shaking LSE techniques using different organic solvents or mixtures, mixtures of water and solvents or aqueous alkaline media [1,3,4,6,8,14,18,22,39,46]. Mechanical shaking or ultrasonic extraction can be at room temperature, which allows analysis of thermolabile pesticides and TPs without altering them. There are many techniques in the literature ranging from those that are extremely long, laborious and complicated to the simplest that shake or sonicate an aqueous solution. Comparison of different methods demonstrated that all of them provided comparable results [38]. One modification to the conventional method consists of placing the soil samples in small columns that are treated with an organic solvent by sonication. This alternative has recently been proposed for the analysis of fungicides [41], and reduces the volume of solvent (ethyl acetate).

To facilitate sample pre-treatment, there have been tests of new extraction procedures based on instrumental techniques such as microwave-assisted extraction (MAE), microwave-assisted Soxhlet extraction (MASE), and pressurized liquid extraction (PLE).

MAE has attracted most interest. Microwave heating is very efficient and involves two mechanisms, ionic conductance and dipolar rotation, so polar solvents can effectively absorb and convert much more microwave energy. It is interesting to note that water can be used for MAE. Better control of the experimental conditions and shorter analysis times prevent problems of degradation. In the last two years, applications of MAE for extracting organic contaminants from soil have increased rapidly. Applications have so far mainly been devoted to the extraction of triazine, acidic pesticides, carbamates, chloroacetanilide herbicides and their TPs [7,9,13,33,36,37]. The high sample throughput and the relatively small extraction times required make this technique quite attractive; together with sonication, it is the most used extraction methodology.

Table 2. Methods for the analysis of pesticides and TPs in soil					
Pesticide and TPs	Extraction	Clean-up	Determination	LOD (µg/kg)	Ref.
Organochlorines	<i>Continuous LSE (Soxhlet)</i>	Florisil	GC-ECD	1–6	[5]
	Hexane-acetone (1:1) 24 h				
	<i>Discontinuous LSE</i>	Partitioning	GC-ECD	1–4	[1,18]
	Methanol-water (4:1) 1 h	CHCl ₃			
	Acetone-hexane 30 min	Florisil			
	<i>PLE</i>	Ph + C ₁₈ + Al	GC-MS	–	[46]
	Acetonitrile-methanol (9:1)	Diol + C ₁₈ + Al			
		CN + Al			
	<i>SPME</i>	–	GC-MS	30–60	[48]
	Acetone-water				
Organophosphorus	Polyacrilate 80 µm				
	<i>HS-SPME</i>	–	GC-ECD	0.06–0.65	[5]
	<i>LPME</i>	–	GC-MS	90–100	[48]
	Stirring in acetone water				
	Hollow fiber with toluene (4 µl)				
	<i>Continuous LSE (Soxhlet)</i>	–	GC-NPD	10	[45]
	Cyclohexane-acetone (1:1) 24 h				
	<i>Discontinuous LSE</i>	Partitioning	GC-FID	6–5000	[3,6,45]
	Ethyl acetate	Hexane	GC-NPD		
	Methanol	–	GC-MS		
Carbamates	Buffered water	Ligand-exchange	(Derivatization)		
		Anion-exchange			
	<i>PLE</i>	Ph + C ₁₈ + Al	GC-MS	–	[46]
	Acetonitrile-methanol (9:1)	Diol + C ₁₈ + Al			
		CN + Al			
	<i>SFE</i>	Diol-silica	GC-NPD	10	[45]
	5% methanol in CO ₂				
	<i>SPME</i>	–	GC-ECD	0.6–7	[34]
	10% methanol in water		GC-MS		
	PDMS, 100 µm				
Carbamates	MSPD	–	LC-UV	100	[20]
	Soil + water + Florisil eluted with hexane-ethyl acetate				
	<i>Continuous LSE (Soxhlet)</i>	–	GC-NPD	10	[45]
	Cyclohexane-acetone (1:1) 24 h				
	<i>Discontinuous LSE</i>	Partitioning	GC-NPD	10–31	[17,19,39,45,47]
	Acetonitrile	Hexane	LC-UV		
	Methanol	CHCl ₃	TLC		
	Water	Methanol-ethylene glycol	LC-Fluorescence		
	Acetone-methanol (1:1)		(Derivatization)		
		C ₁₈			
		On-line C ₁₈			

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Table 2 (Continued)

Pesticide and TPs	Extraction	Clean-up	Determination	LOD (µg/kg)	Ref.
Triazine herbicides	MAE Methanol, 80°C, 6 min	–	LC-UV	–	[37]
	MASE Acetonitrile, 16 cycles, 20 s each cycle	–	LC-Fluorescence (Derivatization)	–	[38]
	SFE 5–10% methanol in CO ₂	Diol-silica –	GC-NPD LC-UV	10	[37,45]
	SPME Acetone–water Polyacrilate 80 µm	–	GC–MS	20	[48]
	LPME Stirring in acetone water Hollow fiber with toluene (4 µl)	–	GC–MS	80	[48]
	Discontinuous LSE Methanol–water (80:20) Acetonitrile–water (60:40) Acetone Acetone–hexane (2:1)	Partitioning CHCl ₃ Lichrolut EN MI-SPE	GC-NPD GC–MS GC-QIT-MS LC-UV MEKC-UV	10–31	[7–11]
	MAE Aqueous extractant 100–105°C, 2–3 min Acetonitrile 80°C, 5 min	SPME CW-DVB, 65 µm Lichrolut EN	GC-NPD GC–MS LC-UV	1–10	[7,9,36]
	PLE Phosphate buffered water, 8 ml, 90°C, 0.5 ml/min	On-line C ₁₈	LC–MS	–	[43]
	SPME 10% methanol in water or water PDMS, 100 µm Polyacrilate, 85 µm	–	GC-ECD GC–MS	0.6–7	[34,35]
	Acidic herbicides				
Acidic herbicides	Discontinuous LSE Aqueous alkaline media Acetonitrile	C ₁₈	LC-TQ-MS/MS Capillary LC-UV	40	[14]
	MAE Aqueous buffer-methanol (50:50), 80°C, 10 min, 150 ml	On-line C ₁₈ RAM	LC-UV LC/LC-UV	5–50	[13,33]
	PLE Aqueous solution, 8 ml, 90°C, 0.5 ml/min Methanol–water (80:20)	GBC On-line C ₁₈	LC-TQ-MS/MS LC-UV	3–30	[23,32,43,53]
Chloroacetanilide herbicides	Discontinuous LSE	Lichrolut EN C ₁₈	GC-NPD GC–MS LC-UV LC–MS	10–31	[9,28,30]

	Methanol–water (80:20) or (75:25) at 75°C 30 min Acetonitrile–water (60:40) in acidic medium				
	MAE Acetonitrile, 80°C, 5 min	Lichrolut EN	GC-NPD GC-MS	1–10	[9]
	PLE Phosphate buffered water, 8 ml, 90°C, 0.5 ml/min	On-line C ₁₈	LC-MS	–	[43]
Dinitroaniline	PLE Acetonitrile–water (7:3), 120°C, 3 × 5 min	–	LC-QIT-MS/MS	–	[25]
Fungicides	Discontinuous LSE Ethyl acetate Ethylacetate–methanol (90:10) Methanol	– C ₁₈ Basic Alumina	GC-NPD LC-UV	2–50	[4,21,40,41]

On comparing water, methanol, acetone–hexane and dichloromethane, it was shown that in MAE water is as efficient as the organic solvents, and it is inexpensive, safe, and environmentally friendly [36]. MAE efficiency has also been compared with a more laborious and costly method based on flask-shaking extraction (with a mixture of phosphate buffer (pH 7)–methanol (50:50)) to analyze triazine, chloroacetanilide, and phenylalkanoic acid herbicides [9,13]. The precision, accuracy, and reproducibility with MAE were better than with the standard procedure.

Focused MASE is a variant of MAE that tries to overcome the main drawbacks of conventional Soxhlet extraction, but maintain its advantages [38]. MASE is a good alternative to determine *N*-methylcarbamate pesticides without degradation, saving both time (2.5 h vs. 6 h required for a US Environmental Protection Agency (EPA) method [47]) and organic solvent (75–80% of the extractant is recycled in MASE), and avoiding tedious manual operations. The recoveries were similar to those obtained by the EPA method and much better than those provided by supercritical fluid extraction (SFE), which ranged between 39.6% and 91.7%.

Among the new extraction techniques, PLE has been introduced most recently. There has been much confusion about its name. Until now, the most frequently used term is accelerated solvent extraction (ASE), but alternatives are PLE, pressurized fluid extraction (PFE), and solid column extraction (SCE). With this technique, a solid sample is packed into an extraction cartridge and analytes are extracted from the matrix with a solvent or a solvent mixture at a particular temperature and pressure. Water, at moderately high temperatures, was found to be an efficient solvent for selectively extracting some neutral herbicides (e.g., triazines [23,43] and different acid herbicides used in cereal crops [29]). The sub-critical water extraction has been effectively coupled on-line to an LC/MS system through a C₁₈ sorbent trap and a relatively complicated system of two six-port valves [23,43]. It is more able to remove chemicals that have aged in soil than traditional Soxhlet extraction [43].

A limitation in extracting with hot water is that it fails to recover compounds that are hydrophobic, thermolabile, or easily hydrolysable. Water (without methanol) is not suitable for extracting herbicides, such as aryl-phenoxypropionic esters [32] or trifluralin [25,43]. This is probably because of their very low solubility in water. Thus, it is not possible to develop a multi-residue method based on sub-critical hot water as solvent.

The limits of detection (LODs) achieved by LSE in any of its variants were in the range 1–30 ppb (Table 2). These methods have been applied to many incubation laboratory experiments.

Navarro et al. [8] determined persistence of simazine and terbuthylazine and appearance of their principal dealkylated chloro-*s*-triazine metabolites in agricultural

Table 3. Comparison of LSE techniques			
Extraction technique	Characteristics	Advantages	Disadvantages
LSE by Soxhlet	Time: 3–48 h Sample: 1–30 g Extractant: 100–500 ml Only organic solvents	• Sample fresh solvent contact during the whole extraction step • No filtration required after extraction • Well-known procedure • Large experience in the extraction field for more than a century	• Long extraction time • Large consumption of solvent • Degradation of some pesticides, such as <i>N</i>-methylcarbamates • Clean-up required
Discontinuous LSE	Time: 10 min–18 h Sample: 1–30 g Extractant: 30–200 ml Organic solvents, water and mixtures Ultrasonic or mechanical shaking	• Multiple extractions • Performed at room temperature to avoid degradation of some pesticides	• Filtration or centrifugation required • Large consumption of solvent • Clean-up required
MAE	Time: 3–30 min Sample: 1–10 g Extractant: 10–40 ml Organic solvents, water and mixtures	• Rapid extraction • Low solvent volumes • Extract multiple samples simultaneously	• Extraction solvent must be able to absorb microwaves • Clean-up required • Degradation of some pesticides
MASE	Time: 10–60 min Sample: 1–30 g Extractant: 10–150 ml Only organic solvent	• Fast extraction • Low solvent volume	• Extraction solvent must be able to absorb microwaves • Clean-up required • Degradation of some pesticides
PLE	Time: 5–60 min Sample: 0.4–30 g Extractant: 1–50 ml Organic solvent, water and mixtures	• Rapid extraction • Low solvent volumes • No filtration required • Automated system • On-line coupling to LC/MS by a sorbent trap (when water is used as extractant)	• Clean-up required • Degradation of some pesticides • When it is coupled on-line, the sorbent trap has a short life (so it should be replaced after 7–8 h)

soil after adding urban sewage sludge as organic material.

Ghadiri and Rose [18] showed the persistence and the degradation of endosulfan isomers and their primary degradation product, endosulfansulfate, in a clay soil from cotton farms of Western Queensland, Australia.

The degradation of pentachloronitrobenzene under different soil storage conditions was exemplified by Mora Torres et al. [40].

Monkiedje et al. [21] studied the degradation of racemic and enantiopure metalaxyl in Cameroonian and German soils.

Sassman et al. [4] estimated the production and the accumulation of *N,N'*-dibutylurea in soils after application of Benalate or from residual benomyl remaining in soil.

Finally, Fava et al. [31] evaluated the mobility, persistence and leaching potential of some degradation products of alachlor and metolachlor.

However, field studies under real conditions are scarce.

Lerch et al. [25] studied trifluralin metabolism pattern in a real contaminated soil.

The formation and the transport of sulfonic acid metabolites of Alachlor and Metolachlor were established by Aga and Thurman [28].

Decomposition in soil and occurrence in ground water of the herbicide glyphosate was studied by Börjesson and Torstensson [6] after its application for weed control on a Swedish railway embankment.

Kodaka et al. [17] examined the unique nitration of the carbamate fungicide diethofencarb in 14 Japanese soils and three types of clays under aerobic conditions.

Degradation of two model insecticides, diazinon and malathion and their TPs was compared and studied in the environment by Bavcon et al. [3].

3.2.2. SFE. SFE involves a fluid, such as CO₂, pumped under supercritical temperature–pressure conditions through the soil. Because CO₂ has a very moderate critical temperature (31.3°C) and is chemically inert, it is recommended for thermally labile compounds. However, the few studies reported in the literature are quite skeptical.

The applicability of SFE in routine analysis of carbamate, organophosphorus, and morpholines has been compared with conventional shaking and Soxhlet extraction [45]. The extraction efficiencies obtained by the three extraction techniques were comparable. However, the extraction of time-aged soils showed very low recoveries (33%), when compared with conventional shaking and Soxhlet extractions that provided recoveries higher than 63%.

Investigations with fortified samples, conducted by Sun and Lee [37] to compare SFE and MAE, led to the same conclusion. Recoveries of carbamates spiked in soil

were in the range 87–98%, except for profam (66%), which probably underwent thermal degradation under the extraction conditions. On comparing MAE and SFE, it was found that, although higher recoveries were obtained by MAE, recoveries by SFE were still good (for propuxur, methiocarb and chlorprophan). However, when extraction was from time-aged soil, MAE exhibited better recoveries (87%) than SFE (65%) [37].

3.2.3. Miniaturized techniques. Only a few references to the application of SPME for extracting pesticides in soil samples could be found.

Most applications are based on the preparation of a soil-distilled water mixture, and analyze target compounds by directly dipping the SPME fibers into the slurry [34]. However, several disadvantages, relating to fiber stability and sensitivity, have been pointed out.

Recently, headspace solid phase microextraction (HS-SPME) has also been used to determine organochlorine pesticides and their TPs in soil [5]. HS sampling presents a significant advantage in terms of selectivity, because only volatile and semi-volatile organic compounds can be released into the HS. Since the fiber is not in contact with the sample, background adsorption and matrix effects can be reduced, and that also enhances the life expectancy of SPME fibers. This technique provided the most impressive LODs (0.06–0.65 ng/g) (see Table 2), which were lower than those obtained using Soxhlet extraction. When the optimized HS-SPME procedure was applied to the analysis of pesticides in CRM 804-050 soil, it provided results in good agreement with those obtained by Soxhlet.

Liquid-phase microextraction (LPME) involves the use of a small amount (3 µl) of organic solvent impregnated in a hollow fiber membrane, which is attached to the needle of a conventional GC syringe. Several pesticides in soil have been studied by this method from a 4 ml aqueous soil sample [48]. The procedure has several limitations (e.g., its LODs are the highest of all reported techniques (see Table 2) and it cannot be easily coupled on-line to GC–MS). Nevertheless, LPME is viable, easy to use, and rapid for analysis of pesticides in soil samples [48]. When the results of LPME were compared with those achieved using SPME, they demonstrated that LPME was fast (within 4 min) and accurate, but much less sensitive [48].

Matrix solid phase dispersion (MSPD) that homogenized soil with water–Florasil and eluting with hexane/ethyl acetate was used to extract phenthoate [20]. Compared with classical methods, the MSPD procedure is simple and less labor intensive, and it does not require preparation and maintenance of equipment. Table 2 shows that the LODs of MSPD are worse than those obtained by LSE or SPME. However, MSPD has been applied to study enantioselective degradation of phenthoate in three soils under laboratory conditions by

Table 4. Comparative study of SPE clean-up techniques

SPE technique	Characteristics	Advantages	Disadvantages
Polar-SPE (silica, alumina, Florisil)	Applicable to organic extracts	• Good clean-up for most apolar pesticides, such as organochlorine and some organophosphorus compounds	• Not suitable for clean-up of compounds covering a wide polarity range
C ₁₈ -SPE	Applicable to aqueous extracts	• On-line coupling with LC • Analyte enrichment • Allows retention of a wide variety of analytes with different polarities • Low cost and low organic solvent consumption • Simple to use • Well-known technology • Wide variety of formats for consumables	• Tendency to plugging • Low pH of the aqueous solution required to retain acidic herbicides • Modified silicas do not resist extreme pH • Partial removal of coextracted compounds • It is difficult to coextract compounds with polarities that are too different
Graphitized black carbon (GBC)	Applicable to aqueous extracts	• Analyte enrichment • Retains acidic herbicides at any pH	• Not suitable for on-line application because it is not pressure resistant • Partial removal of coextracted compounds
Ion-exchange column	Applicable to aqueous extracts	• Ionic analytes	• Recoveries influenced by the extract characteristics
SPME Carbowax-divinylbenzene (CW-DVB)	Applicable to aqueous extracts	• Elimination of organic solvents • Automation of the process with possibility of coupling on-line with GC and LC	• There is no knowledge of the effects that the matrix have on the process
MI-SPE	Works best with organic extracts	• Highly selective for individual compounds or compound class • Stable at extreme pHs	• Custom-made product developed for each analyte • Little usable for enrichment of many different compounds or unknowns

LC silica-gel-column clean-up of MSPD extract followed by chiral LC analysis [5,11,20,34].

Although these techniques seem to provide good results, we should be cautious because there are still few reports to establish their usefulness and to criticize them or compare them with other techniques.

3.3. Clean-up

During the extraction step many interfering (mainly organic) components are co-extracted from soil samples together with target analytes. The aim of the clean-up stage is to remove these substances that can interfere with the identification and the quantitation of target analytes.

Traditional liquid–liquid partitioning clean-up has clearly been displaced from analytical procedures. Solid-phase extraction (SPE), introduced in the 1970s, is still the dominant method for purification of soil extracts. A large number of sorbents are used to isolate organic compounds from the extracted solutions, including alumina, Florisil, ion-exchange resins, silica gel, many silica-based sorbents (e.g., octadecyl-, octyl-, phenyl-, and diol-bonded silica) and graphitized black carbon (GBC). Table 4 compares different SPE procedures.

Dabrowska et al. [46] assessed the use of various sorbents and sorbent combinations (e.g., alumina, C₁₈-alumina, phenyl-alumina, diol-alumina and

NH₂-alumina) for the clean-up of PLE soil extracts. Silica and alumina solvents were not suitable for extract clean-up prior to the final determination of analytes ranging widely in polarity. The combination phenyl-C₁₈-alumina seems to be one of the most useful for extract clean-up prior to the analysis of pesticides.

SPE can be automated on-line with the determination system, especially LC. Such systems typically handle the pre-concentration of analytes from 50 to 250 ml aqueous extracts on a small cartridge packed with C₁₈ or polymer-based material, because the other solid phases, such as GBC, are not pressure resistant. Automated on-line clean-up can be performed with commercially available equipment [13] or with hand-made cartridges and a system of six-port switching valves [23,39,43].

SPE can discriminate between the target compounds and the matrix component to a degree that depends on the selectivity of the solid phase. These conventional phases are not selective enough to achieve a complete separation between pesticides, their TPs and the humic materials.

The most selective and sensitive phases used in SPE are based on immunoaffinity or molecular imprinted polymers (MIPs). The high production cost of immunoaffinity columns explains the lack of recent applications to soil analysis. As an option, MIPs are more economic and highly stable polymers; they have

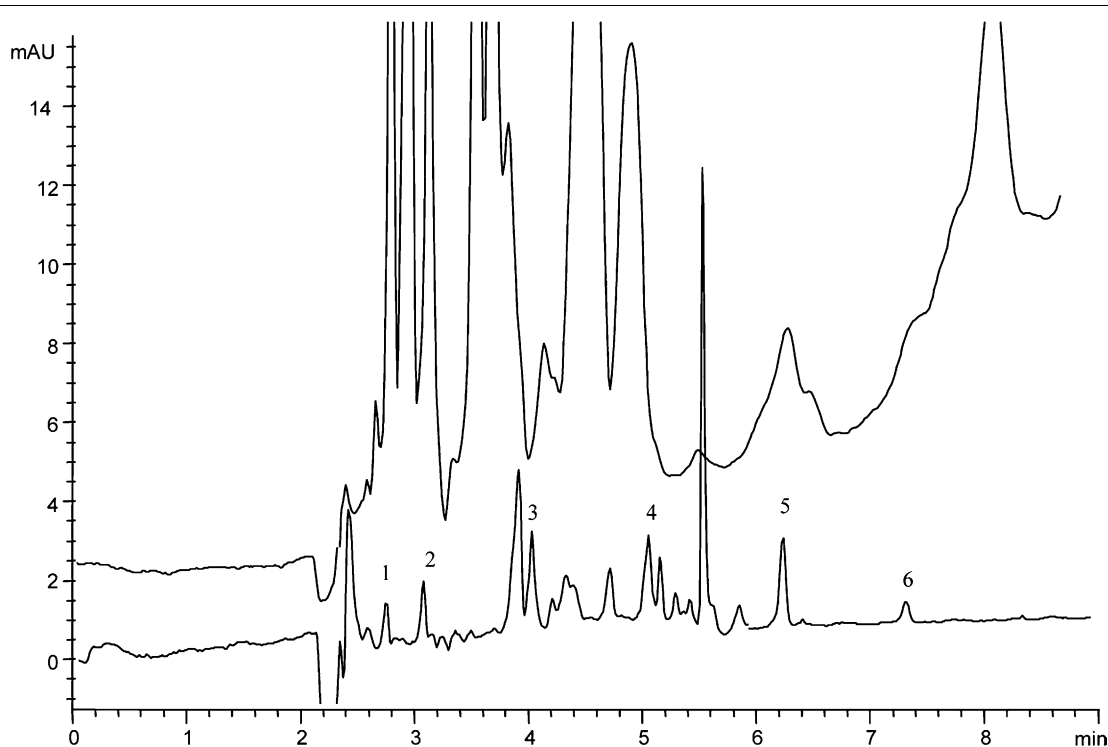


Figure 2. Electropherogram of a soil sample extract obtained at 220 nm, with and without MI-SPE, spiked at 100 µg/l concentration level for each triazine: (1) desisopropylatrazine; (2) desethylatrazine; (3) simazine; (4) atrazine; (5) propazine; and (6) prometryn. (Reprinted with permission from [10]. Copyright (2001) American Chemical Society.)

Table 5. Comparative study of different analytical techniques to determine pesticide residues			
Technique	Advantages	Disadvantages	Solutions
GC	• High resolving power and ability to resolve individual analytes • High sensitivity and good selectivity with element-selective detectors	• Inadequate for polar, thermo-labile and low volatility compounds • High consumption of expensive, high-purity gases	• Derivatization (currently used for only glyphosate and TPs)
GC/MS	• High resolving power and ability to resolve individual analytes • High sensitivity and selectivity • Existence of mass spectrum libraries for screening unknown samples	• Inadequate for polar, thermo-labile and low volatility compounds • High consumption of expensive, high-purity gases	• Derivatization (currently used for only glyphosate and TPs)
LC-UV	• Application to virtually any organic solute, regardless of its volatility or thermal stability • Compositions of both mobile and stationary phase are variable • Can be automated and miniaturized (microchip technology) • Low price, simplicity, robustness and large linear range	• Insufficient separation efficiency and selectivity • Large amounts of expensive, toxic, organic solvent used as mobile phase • Lack of matrix interferences	• Development of more efficient and selective materials for clean-up and separation (immunosorbents, MIPs and restricted access materials)
LC-Fluorescence	• High separation efficiency	• Few compounds are fluorescent	• Derivatization (reported to determine only <i>N</i>-methylcarbamates using <i>o</i>-phthaldehyde and mercaptoethanol)
LC-MS	• Application to virtually any organic solute regardless of its volatility or thermal stability • Compositions of both mobile and stationary phase are variable • Can be automated and miniaturized (microchip technology)	• Strongly affected by matrix interferences (ion enhancement and, most often, ion suppression can be expected). • Identification difficult using interfaces that provides soft ionization • Lack of spectral libraries	• Development of good separations and sample clean-up • Use of isotopically labeled standard • Tandem MS (MS/MS)

recognition sites within the polymer matrix that are adapted to the 3-D shape and functionalities of an analyte. Turiel et al. [10] presented an interesting application of MI-SPE to group-selective extraction of triazine and TPs in soils. They extracted five chlorotriazines (propazine, atrazine, simazine, desethylatrazine and desisopropyl atrazine) and one methylthiotriazine (prometryn) on a propazine imprinted polymer. They loaded 1 ml toluene extracts from soil on the MI-SPE column and eluted the analytes with 8 ml of acetonitrile. Recoveries were higher than 94% for the chlorotriazines and 39% for prometryn. Fig. 2 is an electropherogram depicting the extraction of triazines and the clean-up efficiency. It should be taken into account that MIPs are always less usable for enrichment of many different compounds or of unknowns.

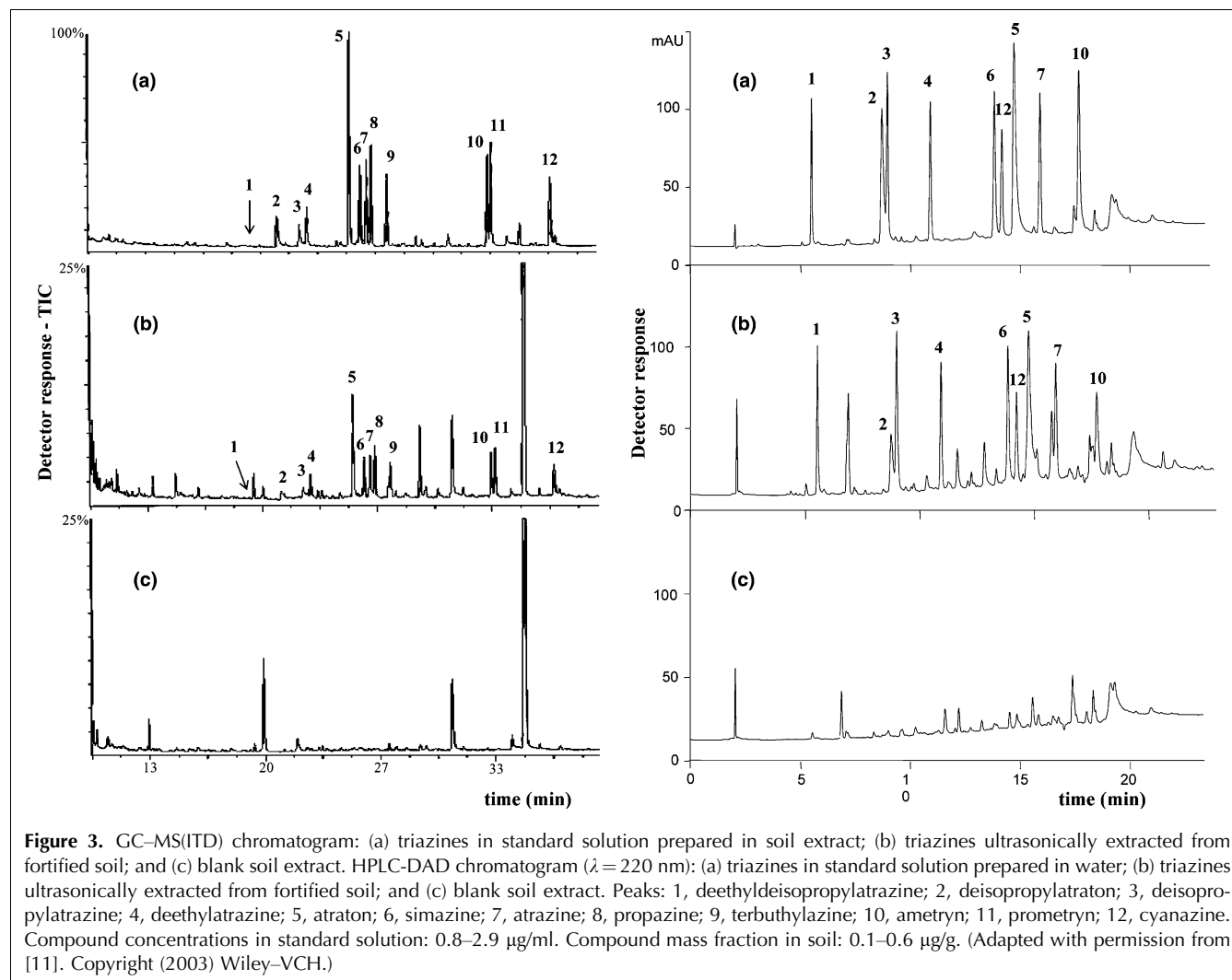
3.4. Determination

GC and LC methods have been published for the determination of different classes of pesticides in soil. Table 5 summarizes their advantages and disadvantages. Pesticide residues have often been analyzed by GC with nitrogen-phosphorus detection (NPD) or electron-capture detection (ECD) [41]. Moreover, GC/MS has been used in

multi-residue methods for pesticide analysis in soil [41]. Fig. 3 illustrates the separation of triazines and TPs by both techniques. GC is very useful for simultaneous determination of several pesticides at trace level, and, in general, higher sensitivity can be obtained using GC rather than LC.

The most widely used ionization technique in GC analysis is electron impact (EI) that produces a collection of fragment ions characteristic of the compounds. This approach has been applied to determine organochlorines, organophosphorus, carbamates, fungicides, triazines, and chloroacetamides [6,8,11,34–36,41,46,48].

Since molecular ions are not always seen in EI spectra, the complementary technique of chemical ionization (CI) is often recommended to determine molecular weights [49,50]. In CI experiments, the ion source is charged with a reagent gas, which undergoes EI ionization to produce an excess of reagent ions. The resulting pseudomolecular ions present little or no fragmentation and provide unambiguous molecular-weight information. However, GC-MS with EI ionization, in the selected ion-monitoring (SIM) mode, offers sufficient sensitivity and selectivity, and the reported applications are less



creative and innovative in that way and do not exploit the CI alternative.

Tandem or MS/MS instruments coupled to GC are exemplified by the ion trap (IT) [49,50]. The principal advantage of the IT is that achieves routine EI analysis of low-molecular-weight compounds [9,11]; at the same time, it is also capable of CI and MS/MS. However, the situation described above for the ionization modes can be extrapolated to IT, the different features of which have not been fully utilized to determine pesticides in soils.

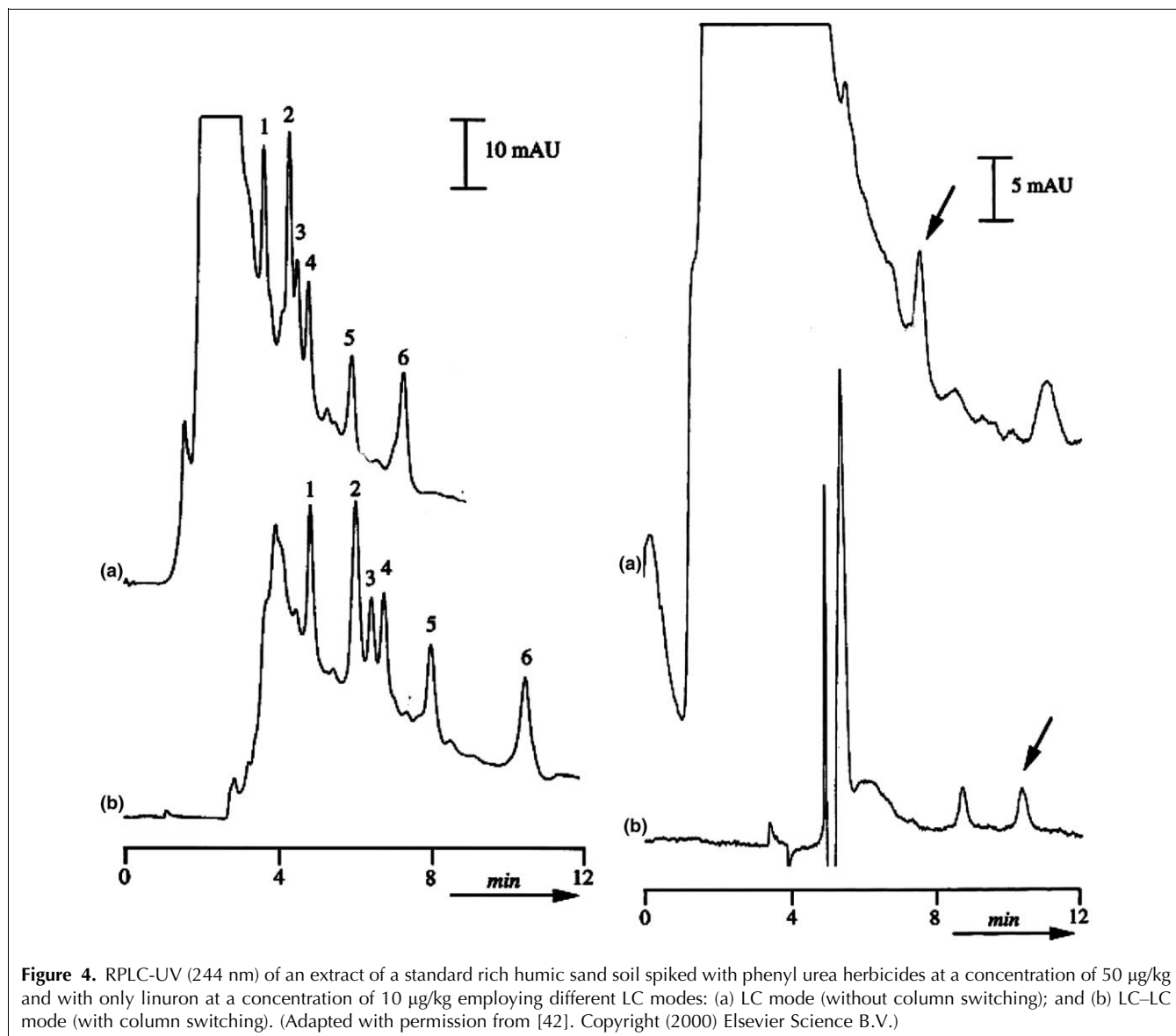
Polar compounds often result from herbicide transformation, so that the polarity range to be covered by the chromatographic method must be extended, and that renders GC analysis less suitable for determining several pesticides and TPs. LC is ideally suited for the analysis of polar compounds. It is a common situation that parent pesticides can be analyzed by either GC or LC, whereas TPs can be analyzed only by LC because of their low volatility and their ionic properties.

The trace analysis of acidic pesticides in soil samples by employing reversed-phase liquid chromatography

with UV detection (RPLC–UV) is blocked by the co-extraction of humic substances. At low wavelength detection, typically at about 220 nm, these interferences show up in the chromatogram as a broad hump that causes baseline deviation and difficulties for the reliable quantification that is required for analytes at low levels.

Coupled-column RPLC (LC–LC) employs one analytical column packed with a restricted access material (RAM) and combines reversed-phase separation of low-molecular-mass analytes and size exclusion of large molecular compounds with ABZ⁺, including a polar embedded functional group. It largely eliminates the bad chromatographic effect of interferences, as shown in Fig. 4 [33,42].

Fluorescence detection has very rarely been used because pesticides and their TPs are not fluorescent and require derivatization [38,39]. This technique is only used for *N*-methylcarbamates, which are hydrolyzed by NaOH at elevated temperatures to yield methylamine, which reacts with *o*-phthalaldehyde (OPA) to produce a fluorescent isoindole.



LC with MS detection (LC-MS) has proved to be an alternative technique for determining pesticides in soil. Several MS reviews have given overviews of the spectral properties of different pesticides and TPs [49–51]. The single quadrupole has been used to determine oxanilic and sulfonic acid metabolites of acetochlor [30], mixtures of pesticides [43], and arylphenoxypropionic herbicides [32]. Fig. 5 shows the absence of interferences in the chromatogram and the mass spectra. The interfaces at atmospheric pressure ionization (API): electrospray ionization (ESI) and AP chemical ionization (APCI), have helped make LC-MS routine. As a disadvantage, these interfaces can obtain the protonated or deprotonated molecule with very rare fragment ions. The MS-fragmentation pattern is a powerful tool, which gives confidence in identifying a compound. MS fragmentation can be achieved using a single quadrupole

by increasing the pre-analyzer extraction (skimmer cone) voltage.

However, by using tandem MS (MS/MS) detection, more selective fragmentation of the protonated or deprotonated molecule is achieved by collision-induced dissociation (CID). Two tandem mass analyzers are used in such studies, triple quadrupole (TQ) and IT. TQ, in which the second fragmentation is carried out in a collision cell between the first and second quadrupole, has been applied to determine different acidic herbicides [14,29] using selected transitions. IT-LC/MS/MS, which achieves consecutive fragmentations in time, has been used in positive and negative ion modes of operation to identify trifluralin metabolites. The procedure was validated for metabolite identification, even in cases for which no standards were available, by confirming the presence of tentatively identified metabolites [25].

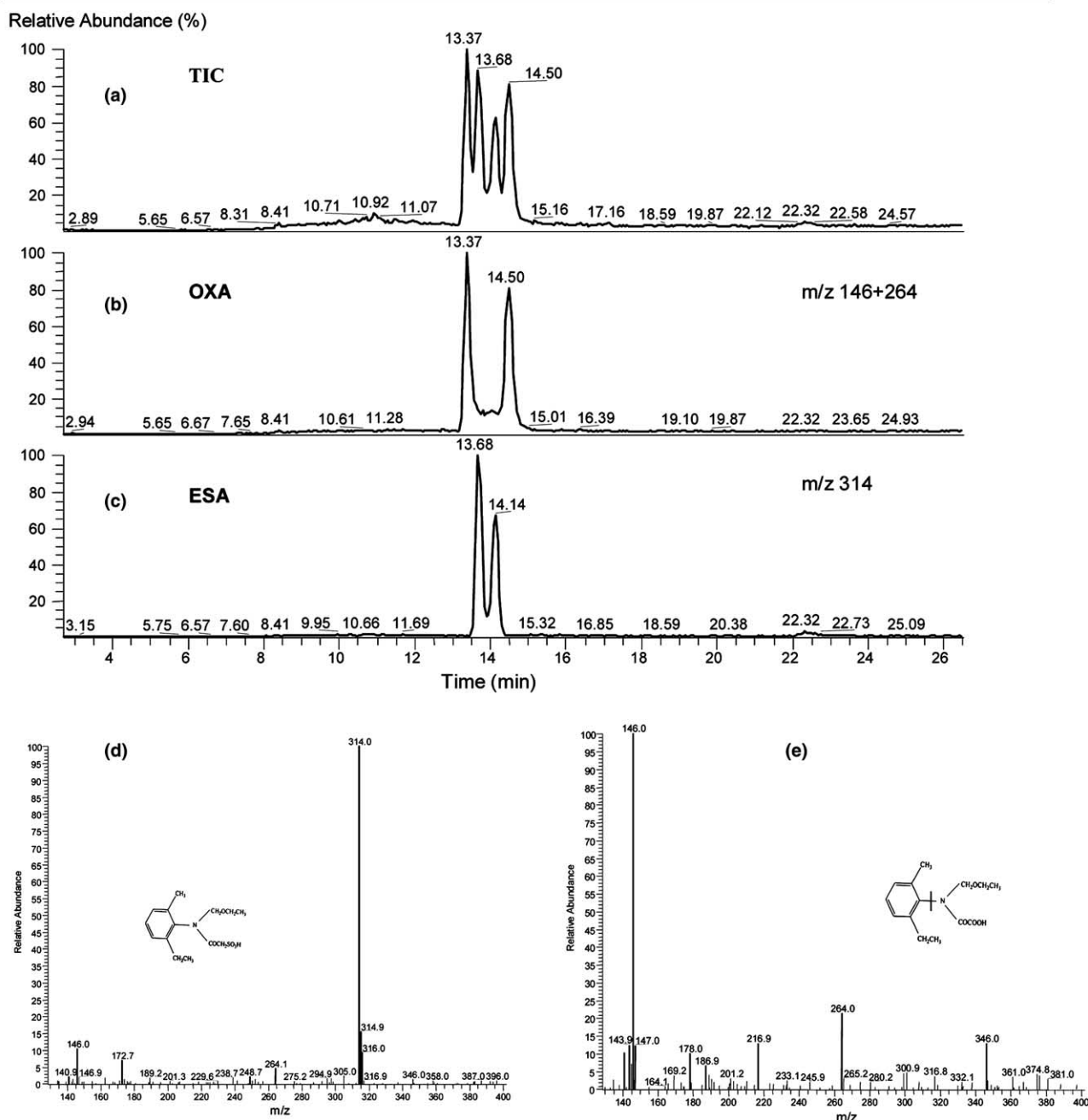


Figure 5. (a) Chromatogram from an LC-ESI-full scan MS analysis of a P-B soil sample; (b) reconstructed ion chromatogram from an LC-ESI-full scan MS analysis (at m/z 146 + 264); (c) reconstructed ion chromatogram for the same LC-ESI-full scan MS analysis (at m/z 314); (d) mass spectra of the ESA metabolite of acetochlor; and (e) mass spectra of the OXA metabolite of acetochlor. (Adapted with permission from [30]. Copyright (2002) Elsevier Science B.V.)

With LC and GC techniques, capillary electrophoresis (CE) [10], enzyme-linked immunosorbent assay (ELISA) [28] or biosensors [52] have been used in recent years. Their more remarkable feature, from the analytical standpoint, is the possibility of fast, direct analysis. However, low sensitivities or the need to confirm positive results still prevent their use in solving most analytical problems relating to pesticide and TPs.

4. Conclusions and future developments

Extraction is surely the most critical step within the total scheme of soil analysis for accurate and reliable determination of pesticides and their TPs. However, because of the peculiarities of these samples – especially the residues “bound” to the organic matter and the presence of thermally or chemically labile TPs in trace or ultratrace

concentrations – selection of the technique for isolating and preconcentrating analytes, as well as careful optimization of the corresponding operational parameters, are extremely important. This overview of the analytical methods shows that there is a divergence between the new trends and developments in analytical chemistry and the determination of pesticides in soil. Some state-of-the-art techniques (e.g., SPME or MSPD, on-line clean-up, or selective phases) have only occasionally been reported for use in determination of pesticides and TPs in soil. The present tendency is to use classical methodologies with newly reported improvements that are quite aggressive to analytes, but well established and achieving a high sample throughput.

From the above conclusions, it is clear that much work still needs to be done on the analysis of pesticides in soil. This particular field of research is also changing each year, since new TPs are being identified and confirmed. Similarly, analytical developments to determine pesticides and the increasing amounts of toxic TPs released into soils need to be made constantly.

Perhaps the next frontier in this area will be field sampling and analysis. The great majority of analytical work on pesticide and TPs in soil is still performed, totally or in part, in the laboratory and/or under incubation conditions. However, appropriate and convincing characterization of the pesticide TPs can be made only in natural conditions.

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