Talanta 114 (2013) 227–234

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Development of a one-step microwave-assisted extraction method for simultaneous determination of organophosphorus pesticides and fungicides in soils by gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 25 February 2013
Received in revised form
2 April 2013
Accepted 6 April 2013
Available online 3 May 2013

Keywords:
Microwave-assisted extraction
Pesticides
Fungicides
Soil
GC–MS

ABSTRACT

A one-step microwave-assisted extraction (MAE) procedure was developed for the simultaneous extraction of organophosphorus pesticide and fungicide residues in soil which have been greatly used in agriculture. Parameters that could influence the MAE efficiency such as irradiation power, temperature, time and solvent were investigated, and extraction efficiencies in the range of 92.6–103.7% were obtained using 400 W (100% output) at 160 °C for 10 min with only 12 mL of acetone–hexane (2:1, v/v). The analytes in extracts were analyzed directly by gas chromatography–mass spectrometry (GC–MS) without any further cleanup. At 5 and 50 ng g^{-1} fortification levels for each analyte, the average recoveries obtained were ranged from 70.0% to 120.0% with relative standard deviation (RSD) between 0.2% and 14%. The method was linear over 1–250 ng g^{-1} with a correlation coefficient (r^2) between 0.9916 and 0.9966. The detection limits (S/N=3) were between 0.10 and 0.12 ng g^{-1}. The applicability of the method was demonstrated by analyzing field soil samples collected from six intensive horticultural sites in Ethiopia.

1. Introduction

The use of pesticides in agricultural systems is possibly the most important factor which has contributed to the massive worldwide increase of food production [1–3]. However, slow degradation in the environment and extensive or inappropriate usage of pesticides by farmers can lead to environmental contamination and adverse effects to humans [3]. Pesticides in soils are continued to be studied more than any other environmental contaminants [4] and therefore, monitoring of their residues in soils were reported by many investigators [5–11].

Organophosphorus pesticides have been extensively used for agricultural purposes in the last five decades, providing well-characterized and cost-effective treatments to prevent, repel or mitigate the effects of pest on a wide range of crops [12]. They have replaced organochlorine compounds because of the persistence and accumulation of the latter in the environment [13]. These organic compounds are frequently found in soil and other environmental matrices, constituting an animal and human health hazard [12]. Fungicides of various chemical classes are also used in agriculture to control plant diseases [14–16]. These compounds can be applied pre- or post-harvest, and a certain proportion of the amount applied may reach the soil where it is transformed or distributed in the environment.

Sample preparation prior to the determination of organic pesticides in soil usually consists of many steps due to the complexity of the matrix [17,18]. Isolation of pesticides from soil samples usually involves conventional solid–liquid extraction methods such as soxhlet extraction [19]. However, these methods are time consuming, labor intensive, and demands large volumes of hazardous organic solvents with high cost of both purchase and disposal [20]. Thus, substantial efforts have been made to develop sample preparation techniques that could alleviate the drawbacks associated with the conventional methods. Accordingly, several new extraction techniques have been developed and applied to extract pesticides in soil matrix, such as supercritical fluid extraction (SFE) [21], matrix solid-phase dispersion (MSPD) [22], headspace solid-phase microextraction (HS-SPME) [3], ultrasonic-assisted extraction (UAE) [23], pressurized liquid extraction (PLE) [7–9,20], and microwave-assisted extraction (MAE) [24–35].

MAE was first introduced in 1986 by Ganzler et al. [24] and has been successfully applied to extract organic compounds from various solid and liquid matrices [25–27]. Several publications have described the use of MAE for reducing solvent usage and

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http://dx.doi.org/10.1016/j.talanta.2013.04.035

analysis time [28–31]. MAE has many advantages over other classical extraction techniques such as reduction of extraction time and solvent consumption as well as the possibility of running multiple samples [34,35]. To the best of our knowledge, however, MAE combined with gas chromatography–mass spectrometry (GC–MS) for the determination of amide and antibiotic fungicides in soil was not reported elsewhere. Therefore, it is of great importance to develop a low-cost one-step MAE procedure for the simultaneous MAE of organophosphorus pesticides and fungicides in soil as they are the two classes of pesticides which were widely used in the Ethiopian agriculture.

The aim of this work was to develop and validate a one-step MAE procedure for the simultaneous extraction of organophosphorus pesticides and fungicides in soil. Under the optimized conditions, the organophosphorus pesticides and fungicides were successfully extracted by MAE, and then determined by gas chromatography–mass spectrometry (GC–MS) directly without further sample cleanup procedure. The proposed extraction procedure is simple, time and cost-effective, which is very suitable for developing countries. The method was verified by analysis of these pesticides in soil samples from an intensive horticultural sites in Ethiopia.

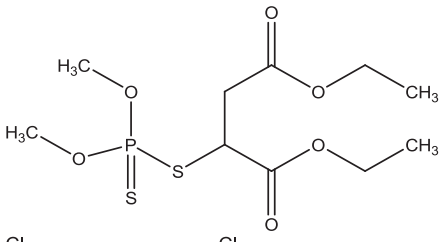
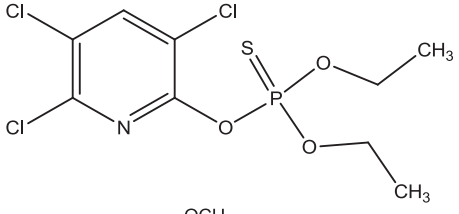
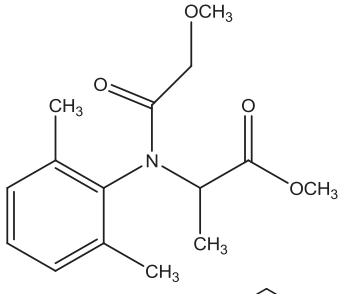
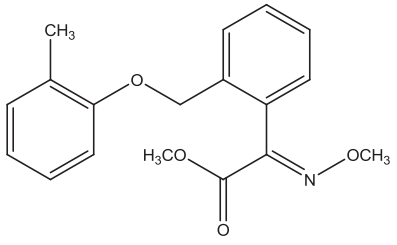
2. Experimental

2.1. Chemicals and reagents

All pesticide standards used were of 98.0–99% purity. Metalaxyl (99.0%), chlorpyrifos (98.0%), and kresoxim-methyl (98.5%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Malathion (98.5%) was obtained from Sigma-Aldrich (Buchs, Switzerland). The structures and physico-chemical properties of these pesticides are listed in Table 1. HPLC-grade acetonitrile, methanol, dichloromethane, acetone and n-hexane were purchased from Fisher Scientific (New Jersey, USA). The water used was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Individual pesticide standard stock solutions (100 μg mL^{-1}) were prepared by dissolving 0.0025 g of each pesticide in 25 mL of methanol and were stored at 4 °C. A working standard solution (10 μg mL^{-1}) was prepared by transferring 2.5 mL from the stock solution to a 25 mL volumetric flask and diluting with dichloromethane to a given volume. Mixed standard solutions containing each of pesticide (0.001–1.000 μg mL^{-1}) were prepared in dichloromethane for the linearity of detector response and the detection

Table 1
Physico-chemical properties of organophosphorus pesticides and fungicides under study.

Pesticide	Class	Structure	Vapor pressure (Pa, 20 °C)	Henry's law constant (Pa m^3 $mo1^{-1}$, 20 °C)	log Kow[b] (20°C)	log Koc[c]	Solubility in water (mg L^{-1}, 25 °C)
Malathion	OPP[a]		1.05×10^{-3}	2.3×10^{-3}	2.75	2.18	145
Chlorpyrifos	OPP[a]		1.43×10^{-3}	3.7×10^{-1}	4.70	3.78	2.0
Metalaxyl	Fungicide		2.93×10^{-4}	1.6×10^{-5}	1.71 (25 °C)	2.61	26,000
Kresoxim-methyl	Fungicide		2.3×10^{-6}	3.6×10^{-7}	3.40 (25 °C)	2.48	2.0 (20 °C)

[a] Organophosphorus pesticide.
[b] Octanol–water partition coefficients.
[c] Organic carbon–water partition coefficients.

limits (LODs) studies. Mixed standard solution (0.5 μg mL^{-1}) for fortification of soil samples was prepared by transferring 0.5 mL from working standard solution to a 10 mL volumetric flask and diluting with dichloromethane.

2.2. Soil samples

Six soil samples were collected in June 2012 from six intensive horticultural sites around the Taji river (T1, T2), Atsebela river (A1, A2) and Ziway lake (Z1, Z2) located in the southwest zone of Oromia region, Ethiopia (Fig. 1). A composite soil sample (10 cores) was taken from each field. Ten holes of 25 cm depth were made randomly using a spade and then slices of 5 cm thickness were taken along the vertical wall of the holes using a small folding spade [6]. All increment collected were pooled on a plastic sheet having an area of 3 m^2 and mixed thoroughly manually. To ensure further homogenization, the soil sample was divided in six places over the plastic sheet and then a small amount was taken from each portion to make a subsample of approximately 1 kg. The subsample of soil was kept in a polyethylene plastic bag after being wrapped in a methanol rinsed aluminum foil, and then transported to the laboratory in a chilled insulating box [6]. The soil samples were air dried, grounded with Q-250A3 high speed crusher (Qijian, China), sieved through a 0.25 mm pore sieve, kept in a polyethylene plastic bag after being wrapped in a methanol rinsed aluminum foil, and then immediately transported to Beijing, China where it was stored in a deep freezer at −14 °C until the time of analysis.

2.3. Preparation of blank soil samples

In order to remove possible traces of pesticides, 100 g of the soil sample was immersed in 200 mL of methanol, acetone, dichloromethane and n-hexane consecutively and shaked for at least 24 h [30]. The treated soil sample was spread out on a tray and air-dried in a fume hood to remove as much solvent as possible. The dried soil sample was stored in an amber glass bottle at room temperature. The blank soil sample prepared was analyzed before spiking and found no detectable levels of the target compounds.

2.4. Preparation of fresh spiked soil samples

A fresh spiked soil samples were prepared by weighing a blank soil sample into an aluminum cup and spiking with an appropriate volume of standard solution (0.5 μg mL^{-1}) containing each of organophosphorus pesticides and fungicides with a microsyringe, ensuring that the solution did not contact the aluminum cup [31]. The fresh spiked soil samples were immediately transferred to MAE vessels after being air dried waiting for solvent evaporation [5].

2.5. MAE procedure

CEM MARS5 microwave accelerated reaction system (CEM Corporation, Matthews, NC, USA) was used for the extraction procedure which allowed up to 40 extraction vessels to be irradiated simultaneously. A 1 g portion of the soil sample was accurately weighed into an aluminum cup and was transferred quantitatively to the extraction vessel followed by the addition of 12 mL of acetone–hexane (2:1, v/v). The extraction vessels were closed and the samples were equilibrated by shaking for 1 h before microwave extraction [5]. Extractions were performed in a temperature-controlled mode using 400 W (100% output) irradiation power at 160 °C for 10 min. The oven temperature program was set up as follows: ramp to 160 °C for 2 min, holding at 160 °C for 8 min. After extraction, the vessels were allowed to cool to room temperature for 15 min before they were opened [31]. The supernatant was filtered through a Buchner funnel packed with a GF/C grade glass microfiber filter obtained from Whatman (Maidstone, UK) to separate suspended solids and fine particulates. Finally, the Buchner funnel was thoroughly rinsed with 3 × 1 mL extraction solvent and then the combined extracts concentrated to dryness under a gentle stream of nitrogen over heating water at 40 °C. The residues were re-dissolved in 100 μL

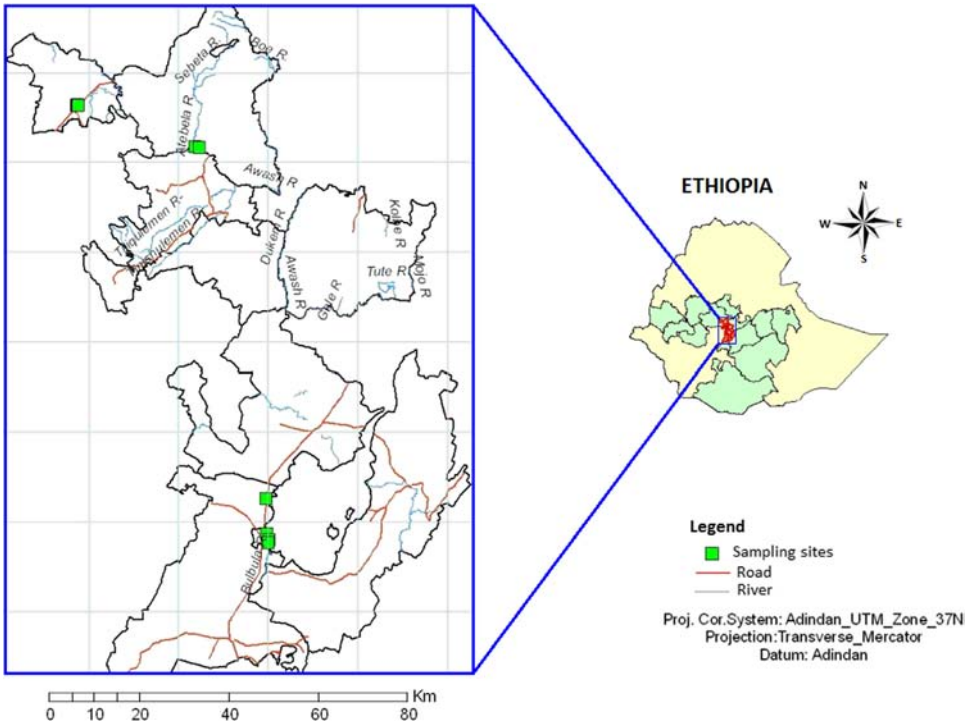


Fig. 1. Description of sampling sites in Ethiopia.

dichloromethane, and 5 μL of the solution was injected into the GC–MS for analysis without a need of further cleanup procedure [2,5,29,34].

2.6. GC–MS analysis

GC–MS analysis were carried out with an Agilent 7890A gas chromatograph (Palo Alto, CA, USA) interfaced to an Agilent 5975C mass selective detector (MSD). The GC–MS system was equipped with HP-5MS fused silica capillary column (30 m × 0.25 mm i. d. × 0.25 μm film thickness) and helium (99.999%) at a flow rate of 1.0 mL min^{-1} was used as a carrier gas. The oven temperature program was set up as follows: start at 80 °C, hold for 1 min; increase at 30 °C min^{-1} to 190 °C; increase at 25 °C min^{-1} to 230 °C; and finally increase at 10 °C min^{-1} to 250 °C, hold for 2 min. The injector port temperature was set at 220 °C. 5 μl of the final concentrated extracts was injected using a 7683B series auto sampler in splitless mode. The electron impact energy was set to 70 eV. The ion source, quadrupole, and transfer line temperatures were set to 300 °C, 200 °C, and 250 °C respectively. All organophosphorus pesticides and fungicides were separated within 10.27 min and the GC–(SIM)MS operating conditions were listed in Table 2. Quantification was accomplished with Agilent G1701EA MSD chemstation.

3. Results and discussion

3.1. MAE optimization conditions

Based on the previous experiences [35–37], the most important parameters that influence MAE efficiency are the type of extraction solvent and pressure or temperature (for closed vessel systems).

3.1.1. Effect of solvents employed for extraction

Preliminary studies were carried out by mixing acetone and hexane to get the necessary solvation characteristics and microwave heating [38]. Acetone–hexane mixture was the most studied solvent system for MAE and the time consumed in the evaporation step is much lower when compared to mixtures of hexane with other polar solvents such as acetonitrile, methanol, ethyl acetate, and tetrahydrofuran. The extraction efficiency was tested with freshly spiked soil samples and the highest extraction recovery (92.6–103.7%) were obtained with a acetone–hexane (2:1, v/v) for all the studied organophosphorus pesticides and fungicides in soil (Fig. 2). Acetone facilitates the extraction of polar analytes in mixed solvent systems and non-polar character of hexane reduces the extractability of interferences [38]. Thus, acetone–hexane (2:1, v/v) was selected for subsequent analysis.

The volume of a solvent is another important parameter that may influence the MAE efficiency [28,37]. The solvent volume must be sufficient to ensure that the entire sample is immersed and it is often in the range of 10–30 mL for a single sample amount between 1 and 5 g [37]. For this reason, MAE efficiency for different volumes of acetone–hexane (2:1, v/v) in the range of 6 to 15 mL (6, 9, 12, 15 mL) were evaluated. The results revealed that extraction recovery for all organophosphorus pesticides and fungicides studied increased with the increase of solvent volume from 6 to 12 mL and decreased with the increase of solvent volume from 12 to 15 mL (Fig. 3). Generally, in conventional extraction techniques a higher volume of solvent will increase the recovery, but in MAE a higher solvent volume may result in lower recoveries. This phenomenon has been shown by several groups [37]. Therefore, 12 mL of acetone–hexane (2:1, v/v) was chosen as an optimum volume for MAE of organophosphorus and fungicides in 1 g of soil sample.

Table 2
GC–(SIM)MS operating conditions for the analysis of organophosphorus pesticides and fungicides.

Pesticide	Retention time, t_R (min)	Segment start (min)	m/z		
			Target ion	Qualifier ion 1	Qualifier ion 2
Metalaxyl	7.405	5.00	206	132	160
Malathion	7.624	7.52	173	125	127
Chlorpyrifos	7.774	7.70	199	197	314
Kresoxim-methyl	8.980	8.70	116	131	206

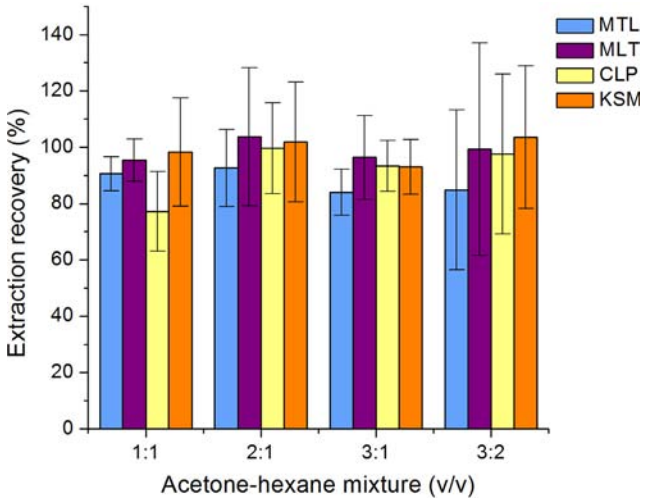


Fig. 2. Effect of type of extraction solvents on the MAE efficiency ($n=5$). Abbreviations: MTL, metalaxyl; MLT, malathion; CLP, chlorpyrifos; and KSM, kresoxim-methyl. Extraction conditions: soil sample, 1.0 g; spiked concentration, 50 ng g^{-1}; solvent volume, 12 mL; irradiation time, 10 min; heating temperature, 160 °C; microwave power, 400 W (100% output).

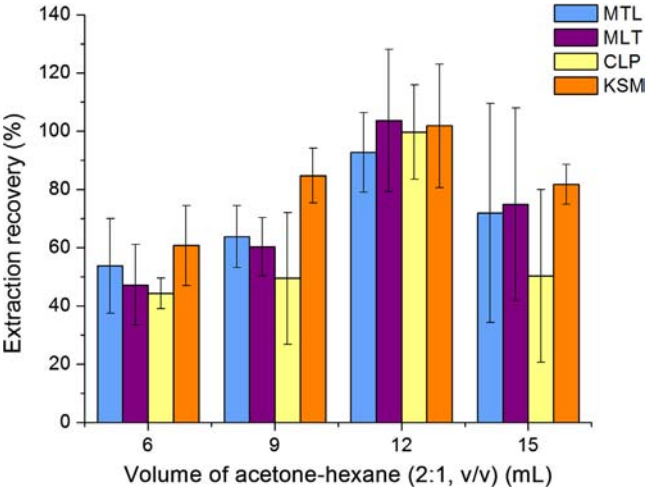


Fig. 3. Effect of extraction solvent volume on the MAE efficiency ($n=5$). Refer to Fig. 2 for abbreviations. Extraction conditions: soil sample, 1.0 g; spiked concentration, 50 ng g^{-1}; irradiation time, 10 min; heating temperature, 160 °C; microwave power, 400 W (100% output).

3.1.2. Effect of microwave parameters

Microwave power, irradiation time and heating temperature are the most important microwave parameters which control MAE

efficiency in closed vessels [36]. These parameters must be carefully selected in order to obtain the highest extraction recovery in the lowest period of time, but also avoiding overpressure effects which can cause leaks and analyte loss. The effect of irradiation power from 400 W to 1600 W (at 50% and 100% output) was systematically studied. For a microwave power equal to 400 W at 100% output, quantitative recovery were obtained for the organophosphorus pesticides and fungicides under study (Fig. 4). Moreover, the use of extremely high irradiation power of 1600 W (at 50% and 100% output) was preferably avoided which can cause leaks in the MAE vessels [35,36]. Thus, a low irradiation power of 400 W at 100% output was selected for further study.

The irradiation time between 10 and 35 min at interval of 5 min (10, 15, 20, 25, 30 and 35) was evaluated. The results indicated that extraction recovery was not significantly affected by irradiation time for the MAE of all organophosphorus pesticides and fungicides in soil (Fig. 5). Thus, a short irradiation time of 10 min was selected which is a typical extraction time for MAE [35].

Temperature is another crucial microwave parameter that influence the efficiency of MAE [9,37]. For this purpose, the effect of temperature on the MAE efficiency of each of the analytes in soil was evaluated from 80 °C to 180 °C. For all organophosphorus pesticides and fungicides, the extraction recovery increased with increasing temperature from 80 °C to 160 °C and dramatically decreased when temperature was increased from 160 °C up to 180 °C (Fig. 6). This result revealed that the highest recovery was obtained with high temperature at 160 °C. At high temperatures, the diffusivity of the solvent into the internal parts of the matrix is increased and desorption of the components from the active sites of the matrix is also enhanced [36,37]. However, the decrease of recovery for temperatures beyond 160 °C might be due to the evaporation losses from extraction vessels [25,28]. Therefore, microwave heating temperature of 160 °C was selected as optimum for further use.

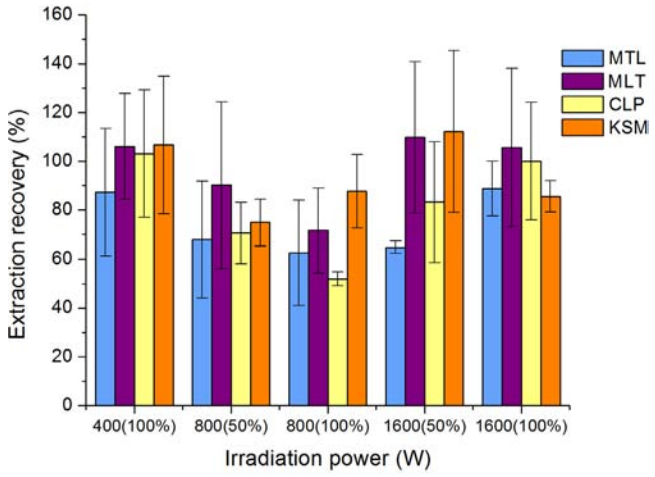


Fig. 4. Effect of irradiation power on the MAE efficiency ($n=5$). Refer to Fig. 2 for abbreviations. Extraction conditions: soil sample, 1.0 g; spiked concentration, 50 ng g^{-1}; solvent volume, 12 mL acetone–hexane (2:1, v/v); irradiation time, 10 min; heating temperature, 160 °C.

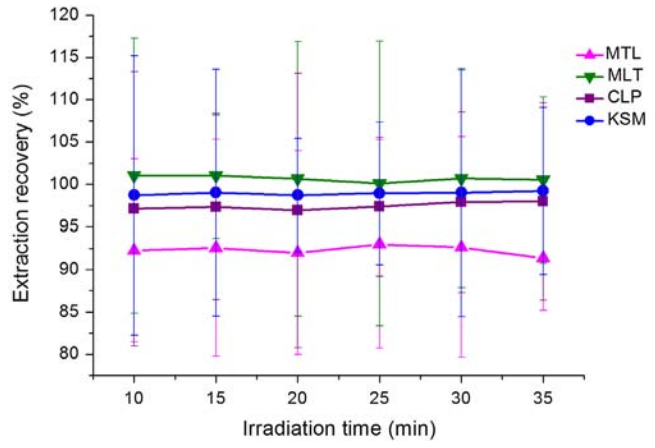


Fig. 5. Effect of irradiation time on the MAE efficiency ($n=5$). Refer to Fig. 2 for abbreviations. Extraction conditions: soil sample, 1.0 g; spiked concentration, 50 ng g^{-1}; extraction solvent volume, 12 mL acetone–hexane (2:1, v/v); heating temperature, 160 °C; microwave power, 400 W (100% output).

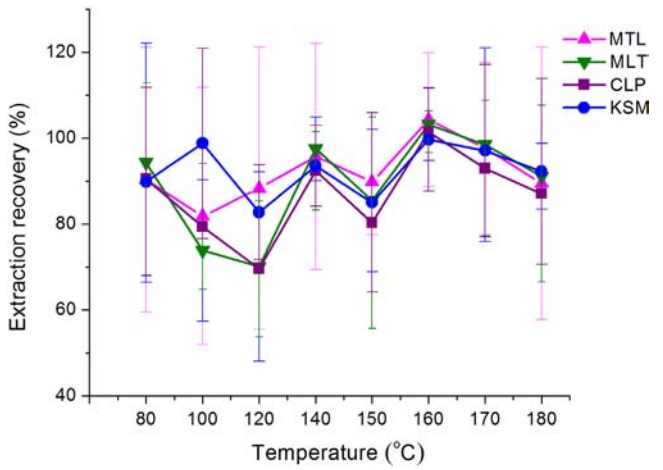


Fig. 6. Effect of temperature on the MAE efficiency ($n=5$). Refer to Fig. 2 for abbreviations. Extraction conditions: soil sample, 1.0 g; spiked concentration, 50 ng g^{-1}; solvent volume, 12 mL acetone–hexane (2:1, v/v); irradiation time, 10 min; microwave power, 400 W (100% output).

3.1.3. *Effect of sample weight and solvet volume proportion*

1 to 5 g of a soil sample were treated with equal volumes (12 mL) of acetone–hexane (2:1, v/v) for the same MAE conditions. In these experiments, the proportion between sample weight and solvent volume was modified from 1:12 to 5:12 (w/v). The results indicated that the extraction recovery decreased with an increase of sample weight from 1 to 5 g. Thus, 1 g of sample in 12 mL of the solvent provided the best extraction recovery (Fig. 7). This could be explained in terms of the bulk of the sample. For the same amount of extraction solvent and capacity of the extraction vessel, a smaller bulk of soil sample is more completely immersed in liquid and extracted [9,32]. Thus, 1 g of soil in proportion of 12 mL acetone–hexane (2:1, v/v) was selected for all organophosphorus and fungicides studied.

3.2. *Method performance validation*

The optimized MAE method as described above was extensively tested in terms of the critical validation parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision which were compiled and presented in Table 3.

3.2.1. *Detection and quantification limits*

In order to determine the sensitivity of the proposed MAE-GC–MS procedure and its appropriateness for environmental pollution monitoring studies, LOD and LOQ for each of the organophosphorus pesticides and fungicides were evaluated. LODs were determined by decreasing concentrations of analytes spiked in soil until obtaining a signal to noise ratio (S/N) of 3, and LOQs were derived from LODs to

give S/N of 10 [2]. The low LODs (0.10–0.12 ng g^{-1}) and LOQs (0.26 –0.37 ng g^{-1}) demonstrated the analytical capability of the proposed MAE technique for the studied organophosphorus pesticides and fungicides in soil samples.

3.2.2. Linearity

Linearity was conducted using soil samples spiked at different fortification levels in the range of 1.0–1000 ng g^{-1} (1.0, 2.5, 5.0, 10, 25, 50, 100, 250, 1000 ng g^{-1}). For each fortification level, five replicate measurements were carried out. The peak areas of each analyte were plotted against the concentrations, and least squares linear regression analysis was performed to determine the slope, *y*-intercept and the correlation coefficient (r^2) of the standard plots [15]. The results confirmed a good linearity between analytical signal and analyte concentration between 1 and 250 ng g^{-1} with r^2 in the range of 0.9916 to 0.9966 for all organophosphorus pesticides and fungicides studied (Table 3).

3.2.3. Precision

The precision of the technique was evaluated in terms of repeatability (within-day RSD) and reproducibility (between-day RSD) in three non-consecutive days. In each case, five replicate soil samples at 50 ng g^{-1} fortification level were analyzed under the same MAE conditions [2]. The repeatability was less than 12% and reproducibility (intermediate precision) was consistently below 9% for all organophosphorus pesticides and fungicides studied (Table 3). Therefore, the results obtained confirm that the precision was satisfactory according to RSD values of intra-day and inter-day variabilities.

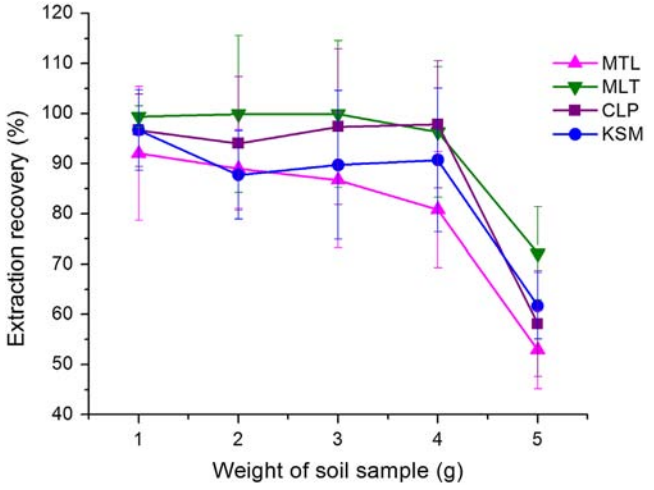


Fig. 7. Effect of sample weight on the MAE efficiency ($n=3$). Refer to Fig. 2 for abbreviations. Extraction conditions: spiked concentration, 50 ng g^{-1}; solvent volume, 12 mL acetone–hexane (2:1, v/v); heating temperature, 160 °C; MAE power, 400 W (100% output); and irradiation time, 10 min.

3.2.4. Selectivity

The selectivity of the method was evaluated by analyzing a blank soil sample to demonstrate absence of interference induced by organic compounds extracted from the soil matrix with analytes. Under these chromatographic conditions, no endogenous sources of interference were observed in soil, and the resolution of all organophosphorus pesticides and fungicides was satisfactory (Fig. 8).

3.3. Method application

In view of the quite satisfactory validation results obtained above, the MAE method developed was successfully applied for the determination of two organophosphorus pesticides and two fungicide residues in field soil samples collected from six intensive horticultural sites in Ethiopia (Fig. 1).

The organophosphorus pesticides and fungicides investigated were widely used in Ethiopia [39] and were detected in all field soil samples (Table 4). The highest mean concentration for metalaxyl (32.0 ng g^{-1}) and malathion (20.0 ng g^{-1}) was obtained in T1, while for chlorpyrifos (17.4 ng g^{-1}) and kresoxim-methyl (6.6 ng g^{-1}) in T2. From these findings, it is possible to deduce that these organophosphorus pesticides and fungicides were intensively used in Taji river area than Ziway lake and Atsebela river areas.

In general, the residual levels of all organophosphorus pesticides and fungicides determined were relatively low (1.9 –32.0 ng g^{-1}) [5,19]. These could be explained in terms of the relative persistence in the soil environment. Consequently, malathion is moderately persistent in soil, with reported field half-lives of 1 to 25 days [40], whereas chlorpyrifos from 60 to 120 days [41], metalaxyl from 7 to 170 days for [42], and kresoxim-methyl 1 to 34 days for [43]. Another possible explanation could be the storage of the samples at −14 °C for four months before time of analysis which may lead to loss of analytes due to high volatilization rates [40–43].

3.3.1. Recovery

Recovery studies were carried out with spiked soils obtained at two fortification levels (5 and 50 ng g^{-1}) [5]. The peak areas were compared with those obtained from blank spiked at the same levels to assess the matrix effects. The relative recovery obtained ranged from 70.2 ± 10 to 109.6 ± 1.1, and 70.0 ± 6.8 to 120.0 ± 6.0 for 5 ng g^{-1} and 50 ng g^{-1} fortification levels, respectively (Table 4). These results could further be used as a basis to draw conclusion that the matrices of the real soil samples do not have significant effects on the proposed MAE method for the extraction of analytes from field soil samples. Therefore, the developed MAE is suitable techniques for extraction of organophosphorus pesticides and fungicides in soil. Fig. 8 shows a typical GC–(SIM)MS total ion chromatogram obtained after MAE of all organophosphorus pesticides and fungicides studied in soil.

Table 3
Analytical performance of MAE-GC–MS for the determination of organophosphorus pesticides and fungicides.

Analyte	Linear range (ng g^{-1})	Regression equation	Correlation coefficient (r^2)	LOD (ng g^{-1})	LOQ (ng g^{-1})	Rept[a] (RSD%, $n=5$)	Repd[b] (RSD%, $n=5$)
Metalaxyl	1–100	$Y=9268x+11884$	0.9957[7][c]	0.10	0.29	9.1	0.3
Malathion	1–100	$Y=3378x-402$	0.9966[7][c]	0.10	0.26	2.5	7.0
Chlorpyrifos	1–250	$Y=5569x-10138$	0.9916[8][c]	0.12	0.37	9.4	2.0
Kresoxim-methyl	1–250	$Y=26625x-76605$	0.9936[8][c]	0.11	0.33	12	8.5

[a] Repeatability at 50 ng g^{-1} spiked level.
[b] Reproducibility at 50 ng g^{-1} spiked level.
[c] Numbers in parentheses indicate the number of concentration points from which the calibration curves were prepared.

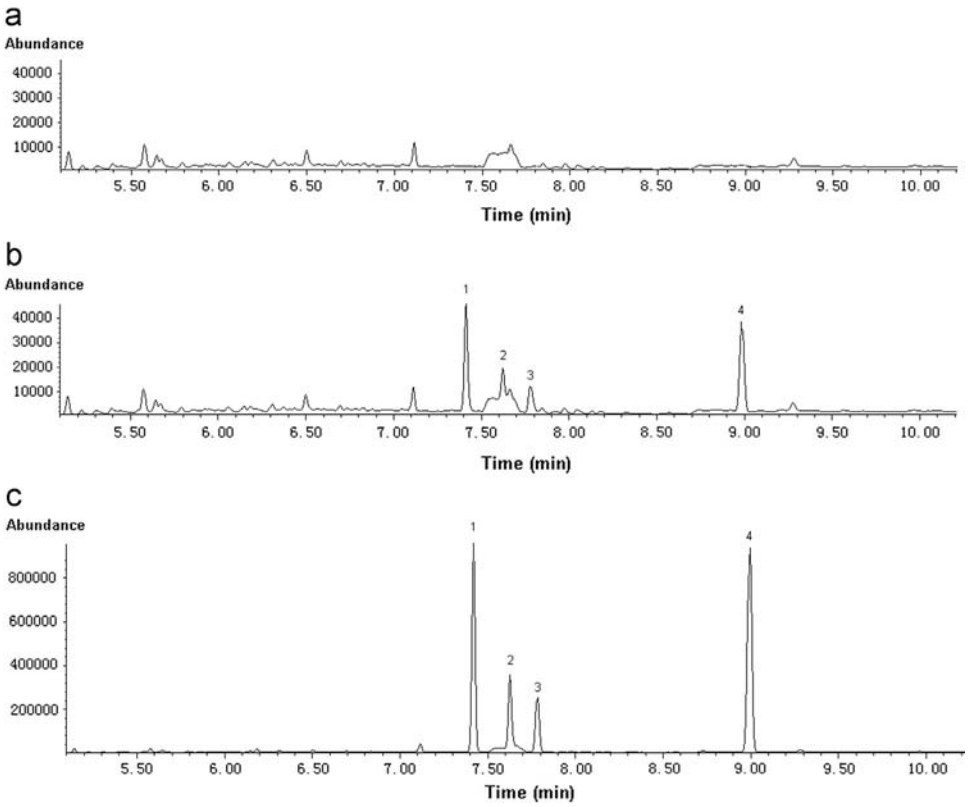


Fig. 8. GC–(SIM)MS total ion chromatogram obtained after MAE of organophosphorus pesticides and fungicides in soil sample extracts (a) blank, (b) spiked at 5 ng g^{-1}, and (c) spiked at 50 ng g^{-1} fortification levels. Peak identities: 1, metalaxyl; 2, malathion; 3, chlopyrifos and 4, kresoxim-methyl.

Table 4
Mean concentrations, relative recoveries (RR, %) and relative standard deviations (RSD, %) for each organophosphorus pesticide and fungicide in dry field soil samples ($n=5$).

Soil samples	Spiked (ng g^{-1})	Metalaxy			Malathion			Chlorpyrifos			Kresoxim-methyl		
		Detected (ng g^{-1})	RR (%)	RSD (%)	Detected (ng g^{-1})	RR (%)	RSD (%)	Detected (ng g^{-1})	RR (%)	RSD (%)	Detected (ng g^{-1})	RR (%)	RSD (%)
T1[a]	0	32.0	–	5.4	20.0	–	9.4	10.8	–	7.2	5.4	–	5.4
	5	4.6	91.2	9.1	4.4	88.7	0.2	4.5	90.4	8.0	3.7	74.2	9.7
	50	36.7	73.4	4.9	58.6	117.0	5.0	40.6	81.2	11	36.2	72.4	12
T2[a]	0	30.4	–	11	19.3	–	8.9	17.4	–	4.9	6.6	–	9.7
	5	4.2	83.7	9.1	4.4	87.6	11	3.9	78.6	11	3.5	70.2	10
	50	37.0	74.0	4.4	59.9	120.0	6.0	55.2	110.0	9.9	46.0	92.0	10
Z1[b]	0	28.0	–	3.7	13.3	–	8.8	7.3	–	12	2.2	–	12
	5	4.5	94.0	12	4.0	80.3	12	4.4	87.7	3.0	3.1	61.1	5.6
	50	39.8	79.5	12	46.1	92.2	12	38.3	76.6	5.7	35.0	70.0	6.8
Z2[b]	0	26.8	–	1.6	17.0	–	9.6	9.0	–	4.7	5.0	–	12
	5	4.8	96.1	7.5	4.2	84.9	8.4	3.5	70.8	6.4	3.65	71.3	9.7
	50	39.6	79.3	8.7	37.8	75.5	10	42.5	85.0	10	35.7	71.5	9.2
A1[c]	0	29.9	–	5.3	18.2	–	10	13.4	–	12	4.5	–	9.1
	5	4.6	91.3	6.5	3.5	70.5	5.3	3.9	77.7	8.0	4.3	86.7	8.1
	50	48.7	97.3	7.3	42.5	85.0	14	36.6	73.3	11	48.2	96.5	11
A2[c]	0	26.6	–	9.2	8.2	–	12	6.4	–	9.3	1.9	–	7.1
	5	5.5	109.6	1.1	4.1	81.3	11	3.8	76.1	12	4.3	85.5	9.0
	50	47.3	94.6	7.0	49.3	98.6	11	57.0	114.0	7.1	43.7	87.3	7.9

[a] Taji river area soil samples.
[b] Ziway lake area soil samples.
[c] Atsebela river area soil samples.

3.3.2. Figure of merits

MAE is a viable alternative to conventional techniques for the extraction of organophosphorus pesticides and fungicides in soil samples with comparable efficiencies and acceptable reproducibilities [24–35]. In this study, 1 g soil sample and 12 mL of acetone–hexane (2:1, v/v) were successfully used for the quantitative extraction at low microwave power 400 W (100% output) for a typical MAE irradiation time of 10 min at 160 °C.

Table 5
Comparison of the proposed MAE (closed vessel) for the extraction of organophosphorus pesticides and fungicides in soil with various MAE extraction conditions.

MAE closed vessel system	Analytes	Sample weight (g)	Extraction solvent		Microwave parameters			Recovery (%)	Ref.
			Type	Volume (ml)	Power (W)	Time (min)	Temp. (°C)		
MES-100	Pyrethroids, carbamates, OPPs[a], OCPs[b]	5	ACE-HEX (3:2, v/v)	35	60	20	120	98–102	[5]
CEM's MARS5	Triazines	10	MeOH-DCM (1:9, v/v)	25	950	20	115	76–87	[9]
CEM MES 1000	OCPs[b]	5	ACE-HEX (1:1, v/v)	30	475	10	115	74–169	[25]
CEM MES 1000	Triazines	10	MeOH-DCM (1:9, v/v)	20	950	20	100	36–107	[26]
CEM MES 1000	Sulfonylureas	10	MeOH-DCM (1:9, v/v)	20	450	10	60	78–102	[27]
Milestone 1200	Hexaconazole	5	ACE	30	1000	15	115	50–96	[29]
CEM's MES-1000	OPPs[a], OCPs[b]	5	ACE-HEX (1:1, v/v)	30	950	10	115	80–120	[31]
CEM's MES-1000	OCPs[b]	6	HEX (20% water)	30	950	20	115	82–101	[32]
CEM Mars Xpress	Herbicides	1	Aqua region and 48% HF (5:1, v/v)	12	800W	20	200	102–109	[33]
MSP 1000	Triazines, herbicides	10	MeCN	20	900	5	80	90–107	[34]
CEM's MARS5	OPPs[a], fungicides	1	ACE-HEX (2:1, v/v)	12	400	10	160	93–104	Current study

Abbreviations: ACE, acetone; HEX, hexane; MeOH, methanol; MeCN, acetonitrile; DCM, dichloromethane.
[a]Organophosphorus pesticides.
[b]Organochlorine pesticides.

In order to evaluate the achievements in this work, it was compared with the previous MAE procedures reported in terms of volume of extraction solvent, heating temperature, amount of soil samples, microwave power and irradiation time (Table 5). The proposed MAE method offers a great reduction in time (10 min) and solvent consumption (12 mL), as well as the opportunity to perform multiple extractions [36]. The use of only a filtration step without further cleanup is another merit of the proposed MAE technique [5]. The technique is easy to use and the systems are cheaper compared to other modern techniques such as SFE and PLE [37].

4. Conclusions

A one-step MAE that uses acetone–hexane (2:1, v/v) as extraction solvent followed by GC–MS has been developed for the simultaneous determination of organophosphorus pesticides and fungicides in soil. The results indicate that the developed MAE procedure is efficient and precise, in which only a small amount of organic solvent (12 mL) was used for each extraction, and the extraction time is quite short (10 min). The method can be generally used, providing very good analyte recovery, and yielding extracts that were mostly very clear, avoiding the need for further cleanup. It proved to be significantly faster, more economical, and produced less waste solvent, compared to conventional Soxhlet extraction or mechanical shaking.

Acknowledgment

This work was supported by the National Science Fund for Distinguished Young Scholars (21025729).

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