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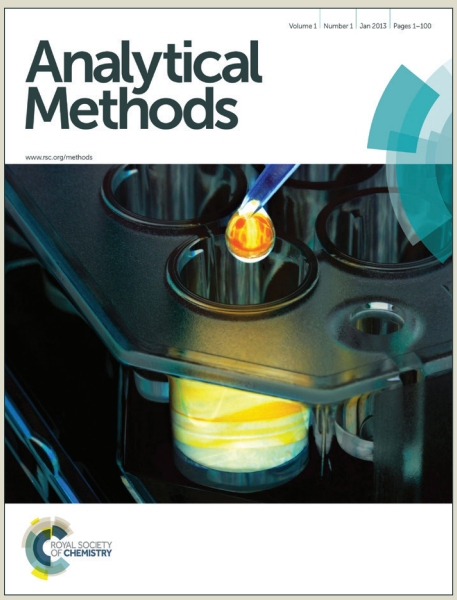

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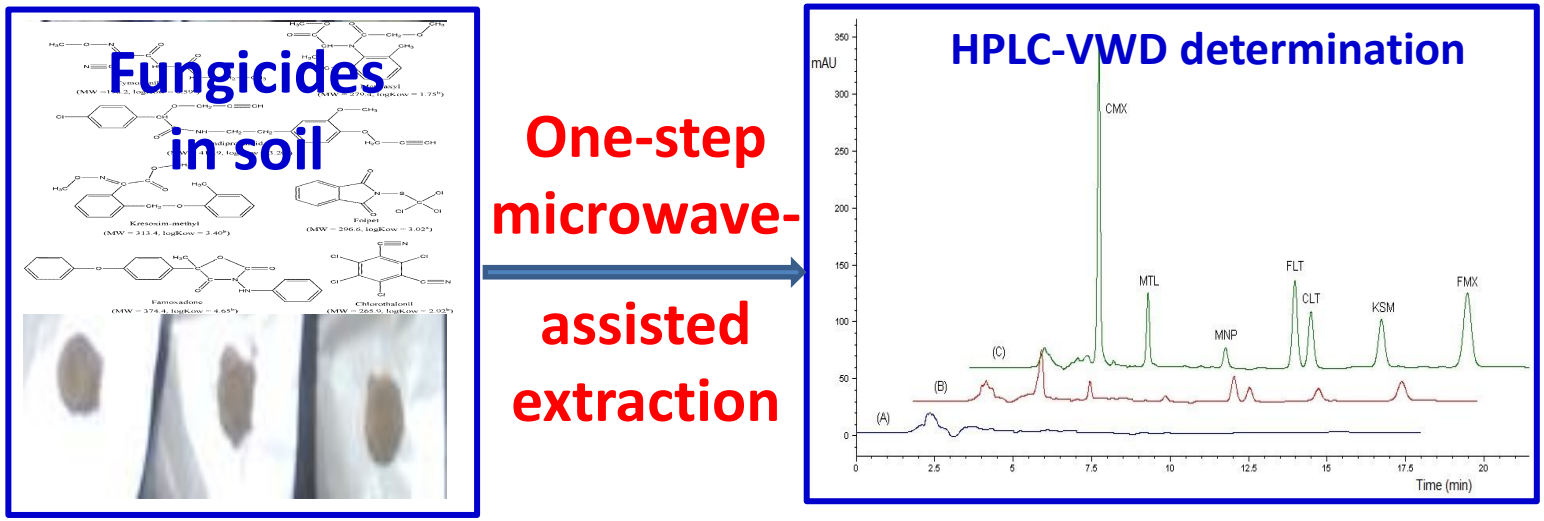

Development of a one-step microwave-assisted extraction procedure for highly efficient extraction of multiclass fungicides in soils

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Abstract

A one-step microwave-assisted extraction (MAE) procedure for highly efficient multiresidue extraction of seven fungicides (cymoxanil, metalaxyl, mandipropamid, folpet, chlorothalonil, kresoxim-methyl and famoxadone) in soil was developed. The trace residue levels in the soil were determined by high performance liquid chromatography (HPLC) with variable wavelength detection (VWD). Parameters affecting the MAE process such as the type and volume of the extraction solvent, irradiation power, temperature, irradiation time, moisture and salt addition were optimized. Under the optimal conditions, extraction efficiencies in the range of 72.4–99.4% were obtained for all the fungicides studied. The method was linear over the range of 0.01–10 μg g^{-1} with correlation coefficients (r^2) between 0.9989 and 0.9999. LODs (S/N = 3) and LOQs (S/N = 10) obtained varied from 0.0006 to 0.0015 μg g^{-1} and 0.002 to 0.005 μg g^{-1}, respectively. The proposed method has been successfully applied to the analysis of real soil samples and acceptable recoveries from 57.5 to 122% with RSDs ≤ 14% were obtained. The overall results have been compared with the Soxhlet, shake-flask and ultrasonic solvent extraction techniques. Thus, the method developed could efficiently be used for selective extraction and determination of the target analytes from the complex soil matrices.

1. Introduction

Fungicides belong to one of the classes of pesticides which are used to control plant diseases caused by various kinds of fungi and play a great role in increasing agricultural productivity.[1-5] They can be applied directly to the soil or sprayed over the crop fields.[2,4] However, the widespread use of pesticides has resulted in the presence of their residues in the environment posing potential risks to both animals and humans.[6]

Analytical methods available in the scientific literature for the selective isolation of fungicides in soil are scarce.[2] In the actual practice, however, the choice of the analytical technique used for the detection of pesticides is strongly dependent on polarity of the analyte. Nonpolar pesticides with high log K_{OW} are preferably analyzed by Gas chromatography (GC)[2,4,7] while polar pesticides are amenable by liquid chromatography (LC).[8,9]

In order to determine the pesticide residues at low concentrations, sample pretreatment methods which usually employ various extraction and clean-up procedures are always challenging and mandatory.[10] Traditionally, extraction of trace levels of pesticide residues from the complex soil matrices mainly employs Soxhlet and shake-flask methods.[2,10] However, these methods usually generate too much solvent wastes and are also labor intensive and time consuming.[10] Recently, a number of alternative methods such as solid phase micro-extraction (SPME),[11,12] supercritical fluid extraction (SFE),[13-15] pressurized liquid extraction (PLE),[16-18] ultrasonic solvent extraction (USE),[2,4,10] and microwave assisted extraction (MAE) [7,8,19-26] are commonly in use for extraction of pesticides in soil. MAE was introduced in 1986 by Ganzler et al.[27] and has been successfully applied to extract organic compounds from various solid and liquid matrices.[20,21] Compared to the traditional extraction techniques, MAE has several

advantages such as reduction of extraction time and solvent consumption as well as the possibility of running multiple samples.[22]

Despite the great number of MAE publications, the MAE for the extraction of cymoxanil, metalaxyl, mandipropamid, folpet, chlorothalonil, kresoxim-methyl and famoxadone in soils was not published elsewhere. These different classes of fungicides were in use in Ethiopia for decades. Therefore, the aim of this work was to develop a faster, efficient, easier, less expensive and sensitive method based on a one-step MAE for the quantitative and selective determination of cymoxanil, metalaxyl, mandipropamid, folpet, chlorothalonil, kresoxim-methyl and famoxadone from soil samples using HPLC-VWD detection. Experimental parameters influencing the MAE procedure were all optimized and its applicability was evaluated using real environmental soil samples collected from intensive horticultural sites in Ethiopia.

2. Experimental

2.1. Chemicals and Reagents

Cymoxanil, metalaxyl, mandipropamid, kresoxim-methyl, famoxadone, and folpet standards with purity > 98.0% were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) while chlorothalonil (purity, 99.7%) was supplied by AccuStandard, Inc. (New Haven, USA). Fig. 1 lists the chemical structure, common name, molecular weight and log K_{OW} of all the fungicides studied. HPLC grade solvents such as *n*-hexane, acetone, ethyl acetate, methanol and acetonitrile were obtained from Fisher Scientific (New Jersey, USA). Sodium chloride (GR grade) and anhydrous sodium sulfate (AR grade) were received from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ultrapure water was produced by a MilliQ water purification system (Millipore, Billerica, MA, USA).

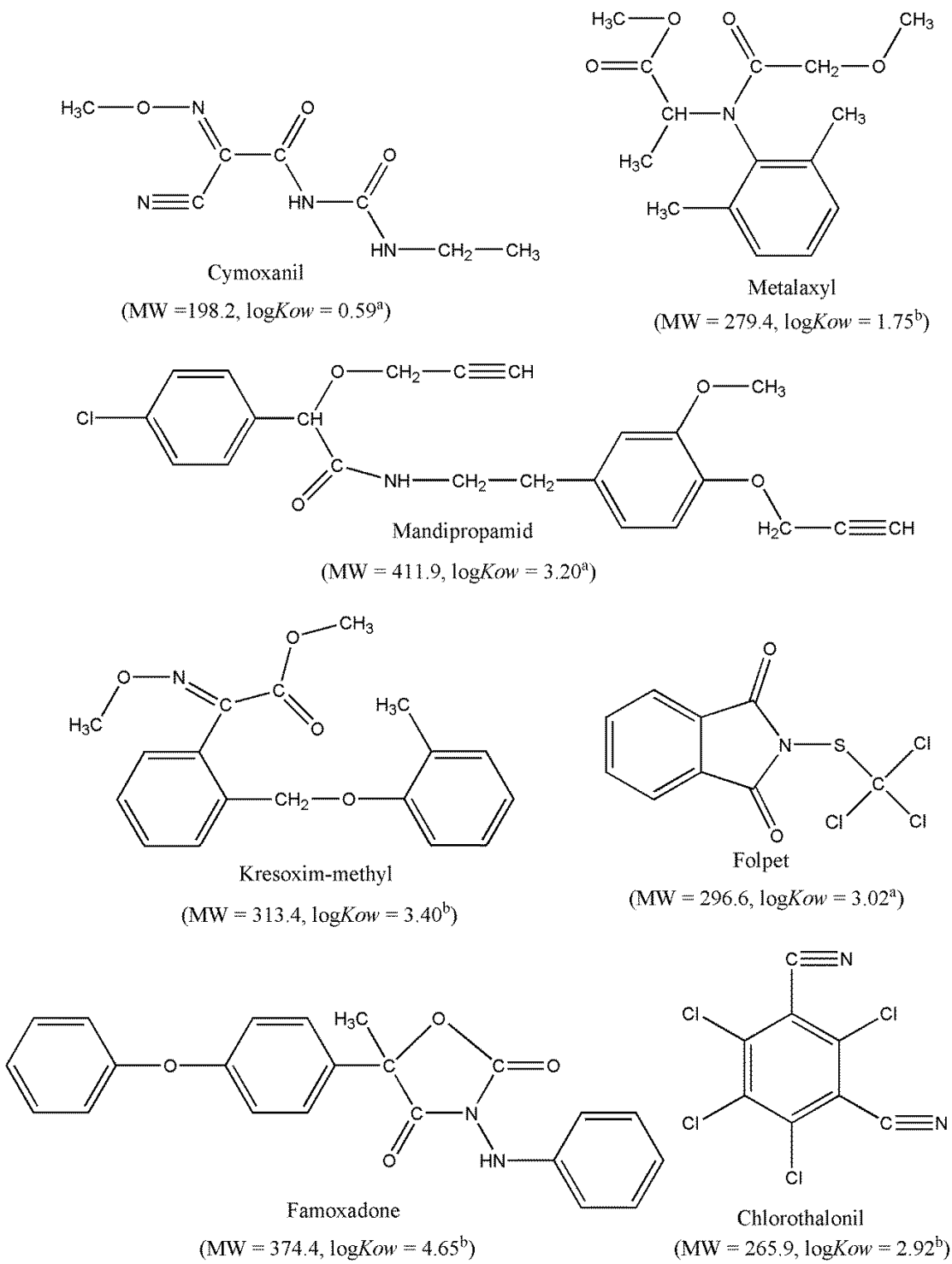

Fig. 1 Structure, common name, molecular weight (MW) and octanol-water partition coefficient (log K_{OW}) of the fungicides studied. [(a)]20 °C [(b)]25 °C

2.2. Instrumentation

An Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, vacuum degasser, auto sampler and variable wavelength detector was employed to perform chromatographic analysis. An Agilent TC-C_{18} column (250 mm x 4.6 mm i.d., particle size 5 µm) was used for separation of the analytes. Data acquisition and processing were achieved using Agilent LC ChemStation software (Rev. B.04.01) throughout the analysis.

2.3. Chromatographic conditions

A mixture of acetonitrile and water (60:40, v/v) was utilized as mobile phase and delivered at the flow rate of 1.0 mL min^{-1} in isocratic mode. The column temperature was maintained at 30 °C. The detection wavelength was programmed as follows: initially held at 232 nm for cymoxanil, 220 nm (5 min) for metalaxyl, 229 nm (7 min) for mandipropamid, folpet, and chlorothalonil, 225 nm (12 min) for kresoxim-methyl, and finally 229 nm (14 min) for famoxadone. The sample volume of 20 µL was injected and eluted for 18 min run time and 2.0 min post run time. For all the target analytes, the baseline separation was obtained under these chromatographic conditions and the peak area was used as an instrumental response. Quantification of the pesticides was performed by external calibration with pesticide mixed standard solutions, using 10 calibration points.

2.4. Preparation of the standard solutions

Stock standard solutions (100 mg L^{-1}) were prepared by transferring 2.50 mg of each of the fungicide standards in 25 mL volumetric flask and dissolving in methanol. The working standard (10 mg L^{-1}) and calibration standard solutions (0.01–5 mg L^{-1}) were prepared by mixing individual stock solution and appropriate dilution with methanol. All the standard solutions were stable and stored in a refrigerator at 4 °C when not in use.

2.5. Sample collection

Six intensive representative horticultural sites in Ethiopia[7] were selected and a real environmental soil samples[9] were collected and processed as described in the previously published work.[28]

2.6. Preparation of the spiked soil samples

All the soil samples collected[7] were tested and no fungicides under study was detected.[29] For recovery determination assays, the spiked soil samples at 0.5 μg g^{-1} spiked level[4] were prepared by adding 25 μL of 10 μg mL^{-1} mixed pesticides working standard solution to 0.5 g portion of soil weighed on the aluminum sheet using 100 μL micro syringe with a blunt needle (Shanghai Gaoge Industrial and Trading Co., Ltd, China). For blanks,[30] 25 μL of methanol was added in a similar way. The samples were allowed to stand to air dry at room temperature and thereafter were extracted by MAE.

2.7. MAE procedure

CEM MARS5 microwave accelerated reaction system (CEM Corp., Matthews, N.C., USA) was used in a temperature-controlled mode which allowed up to 40 extraction vessels to be irradiated simultaneously. To perform MAE procedure, 0.5 g portion of the soil sample was accurately weighed into an aluminum sheet and was transferred quantitatively to the extraction vessel followed by addition of NaCl (10%, w/w) and H_2O (10%, v/w). Subsequently, 5 mL ethyl acetate was added, as an extraction solvent, and the extraction vessels were closed. After the samples were agitated, by shaking manually for 1 min,[23] the extraction was performed using irradiation power of 1600 W (100% output) for 15 min. The oven temperature program was set up as follows: ramped to 90 °C within 2 min, and held at 90 °C for 13 min. After the extraction was completed, the vessels were allowed to cool to room temperature in 15 min before they were

opened.[31] The supernatant was filtered utilizing a Buchner funnel packed with a GF/C grade glass microfiber filter obtained from Whatman (Maidstone, UK) overlaid by 2.0 g of anhydrous sodium sulfate, which had been previously washed with 5 mL of the same solvent.[7] Then, the funnel was thoroughly rinsed with 3×1 mL extraction solvent and the clean extract obtained was evaporated to dryness using N-EVAPTM 112 Nitrogen Evaporator (Organomation Associates, Inc., Berlin, MA, USA) keeping the water bath at 50 °C. The residues were then re-dissolved in 200 µL methanol, and finally 20 µL of the resulting solution was injected into the HPLC-VWD system for analysis without a need for further clean-up procedure.[4,23] The pesticides recoveries (R, %) were calculated from the chromatographic signals.[10]

2.8. USE procedure

A soil sample (0.5 g) and 7.5 mL of ethyl acetate were placed in the 50 mL Erlenmeyer flask. After shaking the contents manually for 1 min, the soil samples were exposed to the USE (80 kHz, 100 W) in KQ-600DE single-frequency ultrasonic cleaner (Kunshan Ultrasonic Instruments Co., Ltd, China) for 10 min and performed in triplicate.[30] Initially, the instrument temperature was set at 30 °C and did not exceed 45 °C in any experiment.[32] After each extraction period, extracts were collected in a vial containing 1.0 g of 400 mesh copper powder and processed as described in section 2.7.

2.9. Shake-flask extraction

To a 50 mL Erlenmeyer flask, 0.5 g soil sample was transferred and 20 mL of ethyl acetate was added.[10] The content of the flask was then shaken mechanically for 5 h using KS 501 digital shaker (IKA®-Werke GmbH & Co. KG, Germany) at room temperature (25 °C).The extracts were collected, filtered and evaporated to dryness, following similar procedure in section 2.7.

2.10. Soxhlet extraction

To the extraction thimble, 0.5 g soil sample was transferred and extracted with 150 mL ethyl acetate for 5 h on an oil bath at 110 °C.[10] The resulting extract was filtered and concentrated to ~5 mL using IKA®RV10 rotary evaporator (IKA®-Werke GmbH & Co. KG, Germany) at 50 °C under a pressure of 250 mbar at 100 rpm and finally processed as described in section 2.7.

3. Results and discussion

3.1. Optimization of MAE procedure

The purpose of this experiment was to establish the optimal MAE conditions using minimum sample and solvent amounts in a short time of analysis.[33] For the closed vessel extraction systems, the major parameters affecting the pesticide extraction efficiency by MAE are temperature, irradiation time, irradiation power, nature and solvent volume.[22,26,34] Experiments were performed in five replicates (n = 5) and the extraction efficiencies were evaluated from recoveries (R, %).[32,35] However, the optimization results obtained couldn't be compared since there is no literature reports available for the same kind of fungicides analyzed using MAE in soil .

3.2. Effect of the extraction solvents

MAE is generally performed with the same solvents used in the traditional extraction.[22] However, the optimal extraction solvents for MAE cannot always be deduced directly from those used in conventional procedures.[26] Hence, MAE efficiency of acetone and ethyl acetate was tested and the results are shown in Fig. 2(A). Compared to ethyl acetate, acetone resulted in the lowest recoveries (< 33%) for cymoxanil and folpet. Furthermore, in order to get the necessary solvation characteristics and microwave heating, the ethyl acetate and acetone were mixed with n-hexane in 1:1, v/v ratio. However, the recovery of folpet drastically decreased (< 13%) in both

ethyl acetate-hexane (1:1, v/v) and acetone-hexane (1:1, v/v). Therefore, for all the analytes tested, ethyl acetate exhibited the highest recoveries (> 57%) and it was selected for subsequent analysis.

The volume of extraction solvent is also another parameter that influences MAE efficiencies[36,37] and it is often in the range of 10-30 mL for a single sample amount between 1 and 5 g.[36] In this work, different volumes of ethyl acetate, in the range of 2.5 to 10 mL (2.5, 5, 7.5, 10), with the solvent-matrix ratio (v/w) of 5:1, 10:1, 15:1 and 20:1, respectively, were evaluated. The results displayed in Fig. 2(B) revealed that the extraction efficiencies of cymoxanil, metalaxyl and chlorothalonil were optimal when 5 mL ethyl acetate was used and significantly decreased when the volume was either increased or decreased. In MAE, a higher solvent volume may result in lower recoveries.[36] However, changing the volume of ethyl acetate has not appreciably influenced the extraction efficiencies of mandipropamid, folpet, kresoxim-methyl and famoxadone. Therefore, 5 mL of ethyl acetate was selected for further studies.

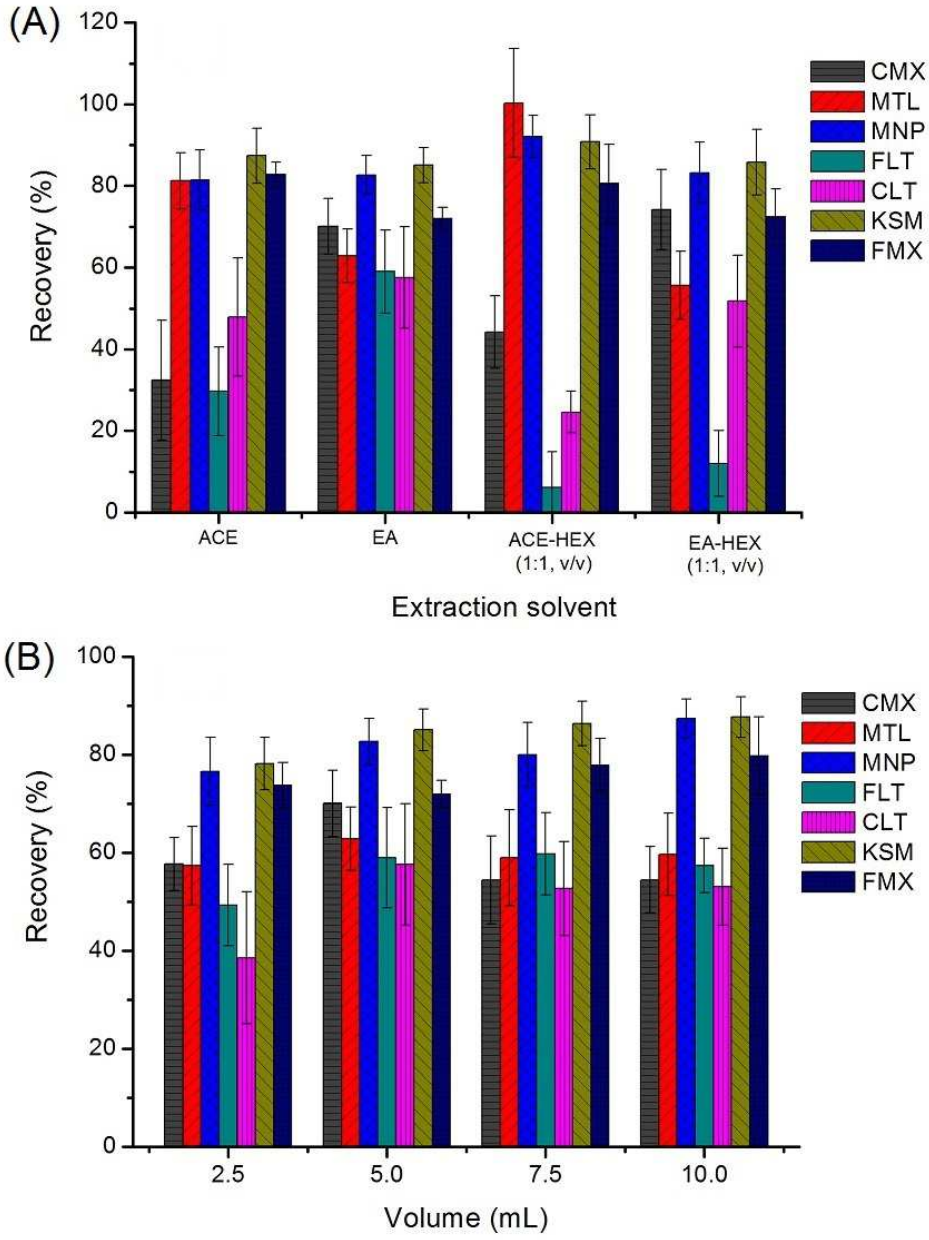

Fig. 2 Effect of (A) nature and (B) volume of extraction solvent on the MAE efficiency (n = 5). Error bar: relative standard deviation, RSD, %. Abbreviations: ACE, acetone; EA, ethyl acetate; HEX, hexane; CMX, cymoxanil; MTL, metalaxyl; MNP, mandipropamid; FLT, folpet; CLT, chlorothalonil; KSM, kresoxim-methyl; FMX, famoxadone. Extraction conditions: soil amount, 0.5 g; spiked concentration level, 0.5 μg g^{-1}; irradiation power, 800 W (100% output); irradiation time, 15 min; and temperature, 90 °C.

3.3. Effect of the microwave parameters

Irradiation power is the most crucial microwave parameter which influences the MAE efficiency in closed extraction vessels[26] and hence it needs to be carefully optimized. To achieve this objective, 0.5 g soil sample was extracted using different microwave power settings between 400 W and 1600 W (100 % output) at 90 °C for 15 min and the observed results are presented in Fig. 3(A). For 400-800 W setting, sample was irradiated at 400 W (100 %) for 8 min and then at 800 W (100 %) for 7 min at 90 °C. Similarly, for 400-800-1600 W setting, sample was irradiated sequentially at 400 W (100 %), 800 W (100 %) and 1600 W (100 %) for 5 min each at 90 °C. A quantitative recoveries (> 60 %) were obtained for all fungicides by using an irradiation power of 1600 W (100% output) and it was selected as optimal irradiation power.[22,26]

Optimization of temperature is also important as it may influence the MAE process.[36,38] In this study, the influence of temperature was studied from 70 to 110 °C at intervals of 20 °C (70, 90, and 110) and the results are displayed in Fig. 3(B). Except for mandipropamid, all the fungicides studied exhibited significant increase in the recoveries when the temperature increased from 70 to 90 °C. This could be due to increase of the diffusivity of the solvent into the internal parts of the matrix which may also increase desorption of the components from the active sites of the matrix.[26,36] However, increasing temperatures beyond 90 °C resulted in the decrease of recoveries which might be due to the evaporation losses from extraction vessels.[21,37] Therefore, an optimal temperature of 90 °C was chosen the successive studies.

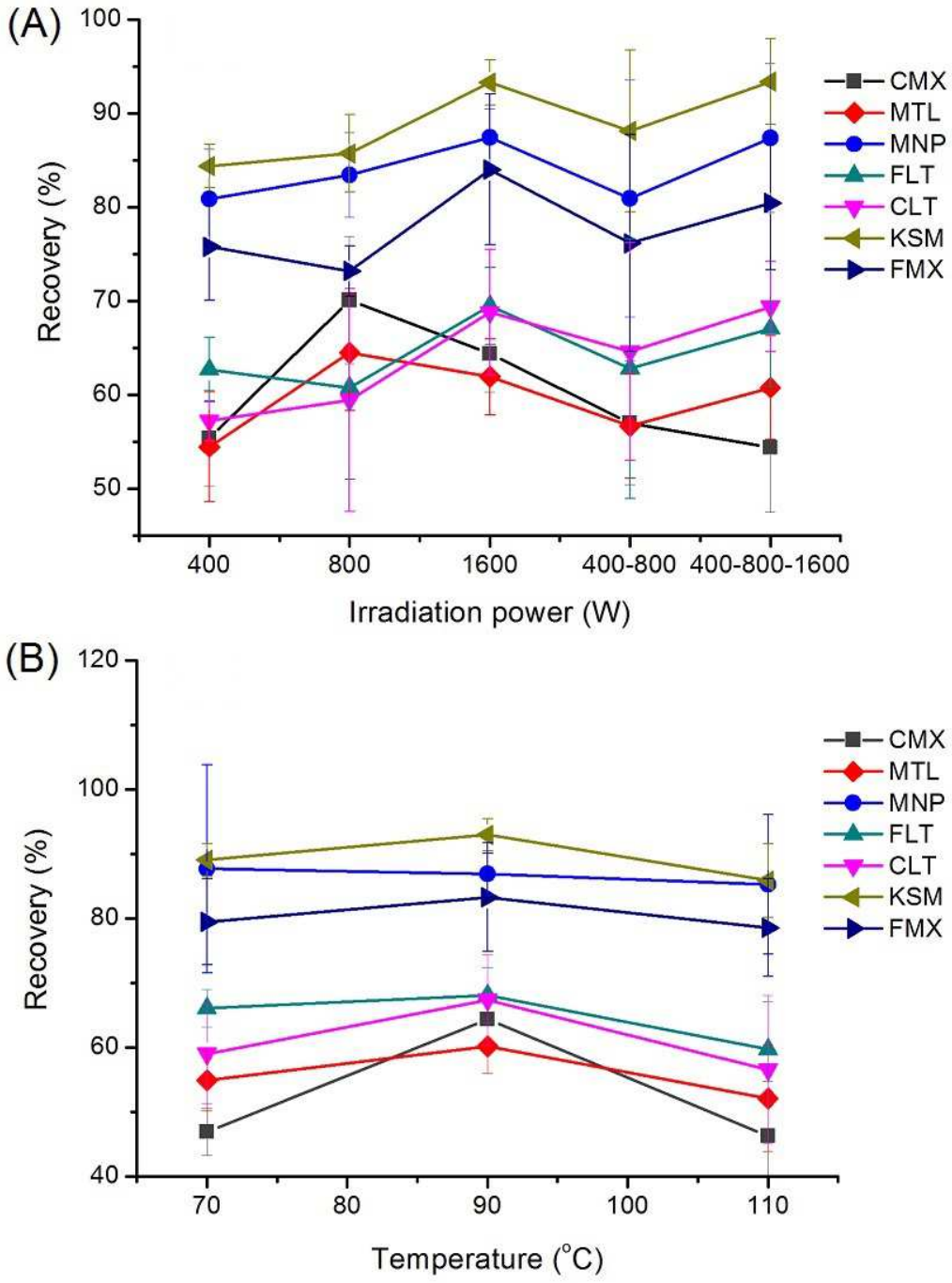

Fig. 3 Effect of (A) irradiation power and (B) temperature on the MAE efficiency (n = 5). For error bars and abbreviations refer to Fig. 2. Extraction conditions: soil amount, 0.5 g; spiked concentration level, 0.5 μg g^{-1}; and solvent volume, 5 mL ethyl acetate.

The influence of time on MAE process needs to be taken into account in a similar manner to the other extraction techniques.[26] Thus, the influence of irradiation time was evaluated by varying the time between 5 and 25 min at an interval of 5 min (5, 10, 15, 20, and 25) and the results obtained are indicated in Fig. 4. For most of the fungicides, increasing irradiation time from 5 to 15 min resulted in the increase of recoveries and further increase beyond 15 min showed decrease in recoveries. The experimental results confirmed that the irradiation time significantly influenced the recovery of the target analytes in soil even though it has been reported that irradiation time is not a significant factor for the MAE of organic compound in environmental matrices.[7,22] Thus, 15 min was used as optimal irradiation time for MAE of all fungicides in soil.

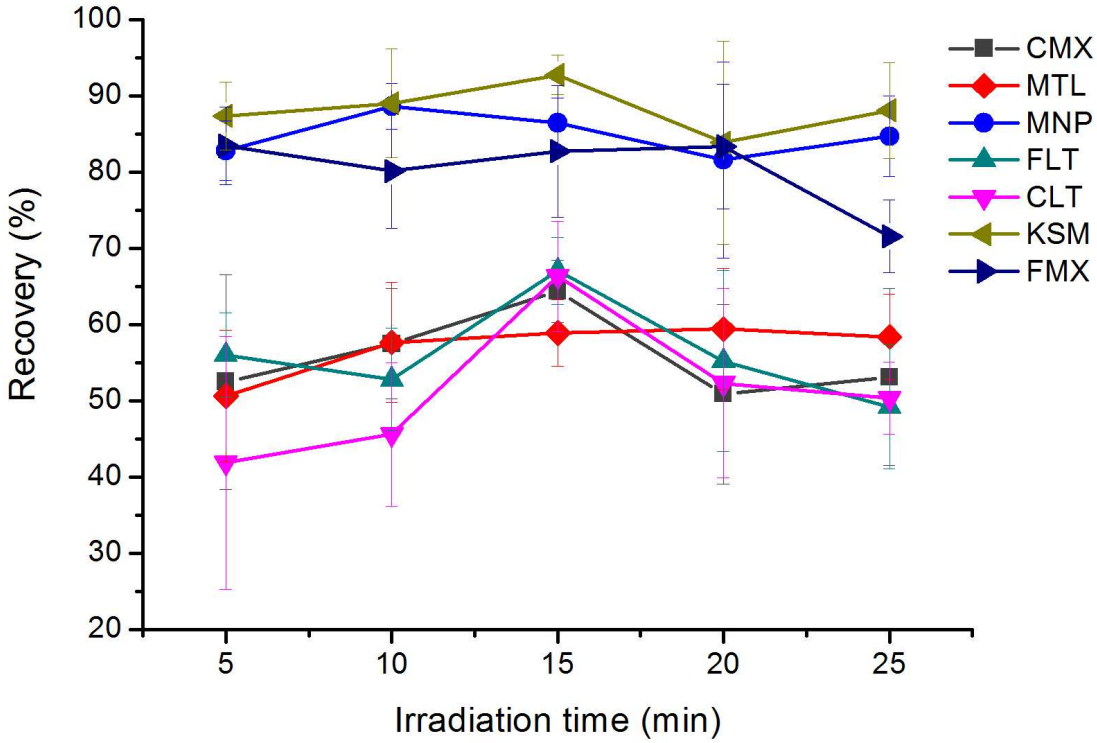

Fig. 4 Effect of irradiation time on the MAE efficiency (n = 5). For error bars and abbreviations refer to Fig. 2. Extraction conditions: soil amount, 0.5 g; spiked concentration level, 0.5 μg g^{-1}; solvent volume, 5 mL ethyl acetate; irradiation power, 800 W (100% output); and temperature, 90 °C.

3.4. Effect of moisture and salt addition

The moisture of the matrix may also influence the MAE efficiency and hence it should be taken into account. For this purpose, soil moisture level between 5 and 20% H_2O (v/w) at an interval of 5% H_2O (5, 10, 15 and 20) was used in order to investigate its effect on the extractibility of the analytes in soil under optimal MAE conditions. In order to do this, an approriate volume of water was added to 0.5 g soil transfred to an extraction vessel. The results in Fig. 5 (A) clearly indicated that 10% moisture level showed enhanced the recoveries (> 74%) of all the studied fungicides except folpet and chlorothalonil (< 63%) which exhibited a slight decrease. In most of the cases, the matrix moisture improved the extraction recovery.[22] Therefore, 10% moisture level in the matrix showed recoveries (> 57%) for all fungicides studied and was selected for optimal MAE efficiency.

In the final MAE optimization procedure, the influence of salt was studied between 5 and 20% NaCl (5, 10, 15 and 20, w/w) was studied keeping the optimal 10% (v/w) moisture level in the matrix. In order to achieve this objective, 0.5 g soil sample was transfered to extraction vessel and an appropriate amount of NaCl was added and the moisture level adjusted to 10 % (v/w) by adding water. As can be seen from Fig. 5(B), addition of a salt generally influenced extractability of the analytes, and the use of 10% NaCl (w/w) at the presence of 10% (v/w) moisture level in the matrix resulted in the highest recoveries (> 72%) and it was selected as the optimal MAE condition.

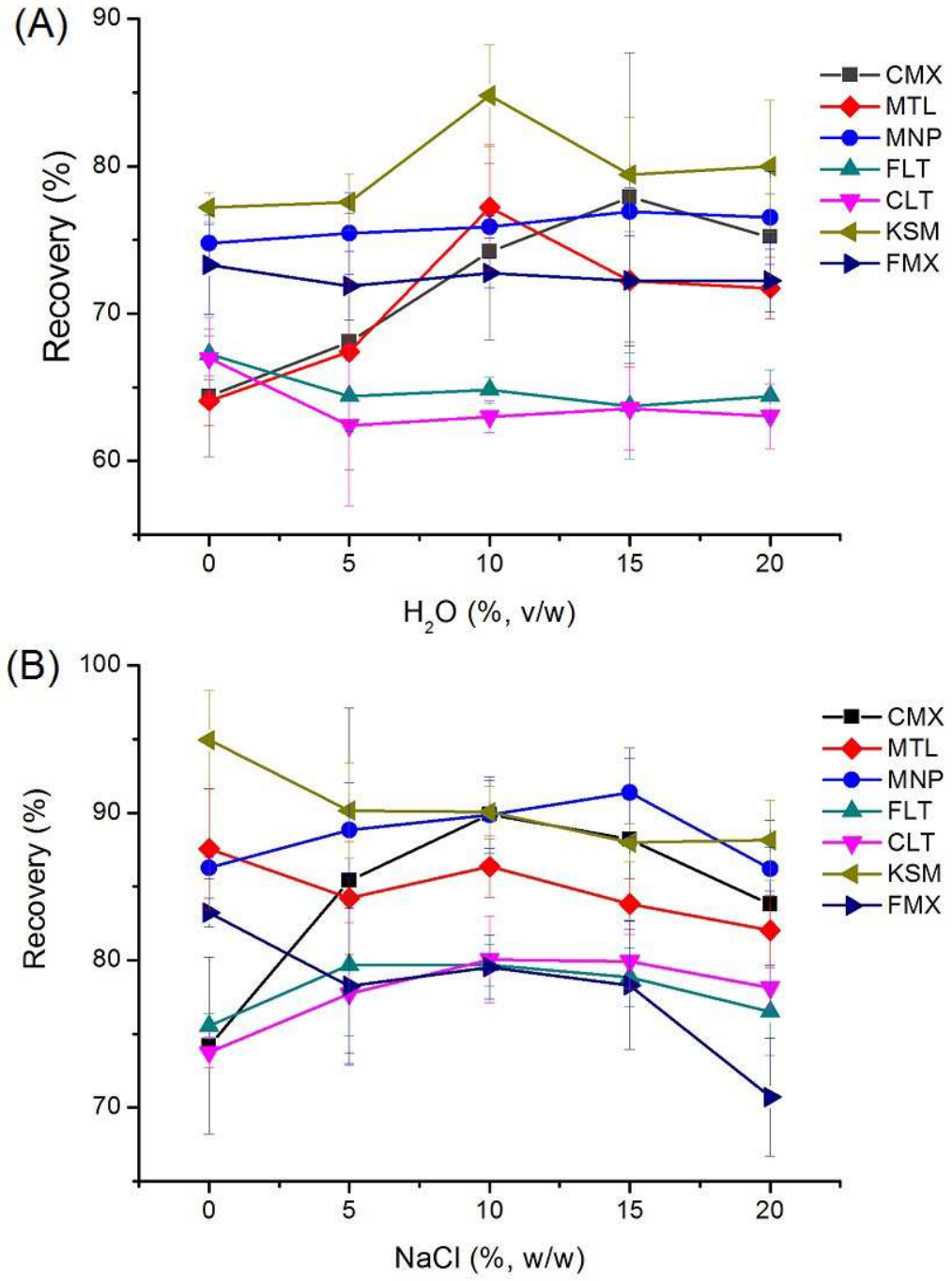

Fig. 5 Effect of (A) water content and (B) salt addition on the MAE efficiency (n = 5). For error bars and abbreviations refer to Fig. 2. Extraction conditions: soil amount, 0.5 g; spiked concentration level, 0.5 μg g^{-1}; solvent volume, 5 mL ethyl acetate; irradiation power, 800 W (100% output); irradiation time, 15 min; and temperature, 90 °C.

3.5 Validation of the proposed MAE method

In order to evaluate the practical applicability of the proposed method, the critical validation parameters such as linearity, limit of detections (LODs), limit of quantifications (LOQs), repeatability and reproducibility were studied and the results are summarized in Table 1.

Table 1 Analytical performances of the proposed MAE method for soil samples

Fungicide	Linearity ($\mu g\ g^{-1}$)	Regression equation	Correlation coefficient (r^2)	LOD ($\mu g\ g^{-1}$)	LOQ ($\mu g\ g^{-1}$)	Rept.[a] (RSD, %)	Repd.[b] (RSD, %)
Cymoxanil	0.01-10	y = 790.7x - 24.58	0.9997[10][c]	0.0006	0.002	3.5	10
Metalaxyl	0.01-10	y = 222.2x + 2.26	0.9999[10][c]	0.0015	0.005	3.7	12
Mandipropamid	0.01-10	y = 91.2x + 5.31	0.9992[10][c]	0.0015	0.005	2.3	5.4
Folpet	0.01-10	y = 389.2x + 18.07	0.9996[10][c]	0.0006	0.002	2.4	9.8
Chlorothalonil	0.01-10	y = 218.1x - 2.88	0.9993[10][c]	0.0006	0.002	3.8	6.0
Kresoxim-methyl	0.01-10	y = 302.3x + 4.89	0.9998[10][c]	0.0015	0.005	3.0	5.9
Famoxadone	0.01-10	y = 546.3x - 7.35	0.9989[10][c]	0.0015	0.005	5.7	7.8

[a]Repeatability and [b]reproducibility (spiked level, 0.5 $\mu g\ g^{-1}$; n = 5).

[c]Numbers in parentheses indicate the number of calibration points from which the calibration curves were prepared.

Linearity study was conducted using the soil samples spiked at ten concentration levels in the range of 0.01−10 $\mu g\ g^{-1}$ and five replicates measurements were carried out for each fortification level. 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.[1,7] The results confirmed a good linear relationship between analytical signal and their corresponding concentration between 0.01 and 10 $\mu g\ g^{-1}$ with correlation coefficients (r^2) in the range of 0.998 to 0.999 for all the fungicides studied.

LODs were determined by decreasing spiked concentrations the of analytes in the soil until the signal-to-noise ratio (S/N) of 3, and LOQs were derived from LODs to give S/N of 10.[4] The low LODs and LOQs obtained in the range of 0.0006 to 0.0015 μg g^{-1} and 0.002 to 0.005 μg g^{-1}, respectively demonstrated the analytical capability of the proposed MAE technique with increased sensitivity.

The precision of the technique was evaluated in terms of repeatability (within-day RSD, %) and reproducibility (between-day RSD, %) in three non-consecutive days.[7] In each case, five replicates soil samples at 0.5 μg g^{-1} fortification level were analyzed under the optimal MAE conditions.[4] The repeatability was observed to vary from 2.3 to 5.7% and reproducibility from 5.4 to 12% for all the fungicides studied. Therefore, the results obtained confirmed that the precision was acceptable based on the RSD, % values of the repeatability and reproducibility.

The selectivity of the method was evaluated by analyzing a blank soil sample to demonstrate the absence of possible interferences introduced from the organic compounds extracted from the soil matrix with analytes.[35] Under these chromatographic conditions, no endogenous sources of interference were observed in the soil, and resolution of all the fungicides was satisfactory (Fig. 6).

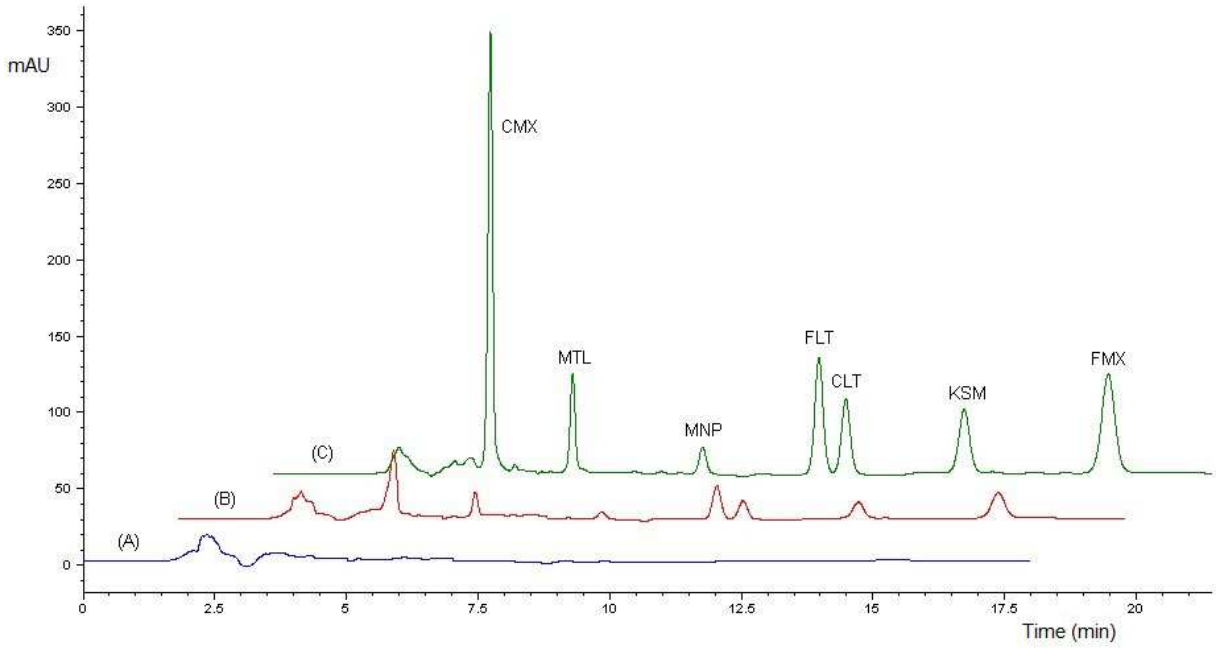

Fig. 6 HPLC-VWD chromatograms obtained from a blank (A) and spiked soil samples at 0.5 μg g^{-1} (B) and 2.0 μg g^{-1} (C) after MAE under optimum conditions. For abbreviations refer to Fig. 2.

3.6. Application of the proposed method to real soil samples

In view of the quite satisfactory validation results described above, the practical applicability of the proposed MAE-HPLC-VWD method was tested using field soil samples collected from six intensive horticultural sites in Ethiopia.[7] None of the analytes was detected in all soil samples. For recovery studies, these soil samples were spiked at 0.5 and 2.0 μg g^{-1} concentration levels,[23] and the recoveries in the range of 60.0 ± 1.0 to 122.0 ± 14.2 and 57.5 ± 0.7 to 111.0 ± 13.6 respectively were obtained (Table 2). 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 method. Therefore, the developed MAE technique is suitable for extraction of multiclass fungicides in soil. Fig. 6 shows a typical MAE-HPLC-VWD chromatogram obtained after MAE of all fungicides studied in soil.

Table 2 Pesticides recoveries (R, %) and relative standard deviation (RSD, %) values for soil samples spiked at different levels under optimum MAE conditions (n = 5)

Sample	Spiked (μg g^{-1})	Cymoxanil			Metalaxyl			Mandipropamid			Folpet			Chlorothalonil			Kresoxim-methyl			Famoxadone		
		Detected (μg g^{-1})	R (%)	RSD (%)	Detected (μg g^{-1})	R (%)	RSD (%)	Detected (μg g^{-1})	R (%)	RSD (%)	Detected (μg g^{-1})	R (%)	RSD (%)	Detected (μg g^{-1})	R (%)	RSD (%)	Detected (μg g^{-1})	R (%)	RSD (%)	Detected (μg g^{-1})	R (%)	RSD (%)
T1[b]	0	nd[a]	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-
	0.5	0.5	102	8.2	0.5	96.0	14	0.6	120	8.4	0.4	88.0	13	0.4	74.0	5.5	0.6	122	10	0.5	102	14
	2	1.5	74.0	6.3	1.6	80.5	12	1.9	93.5	3.6	1.5	77.0	10	1.6	78.5	7.4	1.9	94.5	8.9	1.6	79.0	7.2
T2[b]	0	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-
	0.5	0.4	90.0	2.3	0.4	90.0	5.4	0.5	98.0	6.7	0.4	72.0	3.6	0.4	74.0	7.6	0.5	100	4.6	0.4	72.0	5.6
	2	1.6	80.0	1.8	1.8	90.0	1.1	2.0	97.5	2.4	1.6	81.0	2.9	1.7	85.5	2.1	2.1	104	3.9	1.6	79.5	7.0
A1[c]	0	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-
	0.5	0.4	84.0	5.1	0.4	84.0	9.3	0.5	96.0	13	0.3	64.0	6.1	0.3	62.0	8.4	0.5	106	6.9	0.3	62.0	3.0
	2	1.6	81.0	1.7	1.8	91.0	2.3	2.0	98.5	3.2	1.3	63.0	12	1.8	87.5	2.8	2.2	110	1.8	1.3	65.0	3.8
A2[c]	0	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-
	0.5	0.4	82.0	3.6	0.5	92.0	6.3	0.5	106	2.5	0.3	68.0	9.0	0.3	64.0	5.2	0. 6	118	4.9	0.3	66.0	5.1
	2	1.6	81.5	0.7	1.8	89.0	9.8	2.0	97.4	4.4	1.3	67.0	12	1.8	90.0	2.9	2.2	111	3.7	1.2	62.0	11
Z1[d]	0	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-
	0.5	0.5	98.0	3.0	0.5	100	5.3	0.5	106	3.7	0.3	60.0	2.9	0.4	74.0	3.4	0.6	114	4.3	0.4	72.0	11
	2	1.3	65.5	8.8	1.6	81.0	8.4	1.8	89.5	7.8	1.2	57.5	6.4	1.2	61.5	13	1.9	94.0	8.6	1.3	66.5	14
Z2[d]	0	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-	nd	-	-
	0.5	0.5	102	5.1	0.5	106	1.9	0.6	110	1.0	0.3	68.0	7.6	0.4	72.0	10	0.6	118	2.2	0.4	84.0	2.7
	2	1.3	65.5	8.8	1.7	83.0	7.4	1.9	97.0	2.3	1.3	63.5	9.8	1.2	61.0	13	1.9	97.0	3.5	1.4	69.5	7.6

[a] not detected, [b]Taji river, [c]Atsebela river and [d]Ziway lake area soil samples.

3.7. Comparison of the proposed MAE with other sample preparation techniques

For comparison purpose, recoveries of the proposed one-step MAE was compared with three sample preparation techniques such as shake-flask, Soxhlet and USE as described in sections from 2.8 to 2.10. The results obtained are summarized in Table 3. When compared to all the extraction techniques, MAE provided the highest recoveries for cymoxanil, metalaxyl, folpet, and kresoxim-methyl. However, for mandipropamid and chlorothalonil, Soxhlet extraction gave the highest recoveries followed by MAE. Therefore, MAE demonstrated superior extraction capabilities for most of the fungicides studied from soil using only 5 mL of ethyl acetate and an irradiation power of 1600 W (100% output) for 15 min.

Table 3 Comparison of the proposed one-step MAE method to different sample preparation techniques for extraction of target fungicides from soil samples

Fungicide	Recoveries (spiked level, 0.5 μg g^{-1}; n = 5)			
	Shake-flask	Soxhlet	USE	MAE
Cymoxanil	51.6 (8.7)[a]	84.4 (2.3)	48.2 (6.4)	89.9 (2.3)
Metalaxyl	57.8 (18)	88.6 (5.1)	67.4 (8.1)	89.9 (5.4)
Mandipropamid	86.8 (11)	105.6 (10)	87.2 (3.1)	98.9 (6.7)
Folpet	49.4 (8.1)	56.4 (13)	61.6 (3.8)	72.7 (3.6)
Chlorothalonil	65.6 (7.2)	80.6 (3.0)	71.8 (9.6)	73.7 (7.6)
Kresoxim-methyl	96.3 (7.0)	94.5 (5.1)	88.6 (6.1)	99.4 (4.6)
Famoxadone	75.4 (2.5)	73.7 (2.3)	77.9 (4.2)	72.4 (5.6)

[a]data in parentheses indicate RSD, %.

4. Conclusions

A one-step multiresidue method that combines MAE with HPLC-VWD was proposed for the simultaneous determination of seven fungicides in soil. Over 72% of all the studied fungicides were successfully extracted using only a small amount of organic solvent (5 mL ethyl acetate) in quite short time (15 min). The developed extraction procedure was simple, rapid, efficient, and significantly produced less waste solvent compared to the conventional extraction techniques. Moreover, the method demonstrated low LOD and good analyte recoveries, and provided clean extracts that avoided the need for further clean-up. The applicability of the technique was evaluated and found to be suitable for the efficient and selective extractions as well as quantitative determination of the target analytes.

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