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Assessment of pesticide contamination in soil samples from an intensive horticulture area, using ultrasonic extraction and gas chromatography–mass spectrometry

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Abstract

In order to reduce the amount of sample to be collected and the time consumed in the analytical process, a broad range of analytes should be preferably considered in the same analytical procedure. A suitable methodology for pesticide residue analysis in soil samples was developed based on ultrasonic extraction (USE) and gas chromatography–mass spectrometry (GC–MS). For this study, different classes of pesticides were selected, both recent and old persistent molecules: parent compounds and degradation products, namely organochlorine, organophosphorous and pyrethroid insecticides, triazine and acetanilide herbicides and other miscellaneous pesticides. Pesticide residues could be detected in the low- to sub-ppb range (0.05–7.0 $\mu g\,kg^{-1}$) with good precision (7.5–20.5%, average 13.7% R.S.D.) and extraction efficiency (69–118%, average 88%) for the great majority of analytes. This methodology has been applied in a monitoring program of soil samples from an intensive horticulture area in Póvoa de Varzim, North of Portugal. The pesticides detected in four sampling programs (2001/2002) were the following: lindane, dieldrin, endosulfan, endosulfan sulfate, 4,4′-DDE, 4,4′-DDD, atrazine, desethylatrazine, alachlor, dimethoate, chlorpyrifos, pendimethalin, procymidone and chlorfenvinphos. Pesticide contamination was investigated at three depths and in different soil and crop types to assess the influence of soil characteristics and trends over time.

Keywords: Ultrasonic extraction; Pesticides; Herbicides; Soil; Contamination

1. Introduction

Great productivity gains can be achieved in agriculture using the adequate pesticides. Indeed, they are needed to meet the world's demand on foodstuffs and no other alternative can compete to be used in such a large scale. Slow degradation of pesticides in the environment and extensive or inappropriate usage by farmers can lead to environmental contamination of the water, soil, air, several types of crops and indirectly to humans [1,2].

Chlorinated pesticides (OCPs) are very toxic and persistent compounds in the environment. Although most of them have been banned decades ago, they can still be found in the environment even in remote regions; thus, they are still of great concern [3]. The organophosphorous insecticides (OPPs) and triazine herbicides are among the most commonly used and detected pesticides around the world; thus, monitoring is important from an agricultural and environmental point of view [4–6]. Pesticides is a family of compounds in continuous evolution in terms of chemical synthesis; some of the recent chemicals such as dinitroanilines, chloroacetamides, dicarboximides, acylalanines, regarded as safer to the environment can be found in high-quantities in soils, conversely to the previously referred groups.

Soil is the principal reservoir of environmental pesticides, thus representing a source from which residues can be released to the atmosphere, ground water and living organisms

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[7]. In modern analytical laboratories, the classical methodology for determination of micropollutants in solid matrices based on agitation procedures and Soxhlet extraction [8–10] has been replaced by less time- and solvent-consuming, and often automated techniques, which include high-pressure and/or high-temperature processes: microwave-assisted extraction (MAE) [11], supercritical fluid extraction (SFE) [12,13], accelerated solvent extraction (ASE) [14–16], or vigorous agitation like ultrasonic extraction (USE) [17,18]. Recently, some attempts have been made to enlarge the solid-phase microextraction (SPME) applications to the analysis of solid matrices, which would be beneficial in terms of simplification in sample handling, reduction in sample size and solvent volume, and absence of additional clean-up procedures [19]. Extraction of the soil samples can be achieved by direct dipping of the fibre in a soil/water slurry, indirect extraction in the headspace or by dilution of the extract obtained by other liquid extraction technique with distilled water [19]. Although successful, only a limited number of references can be found reporting the analysis of OCPs [20], OPPs [21] and herbicides [19,22,23] in soil samples, which can be attributed to some limitations in terms of fibre stability or analyte release/volatility.

As common advantages of all the enhanced extraction techniques regarded as environmentally friendly can be referred the improved selectivity, rapidity and automation, however, some particular drawbacks must be considered. SFE cannot handle large sample amounts and recoveries can be somewhat lower for markedly polar pesticides/metabolites [24]. ASE and SFE require expensive instrumentation and laborious optimisation processes [24]. MAE can improve extraction efficiency of thermal stable pesticides particularly of aged pollutants but on the expense of extraction selectivity, thus requiring a further clean-up step [25,26].

The target compounds for analysis in soil and sediment samples have traditionally been highly hydrophobic and persistent contaminants like OCPs [2,3,7,17,20] and, with few exceptions [27,28], little attention has been dedicated either to other type of pesticides or multiresidue analysis. Due to the large number of active ingredients used nowadays, trace analyses of these substances require techniques for the detection of the greatest number of compounds possible, with the fewest number of extraction and clean-up steps [29]. If well-established, ultrasonic extraction has the potential to fulfil the requirements of a multiresidue method. The initial use of ultrasonic energy as a means to extract pesticides from soil was first reported by Johnsen and Starr in 1967 [17]. USE is a very versatile technique due to the possibility of selecting the solvent type or solvent mixture that allows the maximum extraction efficiency and selectivity. The primary advantage of this method is the fact that several extractions can be done simultaneously and no specialized laboratory equipment is required, although it is not easily automated. In combination with state-of-the-art separation and detection techniques good performance can be obtained, eventually in the absence of additional clean-up of the concentrated extract. This paper describes the development and application of an USE technique combined with gas chromatography and mass spectrometric detection for the analysis of OCPs, OPPs, triazines, pyrethroids, acetanilides and other miscellaneous pesticides in soil samples from an intensive horticulture area in North Portugal. This region comprises mainly two types of soil (sand and sandy-loam); thus, their influence on the pesticides behaviour will be investigated. The implemented monitoring program is expected to allow observing temporal trends on pesticide contamination as well as variations according to depth in five sampling points, during two years. Results for the 2001/2002 period will be reported.

2. Experimental

2.1. Chemicals and reagents

All pesticide analytical standards of Pestanal® grade were supplied by Riedel-de-Häen (Seelze, Germany). The OCPs were obtained as a commercial mixture (EPA 608 pesticide mix, 20 mg l^{-1}) from Supelco (Bellefont, PA, USA). Individual pesticide stock standard solutions were prepared by exact weighing of high-purity substances in 10 ml volumetric flasks and filled up with an appropriate solvent as follows: pyrethroids were prepared in ethyl acetate whereas OPPs and triazines were dissolved in methanol; individual solutions of other pesticides were also made in methanol. Four group mixtures at 2.0 mg l^{-1} concentration of each pesticide were then prepared in methanol, while hexachlorobenzene (HCB) and isodrin were added to the OCPs commercial mixture to obtain a stock solution of 100 μg l^{-1}, in methanol. All stock standard solutions were stored in a freezer protected from light at −18 °C. Ethyl acetate used in handling of standards and soil extractions was of Pestanal® grade from Riedel-de-Häen whereas methanol and dichloromethane were of ChromaSolv and Analytical Reagent grade, respectively (Fluka, Buchs, Switzerland). *n*-Hexane was of SupraSolv grade from Merck (Darmstadt, Germany), and acetonitrile HPLC gradient grade was supplied by Panreac (Barcelona, Spain). In the filtration of extracts regular cotton wool with no special treatment and anhydrous sodium sulfate from Merck, were used.

2.2. Soil samples

An uncontaminated bulk soil sample was selected to use in the optimisation and validation experiments. All studies were conducted with spiked soil at 10 μg kg^{-1} concentration level, except for desethylatrazine and dimethoate 10 times higher and γ-chlordane 10 times lower, after evaporation of the residual solvent and aging during a period of not less than 4 days. Once the method was established, real soil samples from five sampling points at three depths (surface, 10 and 20 cm), as depicted in Fig. 1, were collected in four sampling dates and analysed with the USE method.

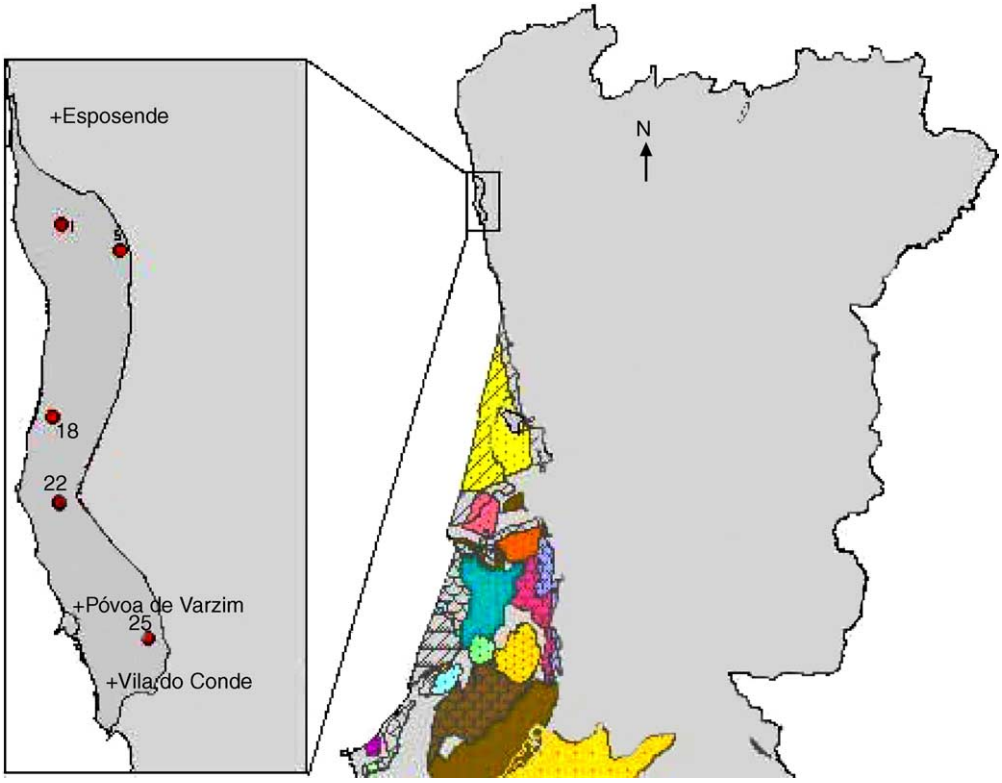

Fig. 1. Map of North Portugal showing a delimited region corresponding to the Vulnerable Area no. 1 where the sampling points were located.

This monitoring program was scheduled to include a sampling event approximately every 3 months. After collection and transport to the laboratory in aluminium foil packets, the samples were dried in an oven at 40 °C during 48 h, sieved at 500 μm, perfectly well homogenized and kept refrigerated at 4 °C before analysis. Table 1 presents the humidity and organic mater content of the reference soil and surface horizon of five soil samples, as indicative of the general characteristics of these soils.

2.3. Ultrasonic extraction procedure

In order to analyse a large number of pesticides from a variety of chemical groups, a simple method was developed to expand the range of applicability of the EPA method 3550C [18]. This method consists on USE and it is indicated to extract non-volatile and semi-volatile organic compounds from solids, such as soil, sludge and waste. The USE conditions consisted in the following: 5 g of soil samples was placed in small Erlenmyer flasks and 5 ml of a suitable organic solvent added. The soil samples were firstly manually agitated and then exposed to USE in a Bandelin RK 100H (80/160 W) ultrasonic bath (Sonorex, Germany) for 15 min, three times. After each extraction period, extracts were collected by pouring the extractant through a funnel plugged with a small piece of cotton wool overlaid by a portion of anhydrous sodium sulfate, which had been previously washed with the same solvent. In order to achieve the adequate concentration factor, 5 g aliquot of sample was submitted to extraction and the final extract (ca. 15 ml) evaporated to dryness under a gentle stream of nitrogen without need of any clean-up procedure and redissolved in 200 μl of ethyl acetate. For higher productivity, up to eight samples could be processed in a row using this procedure.

Table 1
Physico-chemical characteristics of the bulk soil used in method development and the real samples

	Soil type	Humidity (%, w/w)	Organic matter (%, w/w)
Bulk soil	Sandy-loam	1.12	7.44
Soil 1	Sand	0.12	1.69
Soil 5	Sandy-loam	0.73	7.82
Soil 18	Sand	0.33	4.08
Soil 22	Sandy-loam	0.75	8.62
Soil 25	Sandy-loam	0.89	8.01

Humidity was determined by drying the samples to constant weight at 105 °C and the organic matter content determined by loss-on-ignition at 550 °C in a muffle furnace for 3 h [30].

2.4. Chromatographic analysis

Chromatographic analyses were carried out in a gas chromatograph Agilent 6890 (Palo Alto, CA, USA) interfaced to an Agilent 5973N mass selective detector. The GC–MS system was equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and helium was used as carrier gas at 1 ml min^{-1} flow rate.

The injection port temperature was set at 250 °C and a liner with a plug of glass wool was installed. An amount of 1 μl of the concentrated extracts was injected in splitless mode, during 0.75 min. MS temperatures were: ion source, 230 °C; quadrupole, 150 °C; and transfer line, 280 °C. The 44 pesticides were separated with a 33.67 min oven temperature program built as follows: initial temperature 80 °C (hold 2 min), increase at 15 °C min^{-1} to 180 °C (hold 4 min), increase at 10 °C min^{-1} to 230 °C (hold 5 min) and finally increase at 10 °C min^{-1} to 290 °C (hold 5 min). Quantitation was carried out in the selected ion monitoring mode (SIM) selecting characteristic fragment ions for each pesticide while, when needed, confirmation was achieved in a second analysis recording the full scan mass spectra and requiring a minimum spectral fit of 80.

3. Results and discussion

3.1. Method development

In the early beginning of USE, Johnsen and Starr demonstrated that sonic energy could allow a very efficient extraction method for the analysis of OCPs in soils, comparing the performance of the Polytron, ultrasonic cleaner, Soxhlet and other techniques. The authors concluded that the extraction performance was comparable, differing only on the time to achieve complete recoveries [17]. Also, some other authors developed successful methodologies based on this technique [3,4].

One of the most important parameters that can influence extraction efficiency and selectivity in USE is the solvent nature, which is a critical parameter when attempting multiresidue analysis. To minimize the effect of soil co-extractives on the determination of analytes as well as to improve recoveries several solvents were tested: *n*-hexane, ethyl acetate, acetonitrile and dichloromethane. Since the target list of pesticides includes highly apolar to mid-polar compounds and even degradation products the extraction solvent should have polarity properties compatible with all analytes. The complete list of target pesticides can be found in Table 2.

Dichloromethane was discarded because solvent decantation became difficult. Ethyl acetate gave the best recoveries for all analytes except one (dichorvos) and also best precision. Hexane (5.2–81.1% of ethyl acetate) and acetonitrile (14.7–81.3% of ethyl acetate) showed good properties for the extraction of OCPs; however, hexane almost could not recover the OPPs and triazines from soil. In conclusion, ethyl acetate was the most suitable solvent for USE of these target analytes whereas acetonitrile was only acceptable and hexane was unacceptable. In an attempt to further improve the extraction recovery, the soil sample was previously wetted with 15% NaCl aqueous solution. The salting-out effect expected to occur with this strategy did not enhance extraction recoveries, except for dichlorvos (32.0–122.4% of ethyl acetate), which can be explained by water shielding of soil particles. From the evaluation of method performance using 5 ml of ethyl acetate as extractant in ultrasonic extraction during 15 min repeated three times (as described in the Section 2), it was concluded that these conditions exhibited excellent extraction capabilities; therefore, no further optimisation would be needed.

3.2. Method performance

The USE method as described above was extensively tested in order to assess its precision, sensitivity, selectivity and completeness. The critical validation parameters were compiled and presented in Table 2. All the results were calculated based on peak areas obtained monitoring the respective pesticide fragment ions (*m*/*z*) in SIM mode. Chromatographic data acquisition was divided into 11 different MS segments in order to include the lowest number of compounds in each, this way maximising sensitivity (see Table 3).

The precision of the technique was evaluated in terms of repeatability (within-day relative standard deviation, R.S.D.) by the analysis of six replicate spiked soil samples at 10 μg kg^{-1} concentration level, and in terms of intermediate precision (between-day R.S.D.) at the same concentration level in three non-consecutive days (results displayed in Table 2). The precision can be considered very good taking into account a non-automated procedure having a repeatability of less than 8% for the great majority of analytes. Likewise, the intermediate precision was consistently below 15%, only a few exceptions up to 20%. Endrin however presented an anomalous result due to a decrease in chromatographic response with time.

The recoveries were calculated on spiked soil at 10 μg kg^{-1} concentration level after ageing during 4 days. First experiments gave immediately good recovery values for the organophosphorous insecticides, triazine herbicides and miscellaneous pesticides; however, excessively high (above 130%) recovery values were obtained for some organochlorine and pyrethroid pesticides. Even after some attempts to find out the origin of such behaviour, namely changing the injector temperature, matching the solvent in extracts and standards and testing a higher concentration, the problem persisted essentially for the following pesticides: lindane, heptachlor, dieldrin, endrin, 4,4′-DDD, λ-cyhalothrin, α-cypermethrin and azinphos methyl. Soon the cause of this phenomenon appeared to be related with the extract matrix, since the blank extracts were free of interferences at the retention time and quantitation ions of the pesticides and the same concentration injected in pure solvent gave lower peak areas compared to the recovery standards. This observation led us to perform the calculations based on the analysis of matrix-matched standards and the results obtained are those presented in Table 2. In general, the recovery values obtained this way were lower than the ones previously obtained and were acceptable for the majority of the compounds (except dichlorvos and fenamiphos) ranging from 70 to 118%. Nevertheless, some results remained somewhat above 100% essen-

Table 2
Validation parameters of the USE–GC–(SIM)MS method used for pesticide analysis in soil samples: precision, limits of detection, recoveries and calibration data

Peak no.	Pesticides	t_R (min)	Quantitation ion (m/z)	Repeatability (%R.S.D.) $n = 6$	Intermediate precision (%R.S.D.) $n = 18$	LOD ($\mu g\,kg^{-1}$)	Recovery (%R.S.D.) ($10\,\mu g\,kg^{-1}$) $n = 6$	Determination coefficients (r^2)
1	Dichlorvos	7.03	109	16.8	15.4	2.00	58 (12.4)	0.997
2	Desethylatrazine	11.97	172	3.9	12.3	2.00	75 (4.6)	0.999
3	Hexachlorobenzene	13.03	284	5.2	12.3	0.07	78 (7.5)	1.000
4	Dimethoate	13.21	87	4.9	17.0	1.00	79 (17.2)	1.000
5	Simazine	13.36	201	4.8	11.1	0.80	79 (7.0)	1.000
6	Atrazine	13.54	200	5.8	11.6	0.20	78 (6.4)	0.999
7	Propazine	13.68	214	5.1	11.0	0.30	78 (5.3)	0.999
8	Lindane	13.90	181	13.0	19.3	0.50	86 (15.6)	0.991
9	Terbuthylazine	14.03	214	4.1	11.8	0.10	79 (6.4)	0.999
10	Propyzamide	14.14	173	3.9	10.9	0.50	87 (3.5)	1.000
11	Fonofos	14.17	109	4.8	12.4	2.00	88 (13.9)	0.999
12	Diazinon	14.44	179	7.0	13.5	3.00	74 (10.5)	0.999
13	Metribuzin	15.73	198	4.7	14.2	2.00	80 (9.6)	0.998
14	Parathion-methyl	15.93	125	6.0	17.1	4.00	92 (13.7)	1.000
15	Simetryn	16.04	213	9.3	16.9	2.00	69 (4.6)	0.989
16	Alachlor	16.15	160	4.9	12.3	0.50	84 (6.2)	0.999
17	Heptachlor	16.15	272	4.5	7.5	0.10	118 (9.2)	1.000
18	Fenitrothion	16.72	125	5.7	11.8	4.00	90 (12.1)	1.000
19	Malathion	17.02	127	5.5	10.8	4.00	89 (7.2)	1.000
20	Metolachlor	17.08	162	7.4	11.2	0.10	94 (8.1)	0.999
21	Aldrin	17.14	66	5.4	12.9	2.00	78 (11.5)	0.999
22	Chlorpyrifos	17.32	314	4.7	10.4	0.05	79 (3.6)	1.000
23	Parathion-ethyl	17.34	291	6.0	11.1	0.07	90 (8.0)	1.000
24	Isodrin	17.93	193	5.4	10.2	1.00	78 (10.3)	1.000
25	Chlorfenvinphos E	18.12	267	7.5	13.1	0.20	92 (14.5)	1.000
26	Pendimethalin	18.20	252	4.6	9.5	0.30	101 (4.6)	1.000
27	Heptachlor Epoxy	18.25	353	8.3	12.4	0.05	91 (5.7)	0.999
28	Chlorfenvinphos Z	18.46	267	4.8	10.6	0.20	91 (10.8)	1.000
29	Procymidone	18.73	96	7.6	11.5	2.00	110 (4.7)	0.997
30	γ-chlordane	18.95	373	7.8	11.6	0.10	97 (4.4)	1.000
31	Tetrachlorvinphos	19.30	329	5.4	18.5	0.07	76 (17.0)	0.999
32	Endosulfan I	19.35	241	7.1	12.6	1.00	88 (4.6)	1.000
33	Fenamiphos	19.79	303	5.6	15.7	0.20	70 (19.0)	1.000
34	4,4′-DDE	20.15	246	5.3	12.7	0.50	93 (3.9)	0.999
35	Dieldrin	20.25	79	6.0	12.8	1.40	104 (9.2)	0.956
36	Endrin	21.08	263	14.8	33.7	0.30	80 (14.0)	0.991
37	Endosulfan II	21.45	195	9.3	17.4	2.00	87 (8.7)	0.999
38	4,4′-DDD	21.83	235	12.2	16.7	0.50	109 (10.5)	1.000
39	Endosulfan sulfate	23.38	272	9.4	20.1	0.10	90 (10.3)	1.000
40	4,4′-DDT	23.40	235	10.4	17.4	7.00	115 (11.4)	0.989
41	Azinphos-methyl	26.62	160	4.5	19.2	2.00	76 (14.6)	0.998
42	λ-cyhalothrin[a]	27.36	181	5.6	9.2	1.00	103 (10.0)	1.000
43	α-cypermethrin[a]	30.14	181	5.7	10.8	4.00	103 (7.0)	1.000
44	Deltamethrin[a]	33.06	181	12.1	12.9	4.00	111 (9.7)	1.000

[a] Pyrethroid pesticides consist on a mixture of several (R, S) diastereoisomers and although for validation purposes a single peak was used, the pair was considered for quantitation purposes

tially for the compounds listed above. Due to some heating of the ultrasonic bath, the recovery of slightly volatile compounds like dichlorvos can be prejudiced; however, it also helps in the extraction of other compounds.

Influence of matrix co-extractives on the response of analytes is a well-known phenomenon in pesticide residue analysis, which can result in either decreased detection response [30] or increased analytical signal [2,28]. Excessively high-recovery values were observed before and explained by the phenomenon known as 'matrix-induced chromatographic response enhancement' that can occur for particular pesticides, matrix types and depending on the status of the capillary column [31,32].

Although recommended to improve accuracy, use of matrix-matched analysis is still limited due to the wide range of soil types and inherent organic matter content in soil samples [30]. Humic substances constitute the major part of the organic carbon present in soils and especially humic acids play a major role in the environmental behaviour of pesticides. Prosen and Zupancic-Kralj found that a fraction of

Table 3
GC–MS instrumental conditions for operation in the selected ion monitoring mode including the appropriate time segments and respective fragment ions (*m*/*z*)

SIM groups	Pesticides no.	Segment start (min)	Quantitation ions (*m*/*z*)
Group 1	1, 2	5.00	109, 172
Group 2	3–7	12.50	87, 200, 201, 214, 284
Group 3	8–12	13.75	109, 173, 179, 181, 214
Group 4	13–17	15.20	125, 160, 198, 213, 272
Group 5	18–23	16.40	66, 125, 127, 162, 291, 314,
Group 6	24–28	17.60	193, 252, 267, 353
Group 7	29–33	18.60	96, 241, 303, 329, 373
Group 8	34, 35	19.90	79, 246
Group 9	36–38	20.80	195, 235, 263
Group 10	39–41	22.50	160, 235, 272
Group 11	42–44	26.80	181

triazine residues are virtually impossible to extract from environmental samples presumably because of an interaction with humic acids particularly at strongly acidic pH. However, since the natural soil pH is either weakly acidic or weakly alkaline, some doubts still persist about triazine binding to soil organic matter [33].

In order to determine the sensitivity of the technique and its appropriateness for environmental behaviour and pollution monitoring studies, limits of detection (LODs) of the USE–GC–MS procedure were determined testing decreasing concentrations of analytes spiked on soil until obtaining a signal/noise ratio of 3 (S/N 3). Limits of quantitation (LOQs) were derived from LODs to give a S/N of 10. LODs in the low- to sub-ppb ($\mu g\,kg^{-1}$) level were obtained ranging from 0.05 to 7.00 $\mu g\,kg^{-1}$, which can be mainly attributed to the excellent sensitivity and selectivity widely recognised to mass spectrometry.

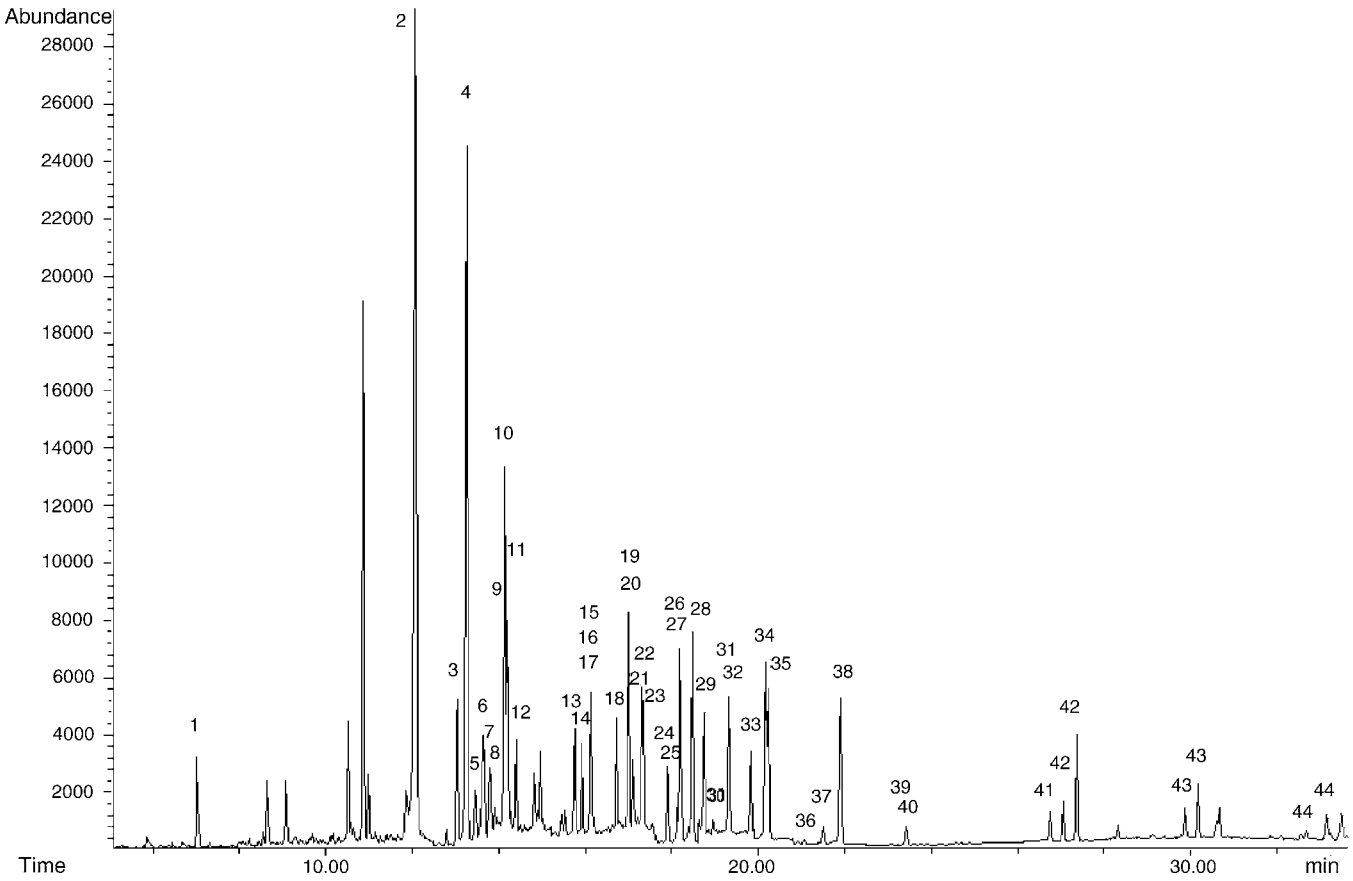

Fig. 2. GC–MS total ion chromatogram (SIM mode) obtained from the analysis of a spiked soil at 50 $\mu g\,kg^{-1}$ concentration level after USE using the optimised conditions. *Note*: Desethylatrazine and dimethoate concentration is 10 times higher whereas γ-chlordane is 10 times lower. For peak assignment refer to Table 2.

Table 4
Pesticide concentrations in μg kg^{-1} of dry soil obtained in a monitoring program of five sampling points at three depths carried out in the intensive horticulture area of Póvoa de Varzim, over 1 year period

	Alachlor			
Soil 1	September 01	February 02	April 02	July 02
Surface	–	–	–	3.7
10 cm	–	–	–	–
20 cm	–	–	–	–

	Atrazine				Alachlor				Chlorpyrifos				Endosulfan I			
Soil 5	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02
Surface	–	–	1.9	4.6	–	11.7	16.9	26.1	–	1.1	2.4	2.0	–	–	–	61.3
10 cm	–	–	–	2.8	–	2.3	–	8.5	–	0.2	–	1.3	–	–	–	18.1
20 cm	–	–	–	1.0	–	–	–	11.6	–	–	–	2.0	–	–	–	4.7

	Endosulfan II				Endosulfan Sulfate			
Soil 5	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02
Surface	–	–	–	135.6	–	–	–	280.8
10 cm	–	–	–	36.9	–	–	–	77.2
20 cm	–	–	–	9.2	–	–	–	16.9

	Dimethoate				Lindane				Chlorpyrifos				Pendimethalin			
Soil 18	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02
Surface	–	–	7.1	–	22.3	–	–	–	–	0.9	53.3	33.8	–	13.4	6906.2	454.8
10 cm	–	–	–	–	31.0	–	–	–	–	0.8	148.8	2.6	–	7.2	9.5	35.3
20 cm	–	–	–	–	60.0	–	–	–	–	0.5	5.3	1.7	–	3.5	232.5	11.5

	Procymidone			
Soil 18	September 01	February 02	April 02	July 02
Surface	–	133.0	–	12.2
10 cm	–	11.8	–	8.6
20 cm	–	7.1	–	–

	Dimethoate				Alachlor				Lindane				Chlorpyrifos			
Soil 22	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02
Surface	–	–	95.6	44.8	12.6	2.1	–	–	–	58.3	–	–	–	–	–	67.3
10 cm	–	–	42.7	–	8.0	–	–	–	–	–	–	–	–	–	–	6.1
20 cm	–	–	35.4	–	1.9	–	–	–	–	–	–	–	–	–	–	1.7

Table 4 (*Continued*)

	Chlorfenvinphos				Pendimethalin			Procymidone			Dieldrin			
Soil 22	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02
Surface	–	–	–	–	–	–	2.9	1.6	–	–	–	11.6	279.3	297.6	132.9	435.3
10 cm	1.4	–	–	–	–	–	2.0	–	–	–	–	9.1	309.6	331.7	254.7	465.5
20 cm	–	–	–	–	–	–	2.6	–	–	–	–	–	279.3	147.1	235.4	407.5
	Endosulfan II[a]				Endosulfan Sulfate				4,4′-DDE				4,4′-DDD			
Soil 22	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02
Surface	18.2	35.1	19.7	20.2	65.3	155.6	86.9	136.9	17.0	12.6	7.1	22.5	4.3	7.4	2.9	9.2
10 cm	13.4	18.5	20.9	21.6	67.3	104.4	109.2	149.1	15.5	15.3	11.2	24.0	6.0	6.0	4.0	8.4
20 cm	13.6	7.1	10.8	15.5	41.7	38.0	61.0	111.6	12.2	10.6	10.8	20.0	3.9	3.0	3.1	6.8
	Desethylatrazine				Atrazine				Alachlor							
Soil 25	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02	September 01	February 02	April 02	July 02				
Surface	12.2	–	–	–	85.4	4.1	11.8	5.2	60100	927.8	225.2	10.4				
10 cm	3.7	–	–	10.8	42.2	11.4	–	76.1	8341.0	300.2	5.9	300.3				
20 cm	–	–	–	–	8.2	7.2	–	3.8	2985.0	8.3	7.4	11.9				

[a] Although endosulfan I was detected in soil 22 it could not be quantified, thus only the endosulfan II isomer was reported.

Method calibration was then conducted using spiked soil standards at different calibration levels: 1.0, 5.0, 10, 50, and 100 μg kg^{-1} and the blank, injected in triplicate. The determination coefficients presented in Table 2 confirm the excellent linearity between analytical signal and analyte concentration. It should be noted, however, that since the data treatment was carried out in the MSD ChemStation, Data Analysis Application ver. G1701DA (Agilent Technologies, Palo Alto, CA, USA) replicates at each concentration level were first averaged and then applied a linear regression. This way, coefficients of determination (r^2) appear somehow better (rounded to three decimal places) even though the remaining linear regression coefficients are the same as keeping replicates separate. Fig. 2 shows a chromatogram of a 50 μg kg^{-1} spiked soil standard where the peaks have been numbered as assigned in Table 2.

From the chromatographic point of view the method presented herein does not require a clean-up step of the soil extracts which was evident from the absence of interfering peaks in the blanks and uncontaminated samples. Though a few matrix peaks exist they do not hamper identification and quantitation of pesticides. Chromatographic resolution was not also affected for a long period of time. The only shortcoming exists when calculating recoveries, which would probably benefit of injecting a cleaner extract. Nevertheless, calibration with matrix-matched standards equivalent to real soil samples minimizes errors and special care has only to be taken when analysing quite different soil matrices.

3.3. Monitoring program

The present method was applied to real soil samples included in a monitoring program of soil and groundwater samples from an intensive horticulture area in Póvoa de Varzim, North Portugal. This program will be extended for 2 years with a sampling schedule approximately every 3 months. The main objective was to identify the pesticides that were contaminating the soil, their quantities and fate. The location of sampling points was selected to include soil with different composition, from sand to loam, and different crops such as vegetables, potatoes, corn, grass and others. A pre-treatment of all samples was employed as described in Section 2.

Results from only four sampling dates will be discussed here: September 2001, February, April and July 2002. New calibration curves were created every time before the soil analyses of each sampling event, and blanks and control standards at 10 μg kg^{-1} level were analysed ensemble.

Pesticide concentrations in soils over 1 year period are reported in Table 4.

Results below the method quantitation limits or in the case the pesticide identity could not be confirmed are not reported. Sampling point no. 1 was a very low contaminated site. Soil 5 was moderately contaminated with the herbicide formulation atrazine and alachlor. There were found traces of chlorpyri-

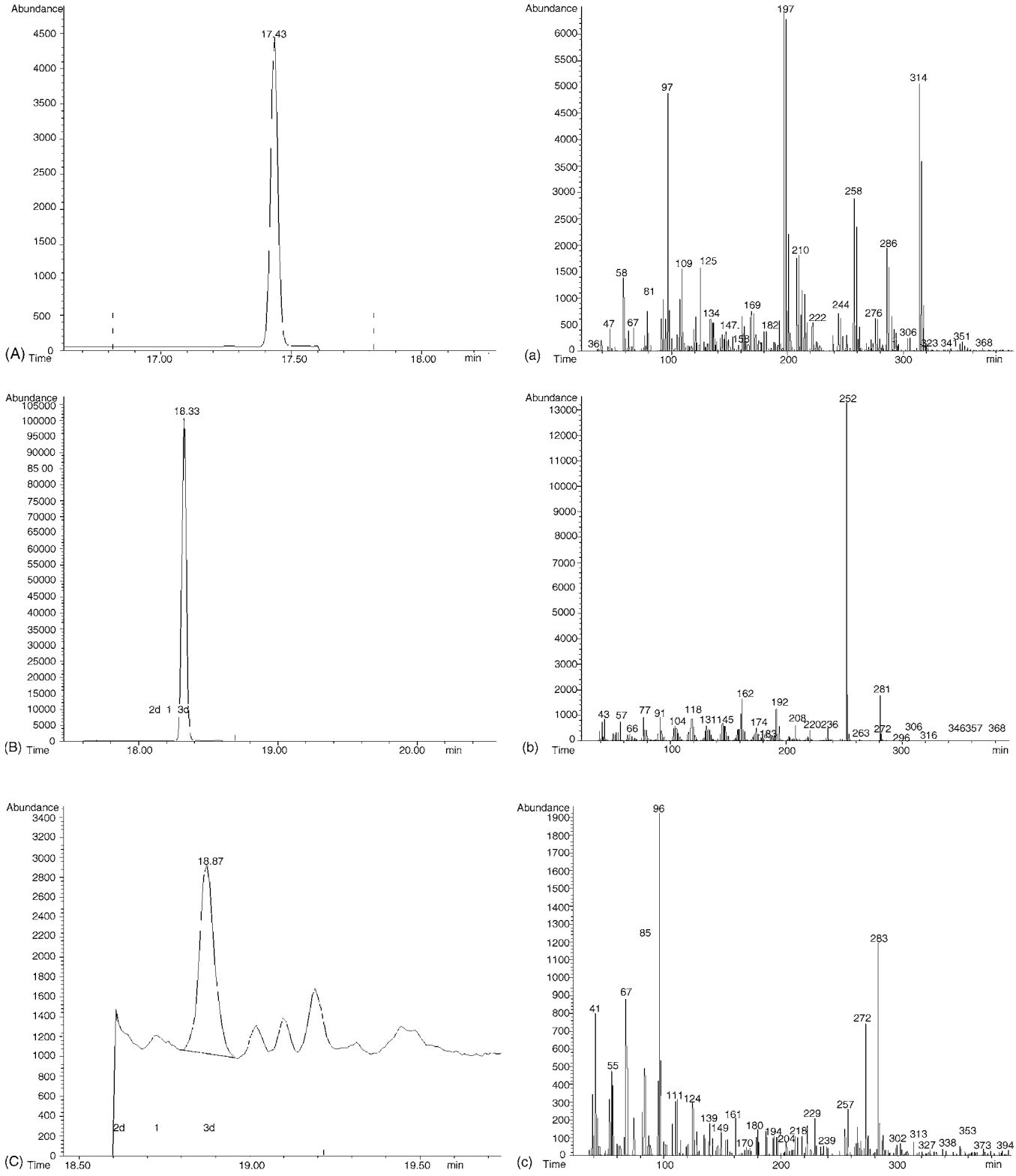

Fig. 3. Analytical peaks (A, B, C) obtained in SIM mode and respective full scan mass spectra (a, b, c) of pesticides detected in the contaminated sample: soil 18, surface, July 2002. Pesticides and respective concentrations were the following: (A) chlorpyrifos—33.8 $\mu g\,kg^{-1}$, (B) pendimethalin—454.8 $\mu g\,kg^{-1}$, (C) procymidone—12.2 $\mu g\,kg^{-1}$.

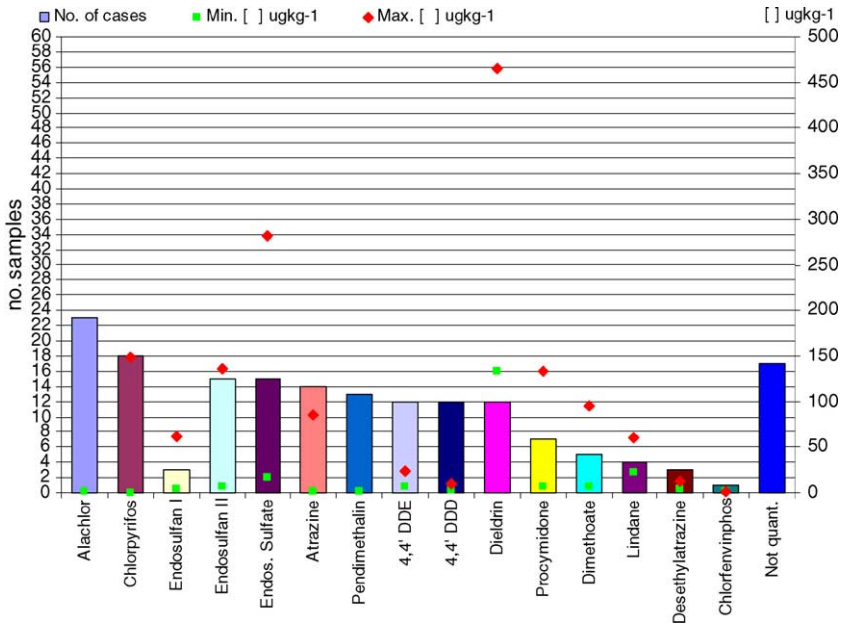

Fig. 4. Schematic representation of pesticide occurrence and concentration range in total 60 soils collected in Póvoa de Varzim from September 2001 to July 2002. The number of uncontaminated samples is also displayed.

fos but fairly high concentrations of endosulfan and endosulfan sulfate. The concentration of these compounds decreased with depth. In soil 18, besides the low contamination with dimethoate and moderate contamination with lindane, chlorpyrifos and procymidone, should be emphasized the very high amounts of pendimethalin found in April 2002. It was mainly present in the surface layer and decreased significantly in a 3-month period. Soil 22 was a highly contaminated site with a large diversity of identified pesticides, in total 12 compounds. The OCPs (except lindane) were present in all samples at different soil depths and sampling campaigns. Currently used pesticides as well as superseded compounds and degradation products were identified. Dieldrin, 4,4′-DDE and 4,4′-DDD contamination was widespread in soil with similar quantities over the soil profiles and sampling dates, which confirms the persistent character of these molecules and their adsorption to soil. Dieldrin was, indeed, present in quite high concentrations, also detectable in water samples. As reported before, DDE was the main contributor to the sum of DDTs, indicating that the transformation of DDT to DDE is favoured in aerobic systems [3]. Endosulfan was detected in all soil samples at medium concentration mainly represented by the β-isomer (or II) (α-isomer (or I) below LOQ), which differs from the technical formulation and can be due to the faster degradation of the α-isomer in soils. Indeed, measured half-lives for α- and β-endosulfan were 60 and 800 days, respectively [34]. The concentration of endosulfan sulfate was always higher than the sum of both endosulfan isomers, as it is the major degradation product by soil bacteria, which raises concerns about its toxicity since it is known to be as toxic as the parent compound [7,35]. The same comments are applied to soil 5. Sample 25 was contaminated with atrazine, its degradation product desethylatrazine (DEA) and alachlor. Traces of DEA were found where the concentration of atrazine was higher. In September 2001, a very high-episode of alachlor contamination occurred. Even it has decreased significantly with time; it did not recover completely after almost 1 year (considering no further input of the herbicide). This finding may be related with corn cultivation during summer. Fig. 3 is representative of a real contaminated sample where the chromatographic peaks obtained in SIM mode and the respective mass spectra obtained under full scan acquisition are shown.

A complete summary of all results comprising the identified pesticides, their individual occurrence and concentration range in soil samples are represented in Fig. 4.

Alachlor, chlorpyrifos and endosulfan are among the most detected pesticides. On the other hand, 17 samples were not contaminated with any of the target pesticides or their presence could not be confirmed. In addition to the pesticides we have been dealing with, some others of diverse chemical families were identified as follows: metalaxyl, benalaxyl, quinalphos, pirimicarb and metolachlor. Since they were not considered in the USE–GC–MS method, only qualitative analysis could be conducted.

4. Conclusions

Monitoring of the widespread distribution of pesticides in the environment requires the availability of efficient and robust multiresidue analytical methods. Some experiments were carried out to develop an USE method which proved to be a fast, reliable and inexpensive technique, therefore,

more applicable to routine analysis than the existing conventional techniques. The sensitivity and precision were excellent, added of confirmation capabilities, however, care should be taken in the calibration to encompass a similar matrix to the samples. Although not automated, simultaneous extraction of up to eight samples can be easily handled. Solvent consumption is reduced, taking into account the large number of compounds analysed with a single procedure.

The above-referred method was applied in a monitoring program of sand- and loam-type soils from Póvoa de Varzim, Portugal. The main contaminants that were identified in soils from this region comprise old persistent compounds, currently used herbicides and insecticides, and degradation products. In total, 19 compounds were identified. The most ubiquitous compounds were alachlor (38% of the samples), chlorpyrifos (30%), endosulfan (25%), endosulfan sulfate (25%), atrazine (23%) and pendimethalin (22%). Twenty-eight percent of the samples were not contaminated with any of the pesticides studied. Very high contamination levels, around 7 and 60 mg kg^{-1}, were found for pendimethalin and alachlor, respectively. Recent contamination predominantly affected surface layers of the soil whereas old persistent contamination was more dispersed in the soil profile. Since on intensive culture areas soil horizons are frequently disturbed, the understanding of the results may become difficult. These are partial conclusions and the data gathered along 2 years will allow a more clear understanding of pesticides dynamics in soil, as well as its prevalence and degradation. A different behaviour could be roughly observed in sandy and loam soils.

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