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Comparison of Ultrasonic and QuEChERS Extraction Methods Efficiency for the Determination of Herbicides Residues in Soil and Maize Cob

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Abstract

In this study, the optimization and application of ultrasonic extraction and QuEChERS methods was conducted for the effective extraction of pesticides from maize and their corresponding soil samples. The characterization pesticides (Atrazine, glyphosate, 2,4-dichlorophenoxyacetic acid and mesotrione) was done with gas chromatography-flame ionization detector. Factors influencing the efficiency of the extraction methods such as the extraction solvent, extraction time, solvent volume and spiking concentration were assessed. Under the optimum experimental conditions, the recoveries of analytes ranged between 100 - 104 % in maize cop and 91 - 97 % in soil for ultrasonic method, while they were 94 - 115 % in maize cob and 89 - 101 % in soil for QuEChERS method. The repeatability, articulated as RSD values were less than 5 % for all analytes in both methods. The limits of detection and limit of quantification ranged from 0.02 - 0.15 µg L^{-1} and 0.2 - 0.5 µg L^{-1} for ultrasonic method and 0.01 - 0.23 µg L^{-1} and 0.13 - 0.8 µg L^{-1} for QuEChERS method. The lower limits of detection and quantification with higher recoveries suggests that both methods can be accurately applied for the determination of the assessed pesticides in soil and maize cop. Herbicides concentrations ranged between 0.33 - 45.10 µg L^{-1} in soil and 6.15 - 34.2 µg L^{-1} in maize cob. In all studied herbicides, maize residues detected were found to be higher compared to maize allowable maximum residue limits however, atrazine in all soil samples was found to be lower compared to soil allowable maximum residue limits. This indicates the importance of continuous monitoring the herbicides residues in maize to safeguard health for the consumers.

Keyword: Ultrasonic, QuEChERS, Herbicides, Maize, Soil, Gas chromatography, MRLs

Introduction

Crops compete with lots of undesired plants that naturally grows along with and compete for carbon dioxide, space, nutrients, sunshine and water. This competition has a negative effect on crop growth and crop yield. Therefore, agrochemicals and herbicides are used to increase crop production while controlling undesired plants growth. Generally, herbicides are categorised as selective and nonselective synthesized chemicals. Selective herbicides disrupt and destroy hormones of the target weeds while nonselective disrupts and damage all growth from soil. Glyphosate is a non-selective herbicide that prevents the plants from making certain proteins that are needed for crop growth, therefore it is applied before maize has emerged. The 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine and mesotrione are selective auxin herbicides that do not affect desired crop. However, they inhibit particular target zone within the target plant physiological and/or biochemical pathway, resulting to lethal and catastrophic magnitudes [1].

In sub-Saharan Africa, South Africa has the high usage of pesticides for crops protection and crop management, [2] essential to meet increasing population demands to date and in years to come. Agricultural sector plays a significant role in food security, production and steady supply by employing herbicides and agrochemicals to achieve high crop yield in a short space of time [3,4]. This efforts consequently promotes the introduction of these compounds in the food structures of the consumer, e.g. seeds, crops and grains [5,6]. Herbicides are applied on the soil and taken-up by plant roots and translocate to other plant tissues, including the edible part of the plant. These compounds build-up in the edible part of the crop in low concentration. With the aim of food quality assurance, the maximum permissible levels for different herbicides have been set by the World Health Organization (WHO) as well as Food and Agriculture Organization (FAO) [7]. Agricultural crops are important components in the diet for human, hence, crop

quality assurance should be evaluated from herbicides toxicity as herbicides contains toxic chemicals that poses a risk to the human health [8].

Different preparation/extraction techniques are used for the determination of pesticides present in the matrix. Sample preparation is important for analytes extractions from different matrix followed by cleaning stage which removes impurities present in the extracts [9]. Typically used extraction techniques for solid samples includes: Soxhlet extraction (SE), ultrasonic extraction (EU), quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction, microwave assisted extraction (MAE), while the sample clean-up is done using the solid-phase extraction (SPE) [10]. The UE is based on the extraction of samples with an organic solvent by applying ultrasound radiation ranging from 20 - 2,000 kHz [11] in an ultrasonic bath. The mechanical effect of ultrasound induces a great penetration of solvent into sample and improves mass transfer, resulting to an enhancement of analyte extraction efficiency. This can be followed by SPE clean-up which is significant for the removal of undesirable impurities leaving the analytes behind. The QuEChERS method is based on the extraction with solvent at room temperature and separation by addition of salt followed by dispersive solid phase extraction (d-SPE) clean-up step to promote pure extract [12]. These methods are studied to assess and compare their sensitivity and accuracy for the determination of the studied residues. In this current study, ultrasonic and QuEChERS extraction methods were optimised and applied to assess the uptake of 2,4-D, atrazine, mesotrione and glyphosate by roots from their corresponding soil and their translocation to the edible maize crops. Their efficiency to extract the studied herbicides was also compared.

Methods and materials

Chemicals, and analytical reagents

The 2.4-D (97 %), atrazine (97.4 %), glyphosate (98.5 %) and mesotrione (97.5 %) were purchased from Sigma Aldrich (Durban, South Africa). All used solvents were of HPLC grade: Acetonitrile (99.9 %), acetone (99.8 %), dichloromethane (99.8 %), ethyl acetate (99.9 %), acetic acid (< 98 %), isopropyl alcohol (98 %) and methanol (99.9 %) and were bought from Merck (Durban, South Africa). Oasis hydrophilic-lipophilic balance (HLB) cartridges, (60 mg, 3 mL) supplied by Waters (Milford, USA) were used as solid phase extraction sorbent.

Instrumentation

The extraction of pesticides from soil and maize crops was conducted using ultrasonic bath purchased from Prestige (Durban, South Africa). The solid phase extraction (SPE) vacuum manifolds obtained from Sigma Aldrich (Steinheim, Germany) was used for the clean-up of maize and soil extracts from ultrasonic and Soxhlet extraction. The vacuum pump connected to the SPE manifold was purchased from Edwards (Munic, Germany). The lyophilizer from Antech Scientific (Durban, South Africa) was employed to freeze dry the maize samples. The chromatographic studies were conducted using a Bruker scion 436 GC from Gibbs Technologies (Durban, South Africa) coupled with a flame ionization detector (FID). The separation of herbicides was done on a capillary column, VF-5ms (a length of 30 m ×0.25 mm i.d. ×0.25 m film thickness) supplied by Gibbs Technologies. The injector temperature and detector temperatures were both 250 °C. The temperature program was as follows: Initial temperature of 60 °C and held for 1 min, a rate of 30 °C min^{-1} to 150 °C held for 4 min, followed by 15 - 250 °C and held for 5 min. The nitrogen was used as a carrier gas and the injection was split/splitless with a purge time of 0.10 min and split of 1:50. The FID was fed with hydrogen (30 mL min^{-1}) and synthetic air (300 mL min^{-1}) and the samples injection volume was 1 µL [13].

Working standard solutions

The working standard solutions (0 - 100 µg L^{-1}) were prepared form 100 mg L^{-1} stock solution in 95 % n-hexane containing a mixture of herbicides (2.4-D, atrazine, glyphosate and mesotrione). These were then used for the calibration of gas chromatography within the concentration range 0 - 100 µg L^{-1} and for spiking samples used for validation studies. The calibration standards were stored at 4 °C until analysis were conducted.

Study area

Soil and maize samples were collected from Buhle experimental field. The study site is located in the Howick, province of KwaZulu-Natal and district of UMgungundlovu where studies on maize, tubers and agronomic crops are conducted for the KwaZulu-Natal community. The herbicides such as atrazine, glyphosate, 2.4-D and mesotrione are used for weed management. It was therefore important to assess the

uptake of these herbicides in the study area to ensure crop quality hence, consumer safety. The exact sampling location can be found through the usage of the Global Positioning System (GPS), represented by the co-ordinates −29.524161, 30.247682. The sampling location is known for its high temperatures in summer (wet season) which normally range from 21 - 38 °C, while these usually drop in winter (dry season) ranging from 10 - 28 °C. On average, precipitation is approximately 569 mm annually. For the purpose of the present study, maize crop was sampled from the maize stalk, corresponding soils were sampled on the topsoil surface of the soil (0 - 5, 0 - 10 cm) and subsoil 0 - 15 cm surface layer. Samples were stored in polyethylene bags and transported to the laboratory.

Sample preparation

Soil samples were air dried for 48 h, pulverized and passed through 2.0 mm sieve. The maize samples were freeze dried on Lyophilizer and milled into fine powder and passed through 100 mm screen mesh. Samples were then stored at room temperature until analysis were conducted. The herbicides were extracted using ultrasonic extraction followed by SPE clean-up and QuEChERS extraction followed by d-SPE clean up.

Optimization of UE

The ultrasonic extraction method published by Ramos [14] was adopted with further optimization to improve the extraction efficiency for all the herbicides studied. In this study the extraction time, extraction solvent, extraction solvent volume and spike concentration were parameters studied to assess their effect on herbicides recoveries from the maize and soil

The extraction time was studied to evaluate the optimum sonication time needed for complete extraction without reaching the degradation point. The extraction time studied were 15, 30, 45 and 60 min. Different extraction solvents (methanol, acetonitrile, and mixture of methanol: Dichloromethane) were studied to evaluate the solubility ability of the studied analytes. The investigated solvent volumes were 15, 25 and 40 mL. Solvent volume is significant for sufficient interaction of sample mass with solvent for complete and effective extraction and to achieve high recoveries.

Under optimum conditions, a 1 g sample (soil and maize cob) was wetted with 5 mL of distilled water to hydrate the active sites. The samples mixture was shaken up with ultrasonic waves for 15 min followed by the addition of 25 mL of dichloromethane (DCM): Methanol (MeOH) (1:1 v/v) solvent and further ultrasonicated for 15 min at 40 °C. The sample and supernatant liquid were separated by centrifuging the mixture for 5 min at 3,000 rpm. The supernatant liquid was reduced to 1 mL by rota-vapor. Then, 1 mL of the extract was subjected to clean up step by SPE.

SPE cleaning method

A batch of sample was subjected to cleaning by SPE while the other batch was not cleaned which was done to investigate the cleaning effect. The SPE sorbent used was Oasis HLB (60 mg, 3 mL) cartridge. A 500 µL of methanol was used to condition the SPE sorbent for effective interaction with the analyte. This was followed by addition of 500 µL of water to equilibrate the SPE sorbent. The extracted sample was then loaded into the SPE sorbent to trap the analyte on the SPE sorbent. A 500 µL of 5 % methanol was used to wash the impurities and the analyte trapped on the sorbent were eluted with 500 µL methanol [15].

Optimization of QuEChERS

The method published by [16] was used with further optimization. The extraction solvents, solvent volume and vortexing time were parameters studied for their effect on recoveries. Different extraction solvents including acetonitrile, methanol, acetone and isopropanol. The investigated solvent volumes were 7, 15 and 30 mL. The vortex agitation time was investigated to allow the homogeneous distribution of the analyte to the axtractant where 0.5, 1 and 3 min were investigated.

Under optimum conditions, a 15 g sample (soil and maize cob) was weighed into 50 mL falcon tube. A 15 mL of acetronitrile acidified with 1 % acetic acid (v/v) was added and mixed with the vortex for 1 min. Then, 1.5 g of anhydrous sodium acetate (NaOAc) and 6.0 g of anhydrous magnesium sulphate ($MgSO_4$) were added to the tube, and this mixture was vortexed for 1 min followed by centrifugation at 4,000 rpm for 5 min at room temperature. This was followed by d-SPE cleaning. An 8 mL of QuEChERS extract, 50 mg of C18-bonded silica (particle size 55 - 105 µm) sorbent, 50 mg of primary secondary amine (PSA; particle size 50 mL) sorbent and 150 mg of $MgSO_4$ were added to a 15 mL polypropylene centrifuge tube, vortexed for 30 s, and then the mixture was centrifuged at 5,000 rpm for 5 min at room temperature. The supernatant was collected and 1 µL was injected into a GC-FID system.

Statistical analysis

The residue data was analyzed using the Statistical Package for Social Science (SPSS version 25.0 SPSS Inc, Chicago, IL, USA). For all repeat measurements, the mean values and standard deviations of all samples were computed and the Kruskal Wallis nonparametric test was used to detect the significant differences in the residue concentration of samples. The Mann-Whitney U test was used to determine the specific differences when the significant difference was identified at a 95 % confidence limit.

Results and discussion

Optimization of EU method

Selection of the extraction solvent

The effectiveness of the UE extraction solvent was studied using, acetonitrile (ACN), methanol, mixture of methanol: ethyl acetate (EA) (1:1) and mixture of methanol: Dichloromethane (1:1). The mixture of methanol: Dichloromethane (1:1) gave higher recoveries ranging from 99.9 - 102 and 95 - 98 % in cleaned and uncleaned maize, 89 - 97 and 85 - 94 % in cleaned and uncleaned soil, respectively (**Figures 1** and **2**). This point out that less polar and more polar analytes solubilized better in the methanol: Dichloromethane mixture (as methanol is polar while dichloromethane is nonpolar), resulting in high extraction efficiency for the target analytes from the sample [17]. This is due to that the polarity of the extraction solvent influence the interaction with the sample consequently allow complete elucidation of the target analyte from the matrix, hence high recoveries [18]. Hence, methanol: Dichloromethane (1:1) was selected as the optimum extractant for UE in this study. Comparable recoveries were obtained before and after application of the clean-up stage, indicating that it had no effect. This revealed that the target analytes solubilized well in the extraction mixture carrying no impurities. It also confirmed that the target analyte can be analysed without clean-up step, hence it was not considered in the other extractions.

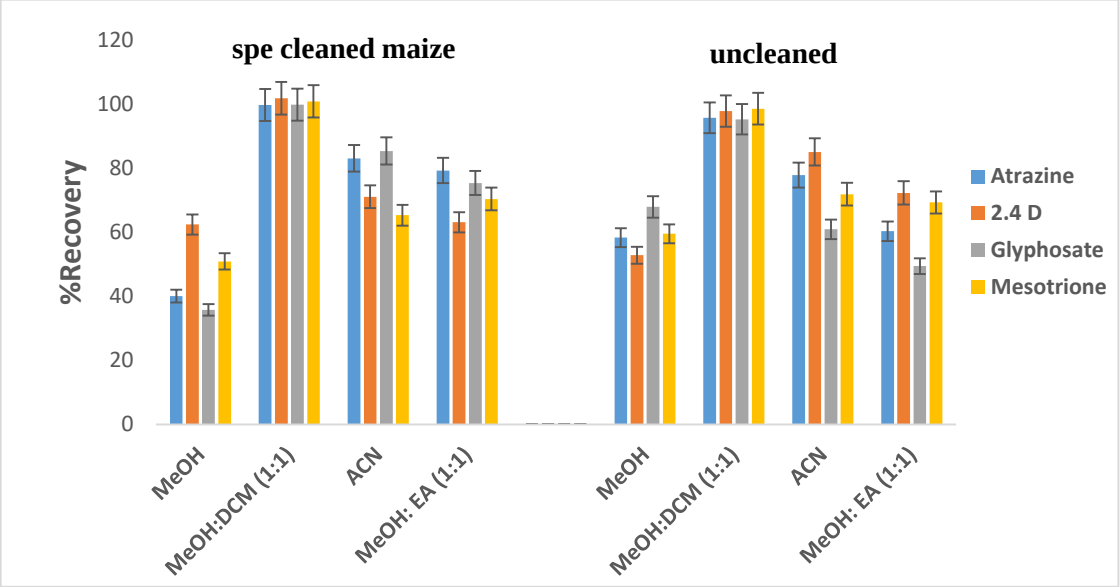

Figure 1 Effect of extraction solvents using UE with SPE clean-up and UE without SPE clean-up methods on the pesticides recoveries from maize.

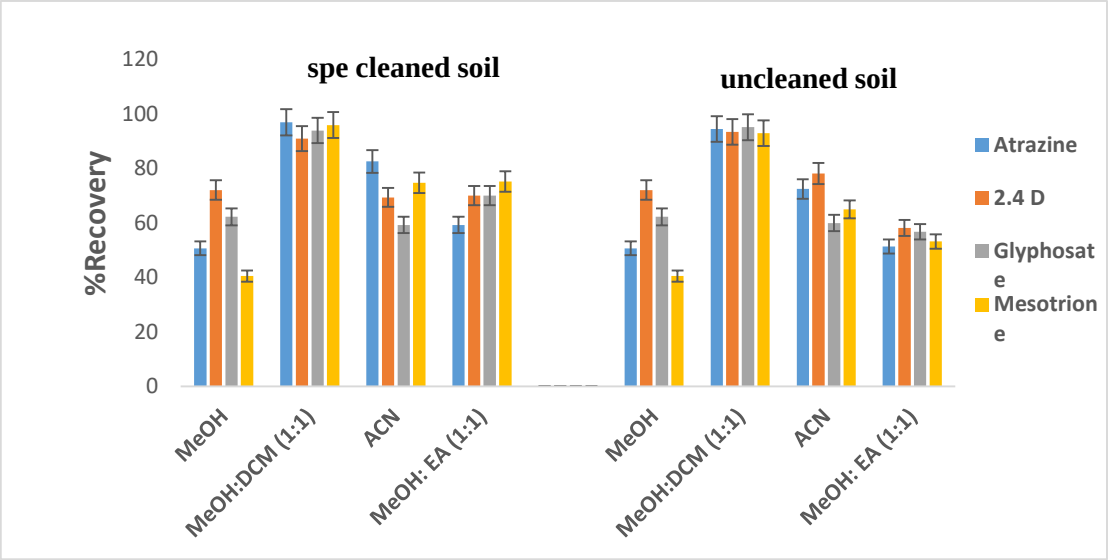

Figure 2 Effect of extraction solvents using UE with SPE clean-up and UE without SPE clean-up methods on the pesticides recoveries from soil.

Effect of the extraction solvent volume

The extraction solvent volume is the element that influence extraction efficiency of the target analytes from the maize and soil samples. To study this factor, different volumes (15, 25 and 40 mL) of methanol: DCM (1:1) were examined. The obtained results (**Figure 3**), showed an increased in extraction efficiency with the increase in extraction solvent volume up to 25 mL and then reduced by further increasing solvent volume. This might be due to the fact that, at low volume of the extraction solvent (less than 25 mL), extraction of analyte was incomplete hence, due to low mass transfer which led to low recoveries [19]. Meanwhile, at more than 25 mL extraction volume, the dilution of target analyte surfaced and led to reduced extraction efficiency. The recoveries obtained with 25 mL ranged from 95 - 98 % in maize and 85 - 94 % in soil. This resulted to 25 mL methanol: DCM (1:1) being selected as an optimum volume. The increase in recoveries with an increase in extraction solvent volume followed by a decrease with further increasing volume is in agreement with the study reported by [17].

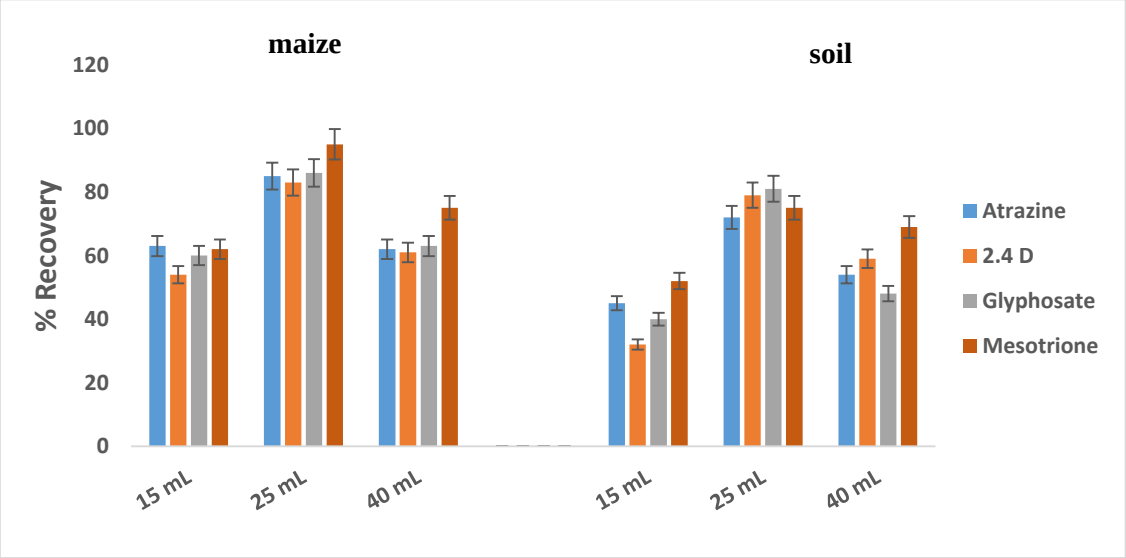

Figure 3 Effect of extraction solvent volume on the recoveries of herbicides from maize and soil samples.

Effect of extraction time

The extraction time is an important parameter as it impacts the quantity of the target analyte recovered. The shorter extraction time prevents the complete extraction process while longer extraction time results into analyte degradation. The effect of extraction time on the analyte recoveries was investigated by employing 15, 30 and 60 min. The recoveries increased from 15 - 30 min and then decreased with further increasing time to 60 min (**Figure 4**) probably due to the degradation of the target analyte. This indicates that 30 min extraction time allowed enough diffusion of the solvent into the sample matrices while breaking matrices membranes and elucidating target analytes. The 30 min yielded 95 - 98 % on maize and 85 - 94 % on soil samples and consequently selected as the optimum extraction time for the UE method. Babić and Petrović [20], also reported the increase in recoveries with an increase in extraction time however, prolonged sonication time caused degradation of compounds resulting to decreased recoveries.

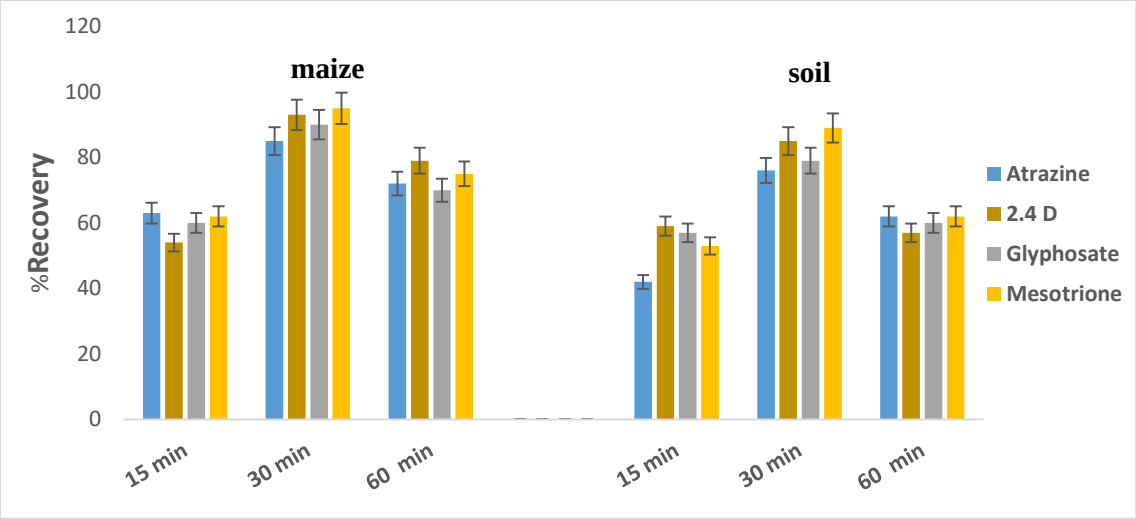

Figure 4 Effect of extraction time on the recoveries of herbicides from maize and soil samples.

Effect of spike concentration

The validity of the studied method was assessed by spiking both maize and soil samples with different spiking concentration 60 and 100 µg L^{-1}. Considering the obtained results (**Figure 5**), the optimum conditions showed recoveries ≥ 99 on both different spiking concentrations in maize and soil samples which determined well developed method.

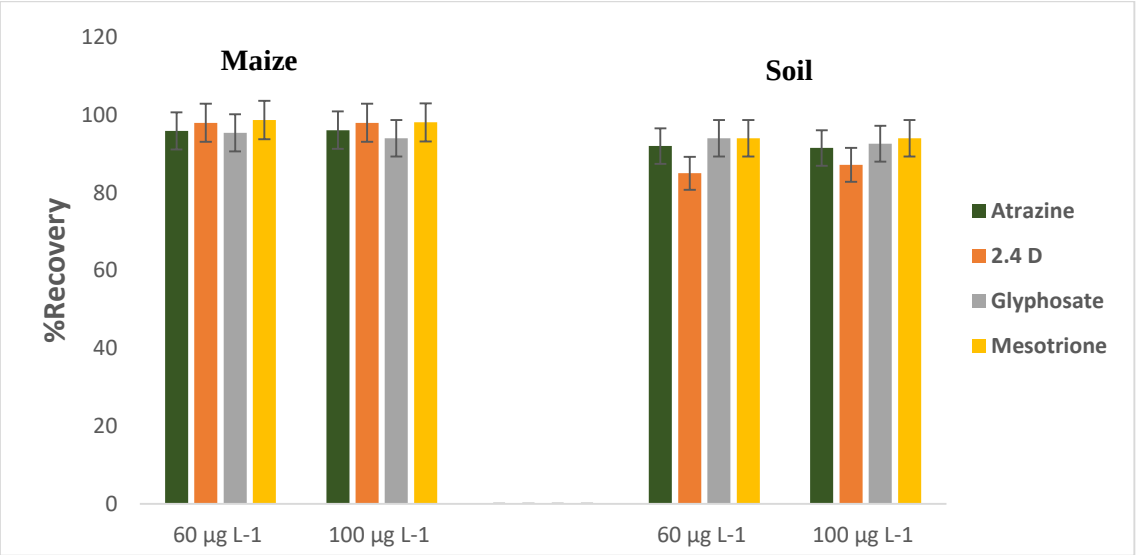

Figure 5 Effect of the spiking concentration e on herbicides recoveries from maize and soil samples.

Optimization of QuEChERS method

Effect of extraction solvent

The effect of extraction solvent on the recovery of analytes were investigated using methanol, acidified acetonitrile, isopropanol and acetone. The Acidified acetonitrile was found to be the optimum solvent with higher recoveries ranging from 94 - 115 and 92 - 101 % in maize and soil, respectively (**Figure 6**). The acidified acetonitrile showed the ability to extract the studied herbicides with their different polarities nature. This is due to that the addition of NaOAc together with 1 % acetic acid allows the buffering of the medium which improves acetonitrile extraction ability. Also, the use of $MgSO_4$ salt during the partitioning step reduced the volume of the aqueous phase by hydration and saturates the molecular spaces with the aqueous solvent, resulting in an increase solubility the analytes [12].

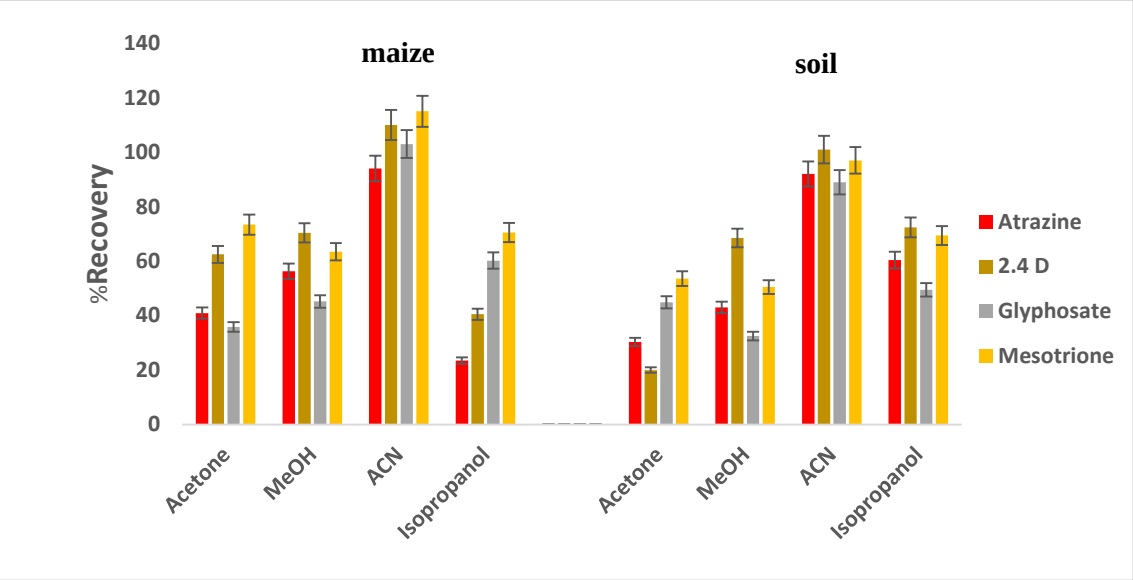

Figure 6 Effect of extraction solvents using QuEChERS with dSPE clean-up method on the pesticides recoveries from maize and soil.

Effect of extraction solvent volume

The influence of extraction solvent volume was examined using 7, 15 and 30 mL. The obtained results (**Figure 7**), showed that 7 mL gave lower recoveries due to incomplete mass transfer of the analyte from the matrix resulting from minimum solvent volume. The increase in volume from 7 - 15 mL promoted complete mass transfer of the analyte hence, increase in extraction recoveries. The recoveries obtained for 15 mL ranged from 94 - 115 and 92 - 101 % in maize and soil respectively. Petrarca, [16] also reported 15 mL as optimum volume for the extraction of pesticides using QuChERS method in baby food samples.

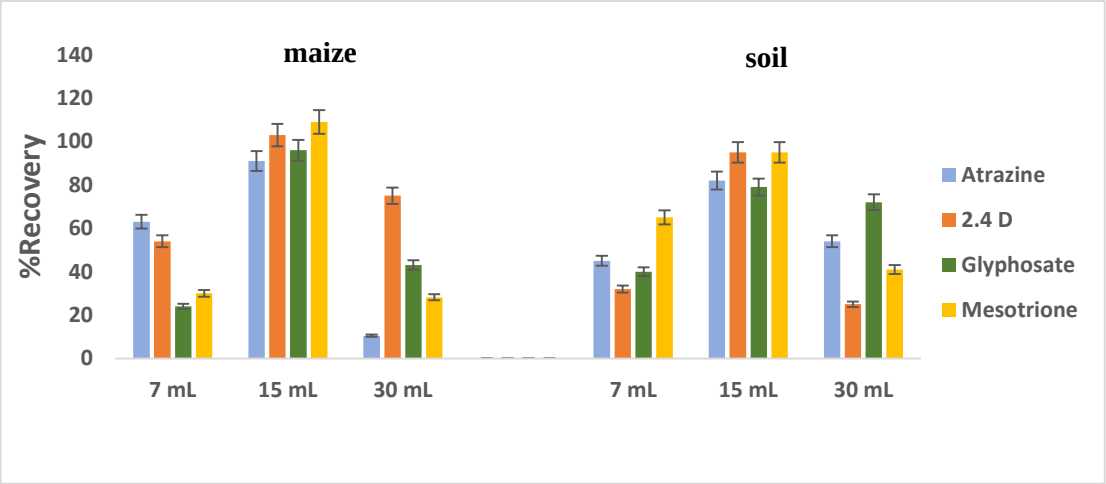

Figure 7 Effect of extraction solvent volume on the recoveries of herbicides from maize and soil samples.

Effect of vortexing time

Vortexing time (0.5, 1 and 3 min) was studied to assess the agitation effect on the recoveries. The obtained results (**Figure 8**), showed no effect of the agitating time on the extraction recoveries of the target analyte which agrees with Farajzadeh and co-workers finding [17]. These results indicated that all the vortexing times used were adequate to allow interaction and transportation of the analyte bound from the matrix into the extraction solvent. Thus, 1 min was selected as a vortexing time for this method.

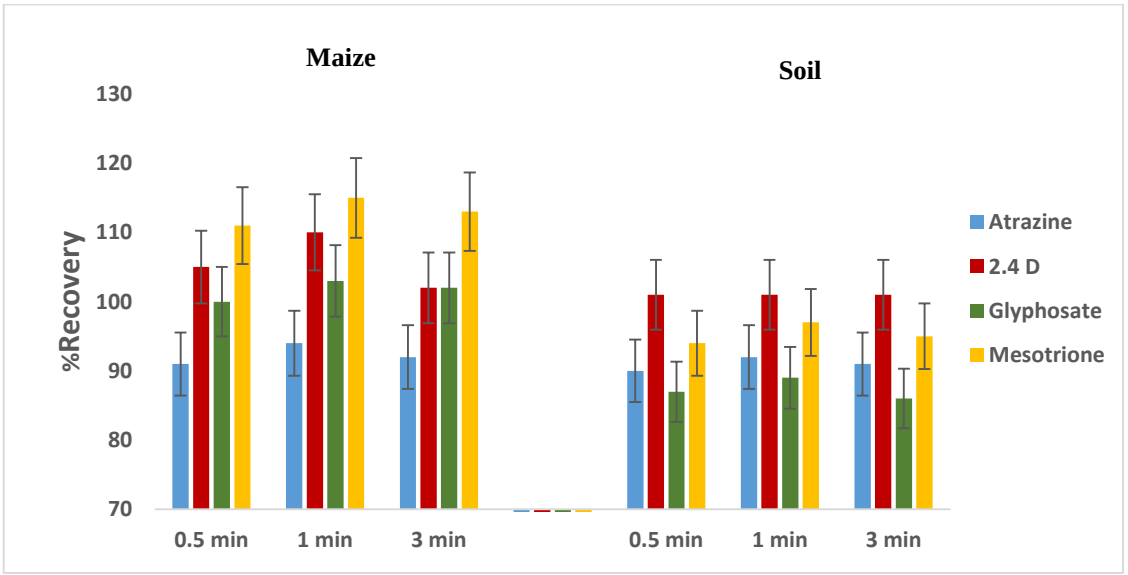

Figure 8 Effect of vortexing time on the recoveries of herbicides from maize and soil samples.

Method validation

The quantitative characteristics of the present method such as relative standard deviation (RSD), coefficient of determination (R^2), limit of detection (LOD), limit of quantification (LOQ) and recoveries were evaluated. The obtained results are listed in **Table 1**. The linearity of all calibration curves showed a good correlation coefficient (R^2) ≥ 0.995. The UE LODs and LOQs ranged from 0.02 - 0.15 and 0.2 - 0.5 µg L^{-1}, respectively (**Table 1**) while QuEChERS LODs and LOQs ranged from 0.01 - 0.23 and 0.13 - 0.8 µg L^{-1}, respectively (**Table 1**). The obtained LODs and LOQs indicated that that the developed methods are sensitive and hence will be able to detect the target analyte at concentration of the real sample, also this

indicate good sensitivity of both methods. The UE maize and soil analytes recoveries were 100 - 104 and 91 - 97 %, respectively, while QuEChERS maize and soil analytes recoveries were 94 - 115 and 92 - 101 %, respectively with the repeatability, articulated as RSD values of less than 6 % for all analyte which indicated good precision of the introduced methods. Zou [21], assessed the gramoxone in 4 edible vegetables (cabbage, lettuce, spinach and Chinese cabbage) with ultrasonic extraction and cleaned up by weak cation exchange solid-phase extraction. The recoveries they obtained (43.6 to 73.5 %) were lower compared to those obtained in the current study, while their LOD (0.94 µg L^{-1}) was higher compared to what was obtained in this study, indicating better accuracy and sensitivity of the our method compared to Zou [21].

Table 1 The maize and soil recoveries (% Rec) and RSD % value (n = 3), LOD, LOQ and R^2 obtained from QuEChERS and UE methods.

Analyte	Maize		Soil		Maize		Soil		R^2
	% Rec, RSD				LOD		LOQ		
	Ultrasonic	QuEChERS	Ultrasonic	QuEChERS	Ultrasonic	QuEChERS	Ultrasonic	QuEChERS	
Atrazine	100 ± 2.1	94 ± 3.1	97 ± 0.9	92 ± 2.0	0.02	0.01	0.2	0.4	0.9988
2.4 D	104 ± 1.0	110 ± 0.9	91 ± 3.5	101 ± 1.6	0.15	0.23	0.5	0.8	0.9985
Glyphosate	100 ± 2.0	103 ± 1.1	94 ± 1.9	89 ± 0.8	0.09	0.12	0.3	0.4	0.9990
Mesotrione	101 ± 0.5	115 ± 2.1	96 ± 4.2	97 ± 1.0	0.02	0.04	0.3	0.13	0.9991

Analysis of real samples

The modified UE and QuEChERS methods were then applied to subsoil (0 - 15 cm), topsoil (0 - 10 cm and 0 - 5 cm) and maize samples. Residues of atrazine, 2.4-D, glyphosate and mesotrione herbicides were detected in all topsoil and maize samples. In the subsoil structures, only traces of 2.4-D were detected with the UE method with higher concentration (2.4 µg L^{-1}) in site 1 (**Table 2**), while 2.4-D and atrazine were detected with the QuEChERS method with higher concentrations of 1.86 and 0.6 µg L^{-1}, respectively. All atrazine detected residues were lower than recommended MRLs. The statistical analysis showed that there was no significant difference between the concentrations obtained with UE and QuEChERS methods. Availability of the 2.4-D and atrazine herbicides to the subsoil is due to their high solubility properties which resulting to their leaching to subsoil structure [22] as compared to the other studied compounds. More pesticides were detected in the subsoil through QuEChERS method as compared to UE method (**Table 3**). Ntombela [23] studied the atrazine herbicide in soil and sediments samples from KwaZulu-Natal. The concentrations detected ranged between 0.032 - 0.93 µg L^{-1} in sediments, while in soil samples they were between 0.12 - 1.03 µg L^{-1}. In both methods, detected concentrations of the herbicides were higher in the topsoil (0 - 5 cm) compared to other soil segments which could be due to the high hydrophobicity properties (low solubility), low log K_{ow} of < 10 and presence of functional group containing O, N, and S [22-24]. This allows connection to soil water molecules through hydrogen bonds hence, bioaccumulation of herbicides to the soil and translocation to the edible crop [25].

In both methods, glyphosate traces were higher 30.52 and 34.2 µg L^{-1} in maize crop sampled from site 1 for UE and QuEChERS method, respectively. However, the obtained maize concentrations are higher than MRLs in all studied sites which makes this finding a great concern as the presence of herbicides traces pose a danger to the environment and the consumer. All detected concentrations were different but there were not significantly different. Farajzadeh [17], extracted diazinon, chlorpyrifos, penconazole, oxadiazon, and diniconazole pesticides from fruits and vegetables using combination of QuEChERS and dispersive liquid-liquid microextraction with GC-FID. Penconazole was found in corn (4 µg kg^{-1}) and other samples were free of the studied pesticides and QuEChERS was found to me effective than dispersive liquid-liquid microextraction. The availability of the herbicides in the tested soil and maize crops maybe due to the application of herbicides to remove growing weeds competing with maize plants which compromises crop effective growth and optimum nutrition levels. This impact greatly food security, therefore, different herbicides were applied to the soil to protect maize crop quality and quantity against competing weeds.

Table 2 Concentrations (µg L^{-1} dry weight) of pesticides in soil and maize crop determined using UE method.

Analyte	Maize MRL (mgL^{-1})	Site 1				Site 2			
		Soil depth (cm)			maize	Soil depth (cm)			maize
		0 - 5	0 - 10	0 - 15		0 - 5	0 - 10	0 - 15	
Atrazine	0.05	10.6 ± 11.2	11.62 ± 3.2	ND	6.15 ± 0.5	5.42 ± 2.0	10.45 ± 1.0	ND	11.36 ± 6.1
2.4 D	0.5	16.5 ± 1.1	6.58 ± 9.1	2.45 ± 1.7	10.28 ± 0.2	10.41 ± 4.6	5.64 ± 0.1.9	1.33 ± 2.8	20.65 ± 2.5
Glyphosate	2	26.1 ± 8.5	9.45 ± 5.2	ND	30.52 ± 2.9	19.5 ± 0.6	15.42 ± 2.5	ND	11.50 ± 1.0
Mesotrione	0.06	8.4 ± 2.3	8.1 ± 0.3	ND	11.23 ± 0.8	30.18 ± 3.1	29.44 ± 3.1	ND	24.61 ± 2.9
	Soil MRL	**Site 3**				**Site 4**			
Atrazine	66	36.30 ± 0.6	20.42 ± 0.9	ND	15.8 ± 1.4	19.41 ± 7.2	5.44 ± 4.3	ND	24.1 ± 4.2
2.4 D	-	28.12 ± 4.6	16.86 ± 0.9	0.33 ± 1.6	18.8 ± 3.5	21.45 ± 3.7	15.26 ± 0.9	1.65 ± 3.2	18.45 ± 2.6
Glyphosate	-	15.66 ± 3.1	11.86 ± 1.6	ND	9.81 ± 2.1	45.10 ± 2.0	30.79 ± 1.2	ND	9.15 ± 0.6
Mesotrione	-	19.40 ± 1.0	20.45 ± 2.1	ND	8.05 ± 1.6	15.4 ± 1.3	8.92 ± 0.6	ND	6.15 ± 0.9

* - Not indicated

Table 3 Concentrations (µg L^{-1} dry weight) of pesticides in soil and maize crop determined using QuEChERS method.

Analyte	Maize MRL (mgL^{-1})	Site 1				Site 2			
		Soil depth (cm)			maize	Soil depth (cm)			maize
		0 - 5	0 - 10	0 - 15		0 - 5	0 - 10	0 - 15	
Atrazine	0.05	11.4± 8.5	8.62 ± 3.2	0.6 ± 0.9	7.6 ± 2.4	5.42 ± 2.0	10.4 ± 1.0	ND	11.36 ± 6.1
2.4 D	0.5	10.4 ± 3.1	9.45 ± 9.1	1.86 ± 1.2	8.4 ± 2.9	7.45 ± 1.6	4.52 ± 3.5	ND	15.42 ± 0.1
Glyphosate	2	28.6 ± 5.8	11.3 ± 0.2	ND	34.2 ± 1.0	15.12 ± 0.6	10.45 ± 2.0	2.56 ± 4.2	18.45 ± 1.6
Mesotrione	0.06	7.5± 1.1	10.4 ± 2.1	ND	10.52 ± 0.9	21.4 ± 0.9	17.41 ± 3.1	ND	14.57 ± 2.3
	Soil MRL	**Site 3**				**Site 4**			
Atrazine	66	33.4 ± 3.1	18.42 ± 0.9	0.2 ± 2.6	18.4 ± 2.0	23.4 ± 2.5	8.42 ± 5.3	ND	19.15 ± 3.5
2.4 D	-	30.5 ± 6.0	23.5 ± 1.3	0.42 ± 0.9	15.2 ± 3.2	28.52 ± 1.9	11.45 ± 3.9	1.6 ± 2.0	22.45 ± 3.2
Glyphosate	-	12.6 ± 0.9	8.46 ± 3.6	ND	6.52 ± 1.2	38.45 ± 6.2	29.23 ± 2.3	ND	16.52 ± 1.3
Mesotrione	-	18.42 ± 2.3	15.45 ± 0.5	ND	9.54 ± 3.6	17.52 ± 4.3	10.32 ± 3.6	ND	7.45 ± 3.6

* - Not indicated

Table 4 Concentration ranges for the pesticides detected in this study against those from previous studies.

Study site	Analytes	Matrixes	Extraction method	Detection method	Concentration	References
Heilongjiang, China	Atrazine	Soil		GC-FID	8.14 μg kg^{-1}	[26]
Bahia, Brazil	atrazine	Environmental water samples	Micro-solid phase extraction (d-μ-SPE)	GC-MS	< 0.51 μg L^{-1}	[27]
Turkey	Acetamiprid	Sour cherry	QuEChERS	LC-MS/MS	4,0 μg L^{-1}	[28]
Lahore, Pakistan	Glyphosate	Spinach and turnip	Solvent extraction	LC-UV/V	0.29 and 0.93 μg L^{-1}	[29]
Pietermaritzburg, South Africa	Atrazine	Soil	UE	LC-PDA	37 8.14 μg kg^{-1}	[30]
Pietermaritzburg, South Africa	Atrazine	Avocado and carrot	QuEChERS	LC-PDA	4 and 38 8.14 μg kg^{-1}	[31]
Pietermaritzburg, South Africa	Atrazine	Vegetables (bell pepper)	UE	LC-PDA	628.14 μg kg^{-1}	[32]
Howick, South Africa	Atrazine, glyphosate, 2,4-D and mesotrione	Soil and maize	Ultrasonic extraction	GC-FID	Soil 5.42 - 36.30 μg L^{-1} Maize 6.15 - 30.52 μg L^{-1}	This study
	Atrazine, glyphosate, 2,4-D and mesotrione	Soil and maize	QuEChERS method	GC-FID	Soil 0.6 - 38.45 μg L^{-1} Maize 6.52 - 34.2 μg L^{-1}	This study

Comparison of pesticides concentrations obtained in this work with those from literature

Information presented in **Table 4** clearly shows that atrazine is the commonly studied pesticide. A summary is presented in **Table 3**. Information presented in **Table 4** clearly shows that the levels of these pesticides in the investigated matrixes are much higher than those reported in previous studies. This was also the case for the same studies conducted in other South African locations. Such an observation applied to all the investigated soil and maize crop. Moreover, a common distribution of the studied pesticides of concern was observed in the soil and crops. In this study, the concentrations found in soil and maize cob did not show any trend. Based on **Table 4**, it can be concluded that the distribution of these pesticides is site specific. Factors that could influence the occurrence of pesticides in soil and maize cob and their concentration include the application source and different physicochemical properties of the pesticides. In a South African study, the authors observed that the site location affects the levels of uptake and translocation in plant tissues due to differences in pesticides physicochemical properties that control the mobility of pesticides at different study location and adsorption capacity of residues by plants.

Conclusions

In the present study, the modified UE-SPE and QuEChERS extraction methods showed effectiveness in sample preparation and pre-concentration of the selected pesticides prior to their characterization by GC-FID. However, the results showed that the UE method can be effectively applied without the additional SPE clean up step, making the method cheaper and more available. The experimental data proved that the proposed methods have low LODs and LOQs, good repeatability and high recoveries, and thus can be presented as applicable for the extraction, pre-concentration and quantification of the selected pesticides in the studied pesticides. However, UE showed higher sensitivity accuracy as compared to QuEChERS method. All detected residues in maize were exceeding the MRLs except for atrazine in all soil samples, due to continuous application of these pesticides in the agricultural fields. Based on the obtained results, it is suggested that a supported program for the assessment of herbicides traces in soil and crops be developed to achieve sustainable farming systems, food quality and promote consumer's wellbeing.

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References

[1] Y Song. Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *J. Integr. Plant Biol.* 2014; **56**, 106-13.

[2] S Horn, R Pieters and T Bøhn. A first assessment of glyphosate, 2,4-D and cry proteins in surface water of South Africa. *S. Afr. J. Sci*. 2019; **115**; 5988.

[3] CJ Burns and GM Swaen. Review of 2,4-dichlorophenoxyacetic acid (2,4-D) biomonitoring and epidemiology. *Crit. Rev. Toxicol.* 2012; **42**, 768-86.

[4] DH Garabrant and MA Philbert. Review of 2,4-dichlorophenoxyacetic acid (2,4-D) epidemiology and toxicology. *Crit. Rev. Toxicol.* 2002; **32**, 233-57.

[5] X Dong, S Liang, Z Shi and H Sun. Development of multi-residue analysis of herbicides in cereal grain by ultra-performance liquid chromatography-electrospray ionization-mass spectrometry. *Food Chem.* 2016; **192**, 432-40.

[6] HS Karapınar. Adsorption performance of activated carbon synthesis by $ZnCl_2$, KOH, H_3PO_4 with different activation temperatures from mixed fruit seeds. *Environ. Tech.* 2022; **43**, 1417-35.

[7] World Health Organization and Food and Agriculture Organization of the United Nations. P*esticide residues in food - 2018: Toxicological evaluations*. World Health Organization and Food and Agriculture Organization of the United Nations, Berlin, Germany, 2018.

[8] F Islam, J Wang, MA Farooq, MS Khan, L Xu, J Zhu, M Zhao, S Muños, XQ Li and W Zhou. Potential impact of the herbicide 2,4-dichlorophenoxyacetic acid on human and ecosystems. *Environ. Int.* 2018; **111**, 332-51.

[9] MT Padrón, ZS Ferrera and JS Rodríguez. Optimisation of solid-phase microextraction coupled to HPLC-UV for the determination of organochlorine pesticides and their metabolites in environmental liquid samples. *Anal. Bioanalytical Chem.* 2006; **386**, 332-40.

[10] HS Karapınar and A Bilgiç. A new magnetic Fe_3O_4@ SiO2@ TiO_2-APTMS-CPA adsorbent for simple, fast and effective extraction of aflatoxins from some nuts. *J. Food Compos. Anal.* 2022; **105**, 104261.

[11] SS Handa, SPS Khanuja, G Longo and DD Rakesh. *Extraction technologies for medicinal and aromatic plants*. International Centre for Science and High Technology, Trieste, Italy, 2008.

[12] M Anastassiades, SJ Lehotay, D Štajnbaher and FJ Schenck. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* 2003; **86**, 412-31.

[13] ADS Pinheiro and JBD Andrade. Development, validation and application of a SDME/GC-FID methodology for the multiresidue determination of organophosphate and pyrethroid pesticides in water. *Talanta* 2009; **79**, 1354-9.

[14] M Asensio-Ramos, J Hernández-Borges, T Borges-Miquel and M Rodríguez-Delgado. Evaluation of multi-walled carbon nanotubes as solid-phase extraction adsorbents of pesticides from agricultural, ornamental and forestal soils. *Anal. Chim. Acta* 2009; **647**, 167-76.

[15] G Bernardi, M Kemmerich, LC Ribeiro, MB Adaime, R Zanella and OD Prestes. An effective method for pesticide residues determination in tobacco by GC-MS/MS and UHPLC-MS/MS employing acetonitrile extraction with low-temperature precipitation and d-SPE clean-up. *Talanta* 2016; **161**, 40-7.

[16] MH Petrarca, JO Fernandes, HT Godoy and SC Cunha. Multiclass pesticide analysis in fruit-based baby food: A comparative study of sample preparation techniques previous to gas chromatography-mass spectrometry. *Food Chem*. 2016; **212**, 528-36.

[17] MA Farajzadeh, H Sohrabi and A Mohebbi. Combination of modified QuEChERS extraction method and dispersive liquid-liquid microextraction as an efficient sample preparation approach for extraction and preconcentration of pesticides from fruit and vegetable samples. *Food Anal. Meth.* 2019; **12**, 534-43.

[18] H Annegowda, R Bhat, L Min-Tze, A Karim and S Mansor. Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. *J. Food Sci. Tech*. 2012; **49**, 510-4.

[19] A Silva, C Delerue-Matos and A Fiúza. Use of solvent extraction to remediate soils contaminated with hydrocarbons. *J. Hazard. Mater*. 2005; **124**, 224-9.

[20] S Babić, M Petrović and M Kaštelan-Macan. Ultrasonic solvent extraction of pesticides from soil. *J. Chrom.* 1998; **823**, 3-9.

[21] T Zou, P He, J Cao and Z Li. Determination of paraquat in vegetables using HPLC-MS-MS. *J. Chrom. Sci.* 2015; **53**, 204-9.

[22] D Mackay, WY Shiu and SC Lee. *Handbook of physical-chemical properties and environmental fate for organic chemicals*. CRC press, Florida, 2006.
[23] SC Ntombela and PN Mahlambi. Method development and application for triazine herbicides analysis in water, soil and sediment samples from KwaZulu-Natal. *J. Environ. Sci. Health Part B* 2019; **54**, 569-79.
[24] A Bilgic, A Cimen, AN Kursunlu and HS Karapınar. Novel fluorescent microcapsules based on sporopollenin for removal and detection of Cu (II) ions in aqueous solutions: Eco-friendly design, fully characterized, photophysical&physicochemical data. *Microporous Mesoporous Mater.* 2022; **330**, 111600.
[25] CR Martins, WA Lopes and JBD Andrade. Organic compound solubility. *Química Nova* 2013; **36**, 1248-55.
[26] Y Chen, Z Jiang, D Wu, H Wang, J Li, M Bi and Y Zhang. Development of a novel bio-organic fertilizer for the removal of atrazine in soil. *J. Environ. Manag.* 2019; **233**, 553-60.
[27] MM Nascimento. GOD Rocha and JBD Andrade. Customized dispersive micro-solid-phase extraction device combined with micro-desorption for the simultaneous determination of 39 multiclass pesticides in environmental water samples. *J. Chrom.* 2021; **1639**, 461781.
[28] T Balkan and K Kara. Determination of pesticide residues in sour cherry used in the sour fruit juice production in tokat provinces. *Turk. J. Agr. Food Sci. Tech.* 2020; **8**, 106-10.
[29] N Khan, G Yaqub, T Hafeez and M Tariq. Assessment of health risk due to pesticide residues in fruits, vegetables, soil, and water. *J. Chem*. 2020; **2020**, 5497952.
[30] PN Kunene. *Method development and application: Solid phase extraction (SPE), ultrasonic extraction (UE) and soxhlet extraction (SE) for the analysis of pesticides in water, soil, sediment and sludge*. University of KwaZulu-Natal - Pietermaritzburg Campus, Pietermaritzburg, South Africa, 2019.
[31] H Mnyandu and P Mahlambi. Optimization and application of QuEChERS and SPE methods followed by LC-PDA for the determination of triazines residues in fruits and vegetables from pietermaritzburg local supermarkets. *Food Chem*. 2021; **360**, 129818.
[32] H Mnyandu and P Mahlambi. Determination of triazines residues in fruits and vegetables: Methods comparison of ultrasonic solvent extraction with and without solid phase clean-up. *Separ. Sci. Tech.* 2022; **57**, 2056-64.