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Determination of heavy metals and pesticide residue in soil, plant and water using QuEChERS method and design of experiment along Asa-River Tributary

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Abstract

The QuEChERS sample preparation method, coupled with gas chromatography-mass spectrometry (GC-MS), was optimized and validated for pesticide determination in soil, water, and vegetables. Experimental parameters, such as mass of sample, volume of solvents, extraction time, and sorbents, were optimized using Minitab statistical software. The GC-MS method showed excellent linearity, selectivity, and recovery, with detection limits ranging from >0.001 μg/L and quantification limits from 0.003 μg/L. The analysis of pesticide samples revealed 17 identified pesticides, with Endosulfan ether showing the highest residue concentration (1.41 mg/L) in soil Sample 2. Similar trends were observed in Vegetable Samples 1 and 2, with residue concentrations ranging from 0.00–870.0 μg/kg and 0.00–110.00 μg/kg, respectively. No pesticide residues were detected in soil Sample 1, water Samples 3 and 4. Additionally, heavy metal analysis (Cd, Pb, Zn, Cu, Ni, Co, As, and Fe) was conducted using Atomic Absorption Spectroscopy (AAS) on soil, water, and vegetable samples collected from Asa-river farmland. The results showed variations in metal content across samples, exceeding WHO guidelines, except for cadmium in water and plant samples and nickel in Soil Samples 4 and 6. These methods offer favorable toxicological, environmental, and economic benefits, making them ideal for routine monitoring of pesticides and heavy metals in agricultural farm products.

Keywords: Design of experiment, heavy metals, pesticide residues, pollution, QuEChERS

1. Introduction

The rapid industrialization, urbanization, and intensification of agricultural practices have led to unprecedented environmental pollution with heavy metals and organic pollutants, such as pesticides. Since the 1940s, the migration rate of these pollutants has increased dramatically, posing significant environmental and health risks [1]. Heavy metal pollution, in particular, has become a major concern due to its harmful effects on ecosystems and human health [2]. Agricultural and industrial activities have compromised natural resources, including soil and water, in many countries. Industrial processes have contributed substantially to elevated concentrations of heavy metals in the environment [3]. These pollutants persist in water, plants, soil, and ultimately, food, leading to adverse human health effects, including carcinogenic and non-carcinogenic risks [4]. Furthermore, exposure to contaminated underground water resources poses significant health risks [5]. The persistence and bioaccumulation of heavy metals and organic pollutants in the environment necessitate urgent attention and effective mitigation strategies to protect human health and ecosystem integrity.

Pesticides are chemical compounds, natural or synthetic, used to control, prevent, or destroy crop pests and vectors of plant diseases. These organic compounds comprise various functional groups and isomeric forms, playing a crucial role in agricultural pest management [6]. The use of pesticides has led to significant increases in food production, improved quality, and reduced incidence of insect-borne diseases. However, the substantial growth in pesticide use has raised concerns about their toxicity [7]. Despite the benefits, occupational and accidental exposure to pesticides has been linked to chronic health effects, including endocrine disorders, blood disorders, and genetic changes. It is essential to address the risks associated with pesticide exposure and explore safer alternatives [4].

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method is a widely accepted technique for analyzing pesticide residues in food chains, particularly fruits and vegetables [8]. This

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method involves a five-step process: crushing the sample, using acetonitrile for extraction and separation, adding $MgSO_4$ and other salts to remove water, and utilizing adsorbents to remove impurities by leveraging interactions between adsorbent fillers and matrix impurities. Finally, the supernatant is analyzed using GC-MS and LC-MS. According to [9], QuEChERS is ideal for multi-class and multi-residue analysis of pesticide residues.

Design of Experiment (DOE) is a chemometric approach that optimizes experimental conditions by identifying significant factors, estimating main and interaction effects, and minimizing experimental runs. This efficient approach streamlines experimentation, reducing analysis time and enhancing sample throughput [10]. This study aims to develop a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method for analyzing pesticides and digestion methods for heavy metals in plant, soil, and water samples. Specifically, Placket-Burman Design (PBD) and Central Composite Design (CCD) are employed to determine the critical factors influencing the effective and efficient extraction of multiclass pesticide residues in vegetables, soil, and water.

2. Materials and Methods

2.1. Sample Collection and Preparation

The accurate analysis of heavy metals and pesticides in environmental samples requires a systematic and precise approach to sample collection and preparation (Fig. 1).

Soil: Three (3) soil samples were collected using soil augers or corers at 15 cm depths, ensuring representation across the study area. Multiple samples were collected from each location to account for spatial variability and air dried. The collected soil samples were grinded in a mortar with pestle to fineness and sieved thoroughly. The collected soil samples were grinded in a mortar with pestle to fineness and sieved thoroughly. Then 2.00 g was weigh using weighing balance with sensitivity of (0.001 mg), and the sieved samples was subjected to QuEChERS and wet acid digestion procedures which were done in the laboratory.

Plants: Three (3) plant samples of *Amaranthus hydridus* were carefully collected, considering factors such as growth stage, and potential for bioaccumulation of contaminants. The collected vegetable samples were blended and homogenized, then 2.00 g was weigh using weighing balance with sensitivity of (0.001 mg) and were subjected to QuEChERS and wet acid digestion procedures.

Water: Three (3) water samples were collected from the river used to irrigate the vegetables. The water samples were collected at various depths and sufficient volume was collected for the analysis and it been kept inside a clean container.

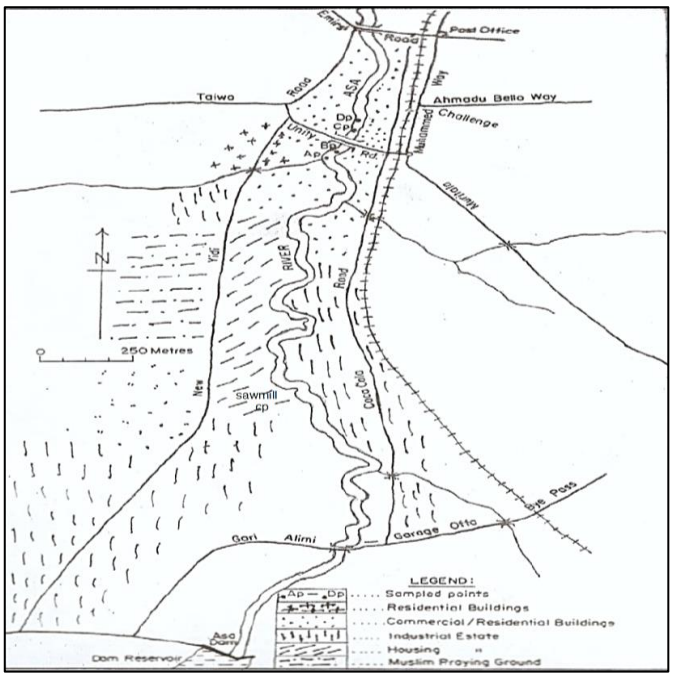

Figure 1. A geographical map of Asa River segment showing study area

2.2. QuEChERS Procedure

A sample preparation protocol was employed to extract and clean up the samples. For soil and vegetable samples, 2.00 g of each was transferred to a 20 mL centrifuge tube, while 2.00 mL of water sample was used. Acetonitrile (10 mL) was added to each sample, and the mixture was vortexed for 1 minute. This extraction step was repeated with an additional 10 mL of acetonitrile. Next, a mixture of salts and an internal standard was added to the sample. Specifically, 6 g of MgSO4, 1.5 g of sodium chloride, 1 g of sodium citrate, and 100 µL of triphenyl phosphate (20 µg/mL) were added. The mixture was vigorously stirred for 1 minute and then centrifuged at 4000 rpm for 5 minutes. Following centrifugation, 6 mL of the supernatant was transferred to a Supel QuEchSPE kit (55437-U) containing 900 mg of $MgSO_4$ and 150 mg of primary-secondary amine (PSA). The mixture was then centrifuged again at 4000 rpm for 5 minutes.

Finally, 5 mL of the supernatant was acidified with 50 µL of 5% formic acid (10 µL/mL of extract) in acetonitrile. The resulting extract was analyzed using GC-MS, adhering to the QuEChERS method and EN 15662-2008 guidelines.

2.3. GC-MS Analysis

The analysis was performed using a (Shimadzu QP2010 Series) GC-MS system operated in split/splitless mode at an injection temperature of 270 ℃. Separation of target analytes was achieved on a DB-5MS fused capillary column (30 m x 0.25 mm i.d. x 0.25 μm film thickness) composed of 5% diphenyl and 95% dimethylpolysiloxane.

The gas chromatography (GC) instrument conditions were optimized for the analysis. A high-pressure Merlin Microseal septumless injection kit and a salinized narrow bore liner (78.5 mm x 6.5 mm o.d. x 0.75 mm i.d.) were used. Helium served as the carrier gas, maintained at a constant flow rate of 1.3 mL/min and linear velocity of 42 cm/sec. The GC column oven temperature program was carefully designed to ensure optimal separation and detection. The temperature program consisted of an initial hold at 60 ℃ for 2 minutes, followed by a series of ramps: 30 ℃/min to 180 ℃, 3 ℃/min to 210 ℃, and 20 ℃/min to 280 ℃. The final temperature was held for 5 minutes, resulting in a total runtime of 24.50 minutes. The MS operating conditions included a transfer line temperature of 300 ℃, ion source temperature of 200 ℃, and electron ionization (EI) of 70 eV. Method optimization was performed in scan mode, while quantitation was done in selected ion monitoring (SIM) mode. A target ion (most abundant ion) and two reference ions were monitored for each target analyte. Pesticide identification was achieved by matching retention times with standards, comparing relative abundance, and matching mass spectra with the NIST library. The NIST library provided a list of best matches based on abundant mass-to-charge ratios, enabling identification of pesticides [11].

2.4. Validations of the Methods

The validation was carried out according to EU commission Detection. The method performance was evaluated by the following parameters: matrix effect study, establishment of matrix – matched calibration, limit of detection (LOD).

2.5. Digestion of Samples

A modified digestion method was employed for preparing soil, water, and vegetable samples for Atomic Absorption Spectroscopy (AAS) analysis. Soil sample digestion was performed using a combination of heat and acid treatment. Specifically, 1 g of oven-dried soil sample was placed in a 250 mL digestion tube, and 10 mL of concentrated nitric acid (HNO_3) was added. The mixture was initially heated at 90 ℃ for 45 minutes, followed by an increase in temperature to 150 ℃, where it was boiled for 6 hours until a clear solution was obtained. During the digestion process, an additional 5 mL of concentrated HNO_3 was added at least three times to facilitate complete digestion. The process continued until the volume of the solution was reduced to approximately 1 mL. After digestion, the interior walls of the tube were rinsed with distilled water, and the contents were thoroughly mixed to prevent sample loss. Once cooled, 5 mL of 1% HNO_3 was added, and the solution was filtered sequentially through Whatman No. 42 paper and 0.45 μm Millipore membrane filters. The filtered solution was then transferred to a 100 mL volumetric flask and diluted to the mark with distilled water.

For water sample digestion, 5 mL of concentrated HNO_3 was added to 100 mL of the water sample, and the mixture was evaporated on a hot plate to a final volume of 20 mL. After cooling, another 5 mL of concentrated HNO_3 was added, covered with a watch glass, and refluxed for 25 minutes. The mixture was then heated on a hot plate while adding concentrated HNO_3 until it became light in color and clear. The beaker wall and watch glass were rinsed thoroughly with distilled water, and the resulting digest was filtered through Whatman No. 1 filter paper into a 100 mL volumetric flask and made up to the mark with distilled water.

Vegetable sample digestion involved weighing 2 g of sample into a beaker, adding 10 mL of analytical-grade nitric acid, covering with a watch glass, and cold soaking for 30 minutes. The beaker was then heated to 120 ℃ for 2 hours, cooled to room temperature, and transferred to a 100 mL volumetric flask. The digest was made up to the mark with distilled water. The resulting digests were subjected to AAS analysis.

2.6. AAS Measurement

The instrument was calibrated with NIST heavy metal standards. Blank solutions were run with each digestion to check for interference and contamination. Heavy metal concentrations are reported as mg/kg dry weight (soil and food) or mg/L (water). All samples were analyzed in triplicate.

2.7. Statistical Analysis

All experiment data was carefully analyzed using MINITAB version 17 statistical software. Microsoft excel was used for the calculation of standard deviation of mean and relative standard deviation. Significant differences between concentrations of the heavy metals following the digestion methods were analyzed by ANOVA using SPSS statistical software (Version 20). Statistical significance was defined as $p < 0.05$.

2.8. Design of the Experiment

In analytical chemistry, Design of Experiment (DOE) plays a crucial role in optimizing key factors, ultimately

enhancing the performance of analytical methods, processes, and products. DOE facilitates the understanding of main interaction effects between factors and models the relationships between factors and responses, all while minimizing the number of experiments required. There are two primary DOE approaches: univariate, which optimizes one factor at a time while holding other factors constant, and multivariate, which includes Central Composite Design (CCD) and Plackett-Burman Design (PBD). These multivariate designs, particularly CCD and PBD, are widely used to investigate multiple factors simultaneously, identifying significant factors and interactions, modeling complex relationships, reducing experimental requirements, and enhancing method robustness [12].

3. Results and Discussion

3.1. Design of Experiment

3.1.1. Placket-Burman Design (P-B design)

A multivariate method was developed for determining pesticide residues in soil, water, and vegetable samples. Plackett-Burman (P-B) design was employed to screen the most critical factors influencing QuEChERS efficiency and recovery [12]. This design enabled the estimation of significant factors impacting efficiency and provided valuable insights into each variable with a minimal number of experimental runs. The factors and level of variable selected for P-B design are shown in Table 1.

Table 1. Levels and factors used in P-B design for QuEChERs

S/N	Factor	Level	
		Low (-)	High (+)
1	Mass of Sample (mg)	1	2
2	Sample/water ratio (mg/mL)	1	2
3	Mass of MgSO4 (mg)	1	6
4	Volume of acetonitrile (mL)	5	10
5	Percentage of acetic acid in acetonitrile (%)	0	2
6	Mass of NaCl (mg)	0	2
7	Centrifugation speed (rmp)	2000	4000
8	Centrifugation time (sec)	2	5
9	Mass of sodium citrate (mg)	0	2

The results of the 12 experimental runs of the Plackett-Burman (P-B) design, examining nine factors at two levels each, are visually represented in a Pareto chart (Fig. 2) and a normal plot of standardized effects (Fig. 3). These plots display horizontal bars for the screened factors, with a red vertical line indicating the significance level. Each factor's levels are denoted as + (higher level) and - (lower level) as presented in (Table 2), providing a clear illustration of the significance of each factor [13].

Table 2. Design Table (randomized) for Plackett-Burman Design

Run	Block (Blk)	A	B	C	D	E	F	G	H	J
1	1	-	+	+	-	+	-	-	-	+
2	1	+	-	-	-	+	+	+	-	+
3	1	-	-	-	-	-	-	-	-	-
4	1	+	+	+	-	+	+	-	+	-
5	1	+	-	+	-	-	-	+	+	+
6	1	-	-	+	+	+	-	+	+	-
7	1	-	-	-	+	+	+	-	+	+
8	1	+	+	-	+	-	-	-	+	+
9	1	-	+	-	-	-	+	+	+	-
10	1	+	-	+	+	-	+	-	-	-
11	1	+	+	-	+	+	-	+	-	-
12	1	-	+	+	+	-	+	+	-	+

NB. A=mass of sample, B= sample/water C= mass of $MgSO_4$, D=volume of acetonitrile, E=mass of NaCl F=% of acetic in acetonitrile, G=centrifugation speed, H=centrifugation time, J=mass of sodium citrate,

The normal plot of standardized effect (Fig. 3) shows that volume of acetonitrile (D) has the most significant effect with about 90 %, followed by mass of NaCl (E) (approx. 80%), centrifugation speed (G) and mass of $MgSO_4$ (C) were below average effect (30 % and 20 % respectively). volume of acetonitrile (D) has been observed to have dual effect on extraction efficiency. It enhances the transport of analytes and also causes a decrease in distribution coefficient and therefore must be carefully optimized.

3.1.2. Central Composite Design (CCD)

The Plackett-Burman design screening experiment identified five factors with negligible impact on extraction efficiency: volume of acetonitrile, centrifugation speed, centrifugation time, mass of sodium citrate, and mass of sodium acetate. These factors were subsequently fixed at their optimal values, determined through univariate optimization. In contrast, five significant variables - sample mass, $MgSO_4$ mass, volume of acetonitrile, acetic acid percentage in acetonitrile, and NaCl mass - were selected for further optimization using a Central Composite Design (CCD) and Response Surface Methodology (RSM), as presented in (Table 3). This optimization significantly enhanced the extraction and cleanup efficiencies of the QuEChERS technique [14]. RSM screening identified significant factors affecting extraction efficiency, visualized in the Pareto chart (Fig. 2 and Fig. 3). Horizontal bars represent screened factors, with the red vertical line indicating the significance threshold It can be observed from (Fig. 2 and Fig. 3) that the mass of the sample (A), percentage of acetic acid in acetonitrile (F) and centrifuge time (H) did not significantly affect extraction efficiency. Therefore, they were fixed according to the optimal value estimated using the one-factor-at-a-time (OFAT) approach. The volume of acetonitrile (D), sample/water ratio (B), mass

of $MgSO_4$ (C), mass of NaCl (F) and centrifugation speed (G), which were found to significantly affect extraction efficiency, were further optimized by the second-order central composite design (CCD), utilizing response surface methodology (RSM). These factors (Table 3) increased the extraction and clean-up efficiencies of the QuEChERS technique.

Table 3. The 2-level significant factors of QuEChERS methods

S/N	Factor	Level	
		Low (-)	High (+)
1	Mass of Sample (mg)	1	2
2	Mass of $MgSO_4$ (mg)	1	6
3	Volume of acetonitrile (mL)	5	10
4	Percentage of acetic acid in acetonitrile (%)	0	2
5	Mass of NaCl (mg)	0	2

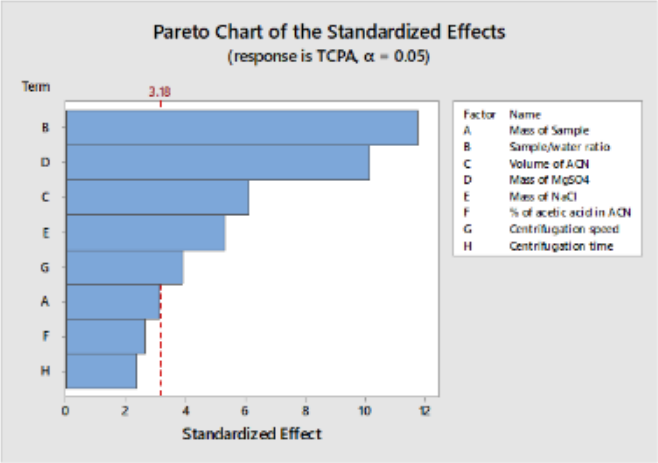

Figure 2. Pareto chart of the standardized effects

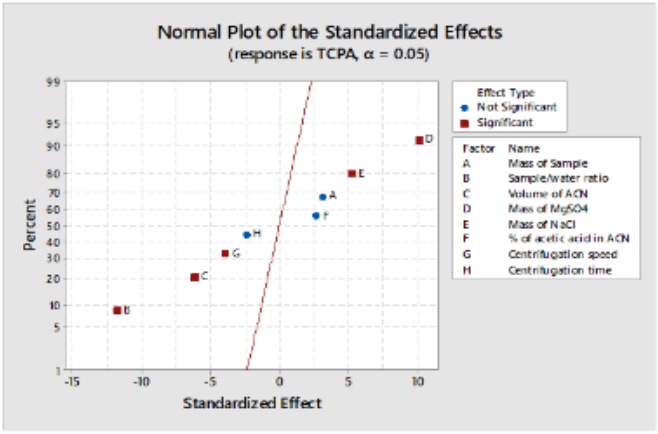

Figure 3. Normal plot of the standardized effects

3.2. Method Validation

The analytical data of the optimized Quencher method was validated for the determination of 17 target pesticides in soil sample (A) and vegetable sample (B) as presented in (Table 4). The limits of quantitation (LOQ) and detection (LOD) were determined experimentally. The LOQ was calculated using a signal-to-noise ratio of 10, while the LOD was calculated using a signal-to-noise ratio of 3. The standard deviation of the y-intercept of the regression line of the calibration curve was used for these calculations. The calibration curve was constructed using the internal standard method. The peak area ratio of each target analyte to the internal standard was plotted against the concentration of each analyte. The resulting calibration curve exhibited the linearity ranges from 5–500 µg/kg, the correlation coefficients (R^2) were around 0.99 for all the tested pesticide residues. The LOD ranges (0.18–6.10 and 0.123–6.10) µg/kg for soil and vegetable samples respectively, while the LOQ ranges from (0.599–20.33 and 0.40–20.313) µg/kg for soil and vegetable samples respectively. Validation procedures were carried out to verify whether the analytical procedure used is suitable. This is essential in ensuring the optimal utilization of analytical resources [15]. The concentration levels of each pesticide residues were analyzed, and the calibration curve was constructed [15]. A set of calibration curves were prepared with concentrations ranging from 5 to 500 µg/kg using an external standard calibration method. The calibration curve was linear over the tested concentration range (Table 4).

Table 4. Linearity ranges (µg/kg), LOD and LOQ (µg/kg) of the developed QuEChERS method

Residues	R^2	Linear range (µg/kg)	LOD (µg/kg)		LOQ (µg/kg)	
			Sample A	Sample B	Sample A	Sample B
.alpha.-Lindane	1	5–500	0.76	0.23	2.53	0.76
.beta.-Lindane	0.99	5–500	0.983	1.0	3.27	3.33
.gamma.-Lindane	1	5–500	1.12	3.12	3.73	10.38
.delta.-Lindane	0.99	5–500	1.0	1.12	3.33	3.72
Endosulfan ether	1	5–500	1.41	8.71	4.69	29.00
Heptachlor	1	5–500	0.27	3.12	0.89	10.38
Aldrin	0.99	5–500	0.28	1.45	0.93	4.82
Heptachlor epoxide	1	5–500	0.28	0.45	0.93	1.49
DDMU	1	5–500	0.46	0.68	1.53	2.26
alpha.-Endosulfan	0.99	5–500	0.18	0.38	0.59	1.26
p,p'-DDE	1	5–500	0.26	0.46	0.86	1.53
Dieldrin	0.99	5–500	0.36	0.36	1.19	1.19
Endrin	1	5–500	0.43	0.123	1.43	0.409
.beta.-Endosulfan	1	5–500	0.2	1.11	0.66	3.69
m,p'-DDD	0.99	5–500	2.14	2.10	7.13	6.993
p,p'-DDT	1	5–500	5.12	5.2	17.06	17.316
Methoxychlor	0.99	5–500	6.1	6.1	20.33	20.31

The accuracy of the developed method was evaluated by determining the relative recoveries of pesticide standards spiked into different samples (Table 5). The chromatograms of the samples, spiked at 100 µg/kg, showed no matrix effect. The relative recoveries for sample A ranged from 95.59% to 115.33%, while those for sample B ranged from 96.40% to 116.13%.

Table 5. Accuracy (relative recoveries) and precisions (relative standard deviation) of the pesticides in samples

Residues	Spiked (µg/kg)	Soil sample		Vegetable Sample	
		%Rec	%RSD	%Rec	%RSD
.alpha.-Lindane	100	97.5	1.2	96.7	0.3
.beta.-Lindane	100	98.2	1.6	99.3	1.6
.gamma.-Lindane	100	98.7	1.8	106.3	5.1
.delta.-Lindane	100	98.3	1.6	99.7	1.8
Endosulfan ether	100	99.6	2.3	125.0	14.3
Heptachlor	100	95.8	0.4	106.3	5.1
Aldrin	100	95.9	0.4	100.8	2.3
Heptachlor epoxide	100	95.9	0.4	97.4	0.7
DDMU	100	96.5	0.7	98.2	1.1
alpha.-Endosulfan	100	95.5	0.2	97.2	0.6
p,p'-DDE	100	95.8	0.4	97.5	0.7
Dieldrin	100	96.1	0.5	97.1	0.5
Endrin	100	96.4	0.7	96.4	0.2
.beta.-Endosulfan	100	95.6	0.3	99.6	1.8
m,p'-DDD	100	102.1	3.5	102.9	3.4
p,p'-DDT	100	112.0	8.4	113.3	8.5
Methoxychlor	100	115.3	10.0	116.3	10.0

RSD= relative standard deviation, %Rec = relative recoveries

These results demonstrate the high accuracy and reliability of the developed method. These are all acceptable according to the SANCO guidelines [16], which state that the method performance criteria require that mean recoveries should be within the range of 70–120% with precisions less than or equal to 20%. The average recoveries obtained in this study align with the report of [17] who obtained average recoveries ranging from 83–99%, but as a result of the fact that the design of experiments was employed to optimize factors, the optimized factors gave improved results.

3.3. Analysis of Real Sample

The analysis of pesticide residues in soil, water, and vegetable samples revealed varying concentrations. Fig. 4 presents the total mean concentration (µg/kg) of pesticide residues in the samples. Notably, pesticide residues were not detected in Soil Sample 1, water samples. In contrast, Soil Sample 2 contained 17 pesticides, with concentrations ranging from 0.00–1.41 mg/L. Endosulfan ether had the highest concentration (141.0 µg/kg), followed by Methoxychlor (60.00 µg/kg), while beta-Endosulfan had the lowest concentration (<LOQ µg/kg). Aldrin and DDMU were not detected, and Heptachlor, Heptachlor epoxide, alpha-Endosulfan, p,p'-DDE, Dieldrin, and Endrin were below the calibration limit.

In vegetable samples (1 & 2), pesticide residues were detected within the range of <LOQ–870.0 µg/kg for Sample 1 and <LOQ–110.00 µg/kg for Sample 2. Endosulfan ether had the highest concentration (870.00 µg/kg) in vegetable sample 1, followed by delta-Lindane (110.00 µg/kg), while Heptachlor epoxide and Endrin below the LOQ. In Sample 2, delta-Lindane (110.00 µg/kg), had the highest concentration, followed by Methoxychlor and p,p'-DDT with concentration of (60.00 and 50.00 µg/kg) respectively, while Heptachlor epoxide and Endrin having the lowest concentrations (<LOQ µg/kg). Heptachlor was not detected in vegetable sample 2, and several pesticides were below the calibration limit in both vegetable samples.

3.4. Heavy Metal Concentration

3.4.1. Method validation

For the heavy metals considered in this study, the Limit of Quantification (LOQ) and Limit of Detection Method (LOD) were calculated using standard formulas: LOQ = 3 × SD and LOD = 10 × SD. The LOQ and LOD values were specifically confirmed by sample and blank atomic absorption spectrometers. The precision and reliability of the heavy metals considered in this study are displayed in (Table 6). The relative standard deviation (RSD%) of the collected samples were analyzed.

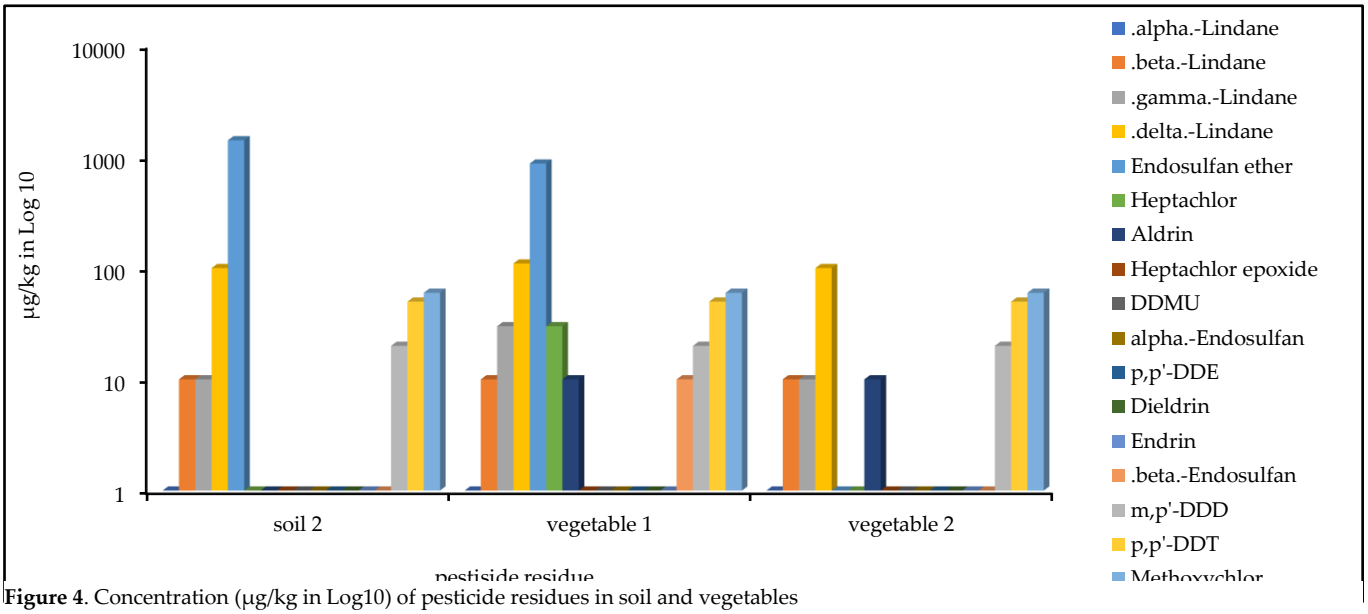

Figure 4. Concentration (µg/kg in Log10) of pesticide residues in soil and vegetables

Table 6. Data specification for AAS, LOD, LOQ and % RSD for the heavy metals considered in this study.

METALS	LOD (µg/L)	LOQ (µg/L)	RSD%	R^2
As	2.1	21.2	7.1	0.9385
Cd	0.1	1.0	1.0	0.9385
Cu	10.6	106.1	3.5	0.9385
Co	6.3	63.6	2.1	0.9385
Fe	12.7	127.2	4.2	0.9385
Ni	8.4	84.8	2.8	0.9385
Pb	6.3	63.6	2.1	0.9385
Zn	8.4	84.8	2.8	0.9385

Table 7. Concentration of heavy metals in water sample

METALS (mg/L)	Mean values ± Std. deviation	WHO (2011) Guidelines for water
As	3.25±0.07	0.03
Cd	0.00±0.00	0.03
Cu	63.25±0.60	0.02
Co	22.25±0.02	0.08
Fe	307.00±4.00	0.3
Ni	10.50±0.02	0.1
Pb	58.25±0.20	0.01
Zn	95.50±0.20	5.00

3.4.2. *The concentration of heavy metal in water*

Heavy metals play a critical role in eco-chemistry and eco-toxicology due to their toxicity at low concentrations and tendency to bioaccumulate in human organs. To mitigate the risks associated with heavy metal exposure, establishing dietary limits for metals in food, vegetables, and soil is essential. The Food and Agriculture Organization/World Health Organization (FAO/WHO) has established permissible limits for metals in soils, waters, and plants.

This study aimed to investigate the mean concentrations of nine heavy metals - cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), cobalt (Co), arsenic (As), and iron (Fe) - in soil, water, and plants within the study area.

Table 7 shows that all the studied heavy metals in water samples exceeded the WHO guidelines for drinking water, except for cadmium (Cd), which was below the limit. The highest concentration was recorded for iron (Fe) at 307±4.00 mg/L, followed by zinc (Zn) at 95.5±0.6 mg/L. Arsenic (As) had the lowest concentration at 3.25±0.07 mg/L.

Water systems, including rivers, streams, and lakes, are vulnerable to contamination through runoff, drainage, and disposal via sediments or wastewater [18]. Groundwater is also impacted through leaching or transport via mobile colloids. The release of heavy metals into water environments poses a significant threat to aquatic ecosystems. Due to their toxic and accumulative properties, heavy metals can alter the diversity of aquatic species and disrupt the delicate balance of ecosystems [18]. This study found elevated levels of copper (Cu) and iron (Fe) in waterholes, likely resulting from copper-containing waste discharge and anthropogenic wastewater effluents, respectively. These findings align with [19], who reported high iron levels in River Omo and Kainji Lake National Park waterholes. Lead (Pb) and nickel (Ni) levels exceeded permissible limits, potentially due to industrial waste discharges and heavy-duty vehicle exhaust [20]. Cadmium (Cd) levels surpassed guidelines, likely caused by fertilizer and sewage sludge runoff from surrounding areas [21]. Statistical analysis revealed a positive correlation between metals ($p = 0.04$, $p < 0.05$), indicating that changes in one metal's concentration are associated with changes in others.

3.4.3. *The concentration of heavy metal in plant*

The analysis of heavy metal concentrations in plant samples revealed that, except for iron (Fe) and nickel (Ni), all studied metals were below World Health Organization (WHO) recommended limits. Notably, iron (Fe) and nickel (Ni) exceeded guidelines at concentrations of 298±1.0 mg/kg and 7.25±0.007 mg/kg, respectively as shown in (Table 8). Iron (Fe) had the highest concentration (298±0.1 mg/kg) in plant samples, followed by zinc (Zn) at 112.75±0.2 mg/kg. The levels of iron (Fe) and zinc (Zn) in plants were significantly influenced by their respective soil content and runoff, as previously reported [21]. The presence of these metals in the study area may be attributed to human activities, including combustion of coal, residential wood combustion, iron and steel production, and power plant operations [22]. These findings suggest that anthropogenic factors contribute to heavy metal accumulation in plants, potentially impacting ecosystem health.

Moreover, Cadmium (Cd) exhibited the lowest concentration of 0.75±0.0007 mg/kg, while nickel (Ni) had a concentration of 7.25±0.007 mg/kg in the study area (Table 9). The presence of these metals in plants can be attributed to factors such as the application of fertilizers and pesticides, industrial waste disposal, and atmospheric contaminant deposition. Furthermore, statistical analysis revealed that the correlation between

Table 8. Concentration of heavy metals in plant sample

METALS (mg/kg)	Mean values ± Std. deviation	WHO recommended limits
As	6.50±0.01	0.2
Cd	0.75±0.00	0.2
Cu	56.75±0.04	10
Co	41.00±0.04	0.2
Fe	298.50±0.10	425.5
Ni	7.25±0.00	67.9
Pb	37.25±0.03	2.0
Zn	112.75±0.20	99.4

Table 9. Concentration of heavy metals in soil samples

SAMPLE	METALS (mg/kg)							
	As	Cd	Cu	Co	Fe	Ni	Pb	Zn
Soil 1	4.50±0.0014	1.50±0.001	97.0±0.002	27.0±0.004	266.25±0.9	17.25±0.004	47.25±0.003	106.5±0.002
Soil 4	30.50±0.002	52.25±0.0007	297.0±0.006	81.0±0.004	19452±14.0	27.0±0.002	106.25±0.004	360.5±0.1
Soil 5	22.0±0.002	42.0±0.004	607.0±0.001	96.25±0002	21390.5±20.0	42.25±0.007	148.0±0.002	547.0±0.004
Soil 6	45.75±0.003	67.75±0,003	638.25±0.01	74.25±0.003	12974.25±19.0	31.25±0.002	91.75±0.003	323.0±0.004
WHO PL	20	0.8	36	0.2	50000	35	85	50

metal concentrations was not statistically significant ($p = 0.87$, $p < 0.05$), indicating a negative correlation. This suggests that an increase in one metal's concentration corresponds to a decrease in another.

3.4.4. The concentration of heavy metal in soil

The analysis of heavy metals in soil samples from areas 1, 4, 5, and 6 revealed varying concentrations. In Soil Sample 1, only iron (Fe), arsenic (As), nickel (Ni), and lead (Pb) were within the World Health Organization's (WHO) maximum allowable limits, with concentrations of 266.25±0.9, 4.50±0.0014, 17.25±0.004, and 47.25±0.003 mg/kg, respectively (Table 9). In contrast, Soil Samples 4 and 6 had only nickel (Ni) within the allowable limits, with concentrations of 27.0±0.002 and 31.25±0.002 mg/kg, respectively. Iron (Fe) had the highest concentration in both samples, with 19,452±14 mg/kg in Sample 4 and 12,974±19.0 mg/kg in Sample 6. Soil Sample 5 exceeded WHO limits for all studied heavy metals, with iron (Fe) having the highest concentration (21,390±20.0 mg/kg) and nickel (Ni) the lowest. These findings are consistent with [23], who noted that soils and sediments serve as primary repositories for metal contaminants in terrestrial and aquatic ecosystems, respectively. Heavy metals in soils pose a significant threat to human and animal health through the consumption of contaminated plants. Analysis of soil samples revealed that mean heavy metal levels exceeded maximum allowable limits. Notably, iron (Fe), copper (Cu), and zinc (Zn) concentrations suggest minimal anthropogenic influence. Zinc, an essential trace element for humans, animals, and plants, plays a crucial role in combating skin issues like acne, boils, and sore throats [24]. Copper enters soil through various means, including contamination from pipes and wires, and algal growth control measures. While copper is vital for organism development, excessive or deficient levels can be harmful [25]. As soil concentrations surpass permissible limits, the risk of heavy metal poisoning through the food chain increases [26]. This highlights the need for monitoring and mitigating heavy metal contamination in soils.

3.5. Pearson correlation Analysis of Heavy Metals

Table 10 revealed the Pearson correlative matrix of heavy metals in all the samples. A positive correlation PC is represented by green color with (PC ≤ 1.00). The correlation matrix of all heavy metals (Cd, Pb, Zn, Cu, Ni, Co, As and Fe) in soil, water, and plants in the study areas as seen in Table 10, shows a strong positive correlation between (Cd, Pb, Zn, Cu, Ni, Co, As and Fe) above (>0.500) and below (<1.00). This implies that there is the possibility that heavy metals are emitted from similar sources. The result agrees with the findings of [27], who explained that common source of metals contamination input is possible across different sampling sites in the same study area.

Table 10. Correlation matrix of all studied heavy metals in the farm areas

	As	Cd	Cu	Co	Fe	Ni	Pb	Zn
As	1							
Cd	0.9810	1						
Cu	0.8477	0.8866	1					
Co	0.7685	0.8586	0.8628	1				
Fe	0.7373	0.8512	0.8147	0.9705	1			
Ni	0.6996	0.8032	0.9224	0.8980	0.9075	1		
Pb	0.6039	0.7394	0.8294	0.9131	0.9517	0.9451	1	
Zn	0.6574	0.7775	0.8664	0.9665	0.9644	0.9542	0.9808	1

4. Conclusion

In conclusion, the importance of monitoring pesticide residues in soil and plant-based foods has led to the development of various sample preparation methods. This study employed the QuEChERS-AOAC technique, a rapid and environment-friendly method, for analyzing pesticide residues in soil, water, and vegetable samples along Asa-river farmland using GC-MS. The results showed that pesticide residues were detected in soil and vegetable samples, but not in water samples, with concentrations below the maximum residue level. Notably, Soil Sample 1 and water samples had no detectable pesticide residues. However, heavy metal analysis revealed variations in metal content across samples, exceeding WHO guidelines, except for cadmium (Cd) in soil and plant samples and nickel (Ni) in Soil Samples 4 and 6. The study highlights the importance of regular monitoring of pesticide residues and heavy metals in soil, water, and vegetables to ensure food safety and prevent environmental contamination. Efforts should be made to reduce contamination in the study area, and safe pesticide usage practices should be promoted among farmers. The QuEChERS method has proven to be a fast, accurate, and efficient sample

preparation technique for pesticide residue analysis, offering a simple and effective alternative to traditional solid-phase extraction methods. Continued monitoring and mitigation efforts are necessary to protect human and environmental health.

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