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Multivariate Analysis of Pesticides in Environmental Samples using High Performance Liquid Chromatography

(Analisis Multivariat Pestisid dalam Sampel Alam Sekitar menggunakan Kromatografi Cecair Berprestasi Tinggi)

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ABSTRACT

In the agricultural regions of Quetta district, Pakistan, the concentrations of pesticide residues (diazinon, malathion and aldrin) were measured in vegetable, soil and water samples. A sensitive and simple method has been developed by using a chemometric approach for quantification of three pesticides, namely diazinon, aldrin, and malathion in leafy vegetables by using high-performance liquid chromatography (HPLC). In the modified ultrasonication method, the factor and responses were optimised through a central composite design. Response surface plots show the importance of sample weight and sonication time factor. This method has linearity range of 0.5 mg/kg-2.5 mg/kg, a correlation coefficient of 0.99, and a detection limit ranges between 0.01 mg/kg-3 mg/kg. The recovery ranges between 85%-93.2%, with relative standard deviation (RSD) between 0.86%-11%. This method was applied to a real vegetable sample in the city of Quetta. Soil and water samples were also collected with these vegetable samples in different seasons. The presence of pesticides in soil and vegetable samples pose a serious risk to people who rely on these foods for sustenance. There was no significant correlation amongst the seasonal distribution of pesticides and physicochemical properties of soil indicating different application techniques and/or mechanisms rather than soil characteristics are responsible for the residue's existence. Therefore, it is advised that continuous monitoring to be conducted in order to control the use of these insecticide in the research area.

Keywords: Chromatography; pesticides; soil characteristics; vegetables

ABSTRAK

Kepekatan sisa racun perosak (diazinon, malation dan aldrin) di kawasan pertanian daerah Quetta, Pakistan, telah diukur dalam sampel sayur-sayuran, tanah dan air. Satu kaedah yang sensitif dan mudah telah dibangunkan menggunakan pendekatan kemometrik untuk kuantifikasi tiga racun perosak diazinon, aldrin dan malation dalam sayur-sayuran berdaun dengan menggunakan kromatografi cecair berprestasi tinggi (HPLC). Dalam pengubahsuaian kaedah ultrasonik, faktor dan tindak balas telah dioptimumkan melalui reka bentuk komposit berpusat. Plot permukaan tindak balas menunjukkan kepentingan berat sampel dan faktor masa sonikasi. Kaedah ini mempunyai julat kelinearan antara 0.5-2.5 mg/kg, pekali korelasi 0.99, dan julat had pengesanan antara 0.01-3 mg/kg. Perolehan semula berjulat antara 85-93.2%, dengan sisihan piawai relatif (RSD) antara 0.86-11%. Kaedah ini digunakan untuk analisis sampel sayuran sebenar di bandar Quetta. Sampel tanah dan air juga dikumpulkan bersama sampel sayuran pada musim yang berbeza. Kehadiran racun perosak dalam sampel tanah dan sayur-sayuran menimbulkan risiko serius kepada pengguna yang bergantung pada sumber makanan ini. Tidak terdapat korelasi yang signifikan antara taburan bermusim racun perosak dan sifat fizikokimia tanah, seterusnya menunjukkan teknik aplikasi dan/atau mekanisme yang berbeza dan bukannya ciri tanah yang bertanggungjawab terhadap kewujudan sisa. Oleh itu, disarankan agar pemantauan berterusan dapat dilakukan untuk mengawal penggunaan racun perosak ini di sekitar kawasan kajian.

Kata kunci: Ciri tanah; kromatografi; plot tindak balas permukaan; racun perosak; sayuran

INTRODUCTION

Pesticides are mixture of substances or any substance, synthetic or natural, used for preventing or organising any pest that competes with human for food, spreading disease and destroying property. The term pest consists of microbes, insects, weeds, and mammals. Pesticides can be biological agents, i.e., virus or bacteria. Before application, pesticides are diluted with water in many commercial formulations and also contain certain chemicals for pesticide retention on shoots and leaves (Yang Farina et al. 2017). Green leafy vegetables are low content in fats, and excellent supply of proteins and vitamins. The nitrates found in green leafy vegetables play an important role in many diseases, i.e., hypertension, blood pressure reducing, healing wounds and reducing cardiovascular diseases. Leafy vegetables also act as an antioxidant to avoid radicals that act on biomolecules like proteins, fats and deoxyribonucleic acids (Bakırcı et al. 2014; Yang Farina et al. 2017). The World Health Organisation (WHO) account approximately 3 million toxic pesticides that cause universal death of about 220,00 people. The human health is also affected by pesticides causing disorders like allergies, nausea, headaches, chronic disorders (cancer, reproductive failure) and neurological disorders (Yang Farina et al. 2017). The organochlorine pesticide (OCP) aldrin can be used to manage a variety of soil insects, including termites, rice water weevils, wireworms, maize rootworms, and grasshoppers having maximum residue limit (MRL) of 0.02 (Kumar, Gupta & Soni 2025). Amongst the registered broad-spectrum insecticides is diazinon (O,O–diethyl–O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate). Since then, diazinon has been used to manage aphids, thrips, red spider mites, whiteflies, Diamondback moths, fruit flies, fruit worms, locusts, and grasshoppers (Oromo et al. 2025). When diazinon is sprayed in accordance with GAPs, the overall dissipation effect, including effectiveness against insect pests, should lower diazinon residues to levels below the Codex maximum residue limit (MRL) of 0.05 mg/kg. Malathion's history begins in the 1940s, during World War II. It is a member of the class of insecticides known as organophosphorus; even minute amounts can have cumulatively harmful effects after repeated exposure. It reduces acetylcholinesterase enzyme activity, neurological and respiratory conditions, liver enzyme abnormalities, and other illnesses that result in cancer are all caused by the long-term cumulative toxic effect (Koca et al. 2024). Due to their careless, extravagant, indiscriminate usage, and improper treatment, these chemicals are also found in the environment and food systems. It is a sad fact that the food and agricultural code has not been amended to address pesticide residues in food products since 1965. In comparison, the use of pesticides has grown by 1,169% over the past 20 years.

In the past 20 years, there has been a growing application of Chemometrics in environmental research, attributed to the extensive exploration of data processing methods in this domain and the presence of suitable software. Chemometric environmental studies can be categorised into three primary areas of focus: Quantitative chemical analysis, environmental quality assessment through monitoring, and the prediction and modelling of toxicological effects (Golge & Kabak 2015; Mas et al. 2010; Zhao et al. 2012). Ultrasonic solvent extraction (USE), a quick and inexpensive sample preparation method, is more effective and crucial for isolating analytes from samples. The USE increases the effectiveness of the organic compound extraction process by using ultrasonic waves to penetrate the solvent and cause cavitation. Cavitation bubbles are created by ultrasonic treatment, and as pressure and temperature rise, these bubbles compress and collapse. Ultrasonic waves from the collapse of the bubbles travel through the solvent and increase the sample matrix and extraction solvent mixing. Consequently, the goal of this work was to optimise the USE for the extraction of pesticides residues from vegetables in order to determine whether their concentration levels are within safe ranges for ingestion by humans (Majlesi et al. 2016; Mnyandu & Mahlambi 2022). It is a widely used tool for pesticide analysis due to its high extraction efficiency, with no purification steps that can lead to the decomposition of organophosphorus pesticides (Zhang et al. 2017).

High performance liquid chromatography (HPLC) is used in many fruits and vegetables samples for quantification and several residual pesticides. The most often use of chromatographic techniques are to detect the data amount and qualify complex mixture (Chen et al. 2020; Lehotay et al. 2010; Sousa, Pinto & de Araujo 2017). The multivariate approach needs experimental parameters in which they provide the quantitative and qualitative methods by the connection of reactions and factors that occupy screening, selecting, modelling and optimisation (Abdulra'uf & Tan 2013; Candioti et al. 2014).

The current study focuses on pesticides usage over the vegetables in Quetta city that serves as the capital of Pakistan's Balochistan province, with a total area of 2,653 km² and a haphazard urbanisation pattern. The infrastructure and service mechanisms that are now in place for waste management, sewerage, water supply, and sanitation are inadequate for the current population needs. Major highways are littered with municipal rubbish, which is a result of the city's windy and dry climate. In addition to causing aesthetic issues, untreated effluent seeps into the earth and contaminates the water supply (Kakar et al. 2010). Due to backward state of Pakistan, there is a lack of awareness and availability of adulterated and banned pesticides in the market, this recommendation has led to their excessive use in agriculture. Also, by using the pesticide residues of organophosphorous (OPs), and organochlorines (OCPs) classes were monitored in water, soil, and vegetable samples collected from vegetables growing areas. In Quetta, pesticide contaminated foodstuff data is scarce due to non-mandatory public institution.

The short-term advantages of a low-cost irrigation source may be outweighed by the long-term financial costs of environmental harm and health issues. Ground water contamination may be caused by agricultural practices that primarily rely on the use of pesticides to boost crop yields, particularly in Balochistan (Jabbar & Mallick 1994). On crops, persistent pesticides (organochlorines) were frequently applied. There is less information about the topic in Pakistan, therefore analysis of aldrin is important as some trace amount of aldrin were detected in cooking oil and dairy products of Quetta city indicating that it is somehow reaching the farmers (Jabbar & Mallick 1994).

MATERIALS AND METHODS

Certified pesticides standards, diazinon, malathion, and aldrin were purchased from Dr Ehrenstorfer (Augsberg, Germany). The purities of all pesticide standards were greater than 98%. Individual pesticide stock solutions (1,000 mg/L) were prepared in pesticides grade methanol and stored in brown glass bottles at 16 °C. Acetonitrile, ethyl acetate, methanol and anhydrous sodium sulfate (pesticides grade) were purchased from Merck (Darmstadt, Germany). Ultrasonic solvent extraction experiments were performed in an ultrasonic cleaning bath bought from Shanghai Pu Chao Ultrasonic Equipment Co., Ltd (Shanghai, China). The working frequency and power were set at 28 kHz and 300 W, respectively.

Samples of pesticide-free vegetables were bought from Kalat district local markets in order to create the methodology. For optimisation process, spinach samples were used as a matrix. A methodical and exact strategy to sample collection and preparation is necessary for the proper measurement of pesticides in environmental samples (Figure 1). Samples of vegetables were spiked with a mixture of a standard solution (1 mL). Prior to extraction and optimisation, these vegetable samples that had been spiked were left for 1 h. In a 100 mL beaker, 5 g of the defrosted sample, 8 g of anhydrous sodium sulphate, and the proper amount of extraction solvent were added. The water level within the beaker was maintained above the solvent level while it was submerged in the ultrasonic bath. The distance between the bottom of the beaker and the bath container was 2.2 cm. Following a period of sonication (12 min), the extract was filtered through filter paper, and two separate 10 mL amounts of extraction solvent were shaken to wash away the solid residue. The filtered liquid was collected in a 100 mL spherical flask and evaporated to dryness by a gentle stream of nitrogen. One mL of methanol:water (40:60, v/v) was used to re-dissolve the extract. Twenty µL was injected into the LC-10AT vp detector after passing through a 0.45 µm filter. The parameters sample amount (5 g), solvent volume (60 mL), sonication time (12 min), room temperature was predetermined in the multivariate optimisation section through multivariate experimental design. The optimisation process has two steps (screening and optimisation). In the first screening step, parameters, such as sample weight, solvent volume, temperature and sonication time amounts were applied to 2^{4-1} fractional factorial design to assess the significance of all variables on the extraction effectiveness of all pesticides (Tables 1 & 2). In the second step, the variables that were important (as indicated by Pareto chart Figure 1(a)) were further optimised through the central composite design by response surface methodology (Table 3). Once the technique was established, the vegetables along with its soil samples and agricultural water were taken from the sampling points as shown in Figure 1, and analysed for pesticides detection. Approximately every three months of four seasons, a sampling event was planned as part of this monitoring programme. Followed by collection and transportation in aluminium foil packets to the laboratory.

The soil samples were taken from the vegetable farms in the agricultural region. A 25 mL screw-capped flask containing 5.0 g of analytical sample was weighed, 20 mL of ethyl acetate then added, and the flask was shaken for 50 min in a mechanical shaker (Thermolyne, Dubuque, Iowa, USA). After letting the soil to settle, the supernatant was transferred to a different flask holding 1 g of anhydrous sodium sulfate. Using a rotary evaporator, 10.0 mL of the organic layer was transferred to a round-bottom flask and concentrated to around 0.5 mL. Ethyl acetate was used to reconstitute the final extract to a sufficient volume (1.0 mL). The soil pH value was determined from a water:soil (5:1) slurry. The organic matter content was ascertained by using the loss on ignition method, which involved drying soil samples in an oven at 104 °C for 24 h before combusting the organic matter to produce ash and CO_2 at a temperature of 500 °C - 550 °C. The loss on ignition (LOI) was then computed by using Equation (1) (Yang Farina et al. 2016).

$$\mathrm{LOI}_{550} = \mathrm{DW}_{105} - \mathrm{DW}_{550}/\mathrm{DW}_{105} \times 100 \qquad (1)$$

Water samples were taken from the vegetable irrigation water. These water samples were kept in a sterile container after being taken at different depths and in adequate volume for analysis. 1 L of n-hexane were used to extract 2 L of water sample, which was shaken repeatedly in a separating funnel. The extraction process was carried out in triplicate. To eliminate the water content, the organic layers were collected and run through anhydrous sodium sulfate. Using a rotary evaporator, the collected fraction was reduced to 1 mL. After adding 2 mL of acetonitrile, the samples were filtered through a 0.22 mm syringe filter before being analysed. In all, 52 surface soil samples and 52 water samples were gathered from agricultural polluted region of outskirts of city in four different seasons of spring, summer, autumn and winter season.

INSTRUMENTATION

High performance liquid chromatography was used to analyse the real sample vegetables for pesticides residues. Liquid chromathography system equipped with LC-10 as pump and symmetry C18 analytical column was operated at 40 °C. High performance liquid chromathography (HPLC) couple with UV detector analyses by using a Shimadzu (shimadzu\QP5050A) Chromathography. The HPLC system used for the quantification and identification of pesticides carried an acetonitrile-water (60;40v\v) as the mobile phase. The mobile phase worked as binary gradient condition in HPLC. The HPLC detector was connected to the computer for data analysis of the column (4.6ID × 250 mm) used for the separation of samples. During the experiments the samples were injected through the Rhodyne injection system. Injection volume of 20 µL was used in the HPLC system.

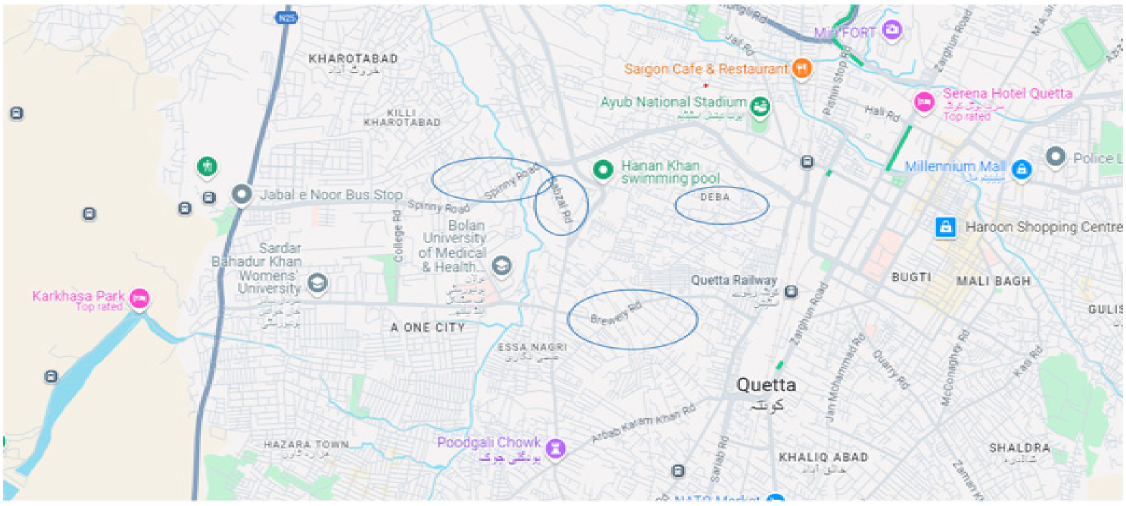


FIGURE 1. Sampling location of vegetables from Quetta city

TABLE 1. Factors and levels considered in the experimental design

Variables	min	Max
weight of sample	5 g	100g
Sonication time	15 min	60 min
Temperature	Room temp	55 °C
Solvent volume	5 mL	60 mL

TABLE 2. Factors and levels considered in the experimental design*(A) Sample weight*(B) time*(C) temperature*(D) volume

StdOrder	RunOrder	CenterPt	Blocks	sample weight	sonication time	temperature	solvent volume
7	1	1	1	-1	1	1	-1
4	2	1	1	1	1	-1	-1
6	3	1	1	1	-1	1	-1
2	4	1	1	1	-1	-1	1
3	5	1	1	-1	1	-1	1
5	6	1	1	-1	-1	1	1
1	7	1	1	-1	-1	-1	-1
8	8	1	1	1	1	1	1

*Generated by Minitab statistical software

TABLE 3. Central composite design matrix fraction design*(A) Sample weight*(B) time*

StdOrder	RunOrder	PtType	Blocks	A	B
9	1	-1	2	1	0
10	2	-1	2	0	-1
14	3	0	2	0	0
13	4	0	2	0	0
8	5	-1	2	-1	0
11	6	-1	2	0	1
12	7	0	2	0	0
3	8	1	1	-1	1
4	9	1	1	1	1
2	10	1	1	1	-1
5	11	0	1	0	0
1	12	1	1	-1	-1
7	13	0	1	0	0
6	14	0	1	0	0

*Generated by Minitab statistical software

RESULTS AND DISCUSSION

SCREENING

The fractional factorial design of experiment was used for screening design to determine the important effects and interaction of variables like solvent volume, sample amount, sonicating time and suitable temperature. In the fractional factorial design, the Pareto chart obtained is shown in Figure 2(a), where sample weight and sonication time plays a major role in enhancing the extraction efficiency of the analytes. This is explained with the length of the bar where the vertical line shows the 95% confidence level indicating that the sample weight and sonication time have significant effect. Where, A and B are sample weight and sonication time, respectively, and sum is the response of peak area of all pesticides. The extraction conditions were evaluated by using analysis of variance (ANOVA) to evaluate the relationship between the significant variables and response. The result showed the significance of model ($p < 0.05$) indicating the model of study. The model equation and response surface plot showed that the optimum values were 2.5 g and 5 min for sample weight and sonication time, respectively. It can be observed that decreasing the sample will enhance decrease in sonication time as well increase the recovery of analytes due to cellular transfer of pesticides from plant tissues to ethyl acetate through osmosis and diffusion as increase in time might cause the degradation of pesticides (Babic et al. 1998). The normal, probability and the residual plot in Figure 2(b), Figure 2(c) and Figure 2(d) showed that the response increases as the values of A and B decreases.

VALIDATION OF OPTIMISED METHOD

Spinach, mint, celery and lettuce were used as representative to validate the methods of optimisation, LOD, and LOQ. The linearity was determined in the concentration range of 0.5 mg/kg-5 mg/kg by triplicate analysis. The limits of detection (LOD) and the limits of quantification (LOQ) were used as sensitivity evaluation. The IUPAC recommended method has been used to evaluate the LOD three times the standard deviation of the seven vegetable samples that were spiked at the lowest concentrations while the LOQ as 10 times the standard deviation of the seven vegetable samples that were spiked at the lowest concentration. The precision and accuracy were evaluated by recovery and repeatability performed by triplicate analysis. The limits of detection and limits of quantification of the experiment were significantly lower or equal to the MRL recognised by the EU (European Union). A good linearity range were obtained for all the pesticides ranging from 0.999-0.997. The relationship of sample weight and sonication times was very important to explain the LOD, LOQ, repeatability and recoveries values. The LOD for all pesticides in these vegetables' samples ranged from 0.01 mg/kg-0.3 mg/kg. Recoveries range in spinach were 85%-93.2%. In case of repeatability, the relative standard deviations (RSD%) ranged from 0.9%-11.6% in spinach sample which verified that the precision of the method is acceptable.

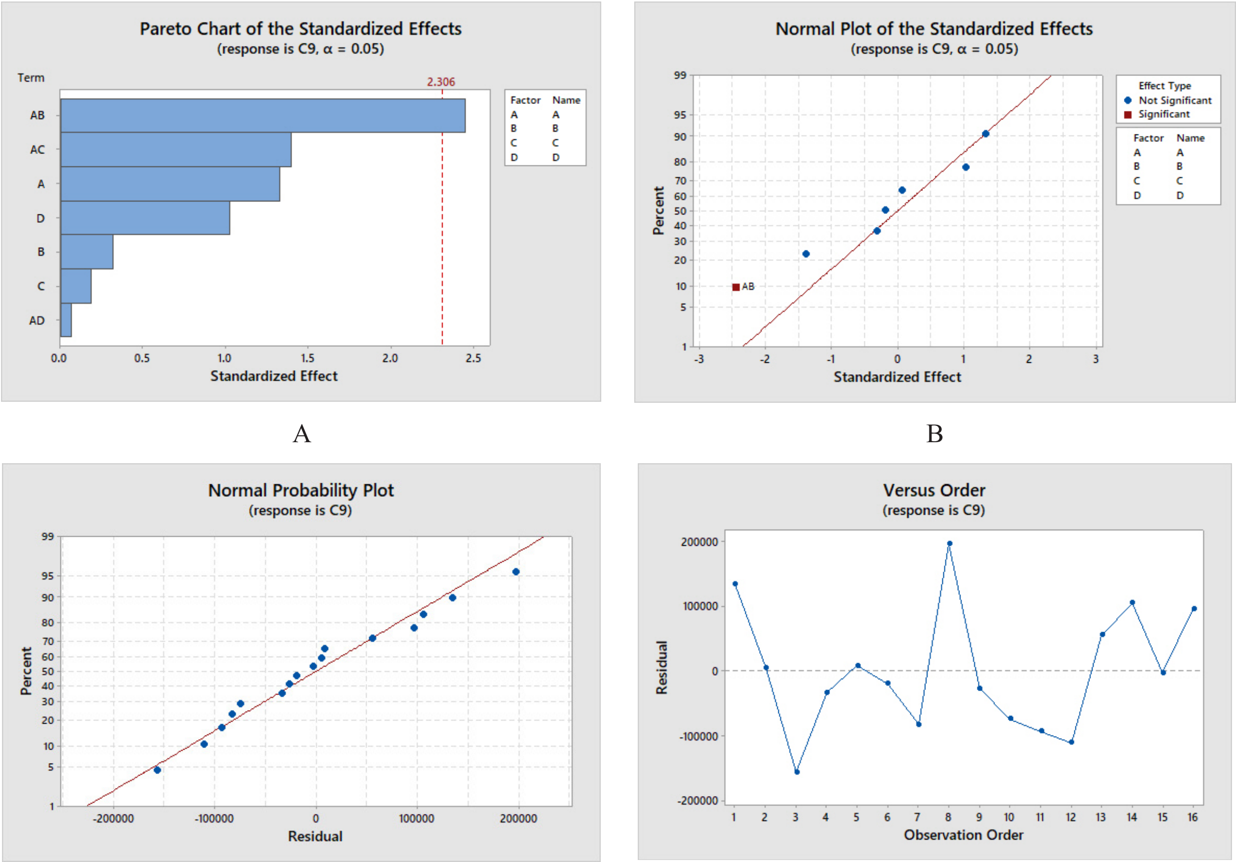
For soil samples, the recovery ranged between 78%-94% with RSD % that ranged as 0.4%-9%. The limit of detection ranged between 0.01 mg/kg-0.4 mg/kg, in the case of water the recovery ranged between 82%-96% with RSD% as 0.8%-4% and the LOD ranged between 0.01 mg/kg-0.2 mg/kg.

CENTRAL COMPOSITE DESIGN

The CCD was used to observe the optimal value of sample weight, solvent amount and sonication time. These variables were calculated at many levels in which high, central and low (+1, 0, -1) were implicit in the following table. Table 3 shows the CCD matrix, factors and levels. In the CCD matrix, the plot showed the response surface which is making experimental response. The following equation shows the sum of A and B response, respectively, where A is sample weight and B is time of sonication. The response surface plot is shown in Figure 3(a), main effect plot (Figure 3(b)) and the interaction plot (Figure 3(c)) which was built through the experimental responses. The model was governed by the following equation, where A and B are sample weight and sonication time, respectively, and sum is the response of peak area of all pesticides. The following equation governed the model:

$$\text{Sum} = 56168 - 453683\,A - 456234\,B + 555109\,A{*}A + 570025\,A{*}B. \tag{2}$$

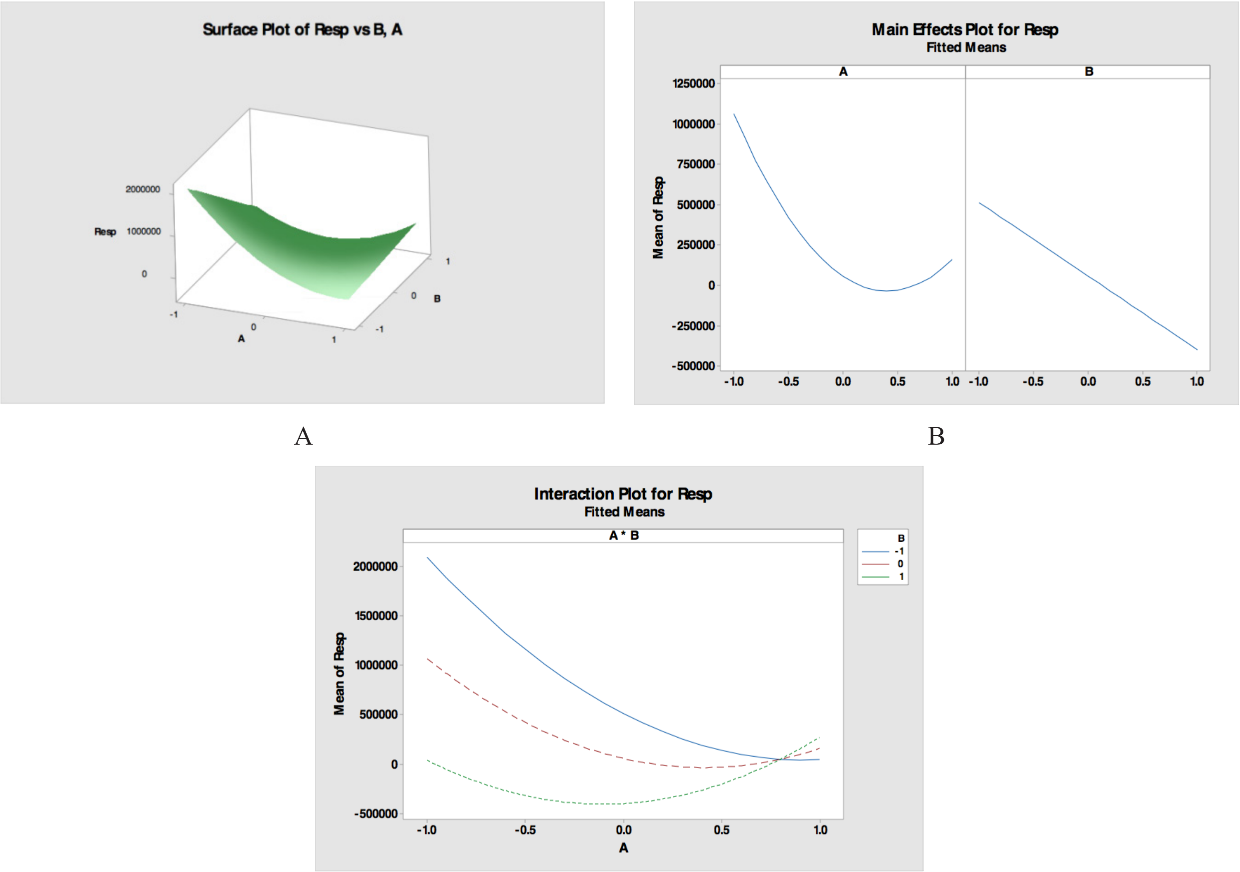
The extraction conditions were evaluated by using analysis of variance (ANOVA) to evaluate the relationship between the significant variables and response. The result showed the significance of model ($p < 0.05$) indicating the model of study (Table 4). The model equation and response surface plot showed that the optimum values were 2.5 g and 5 min for sample weight and sonication time, respectively. It can be observed that decreasing the sample will enhance the extraction efficiency by reducing the deposits accumulation in instrument, reducing the matrix effects, enhancing the sensitivity of instrument (Yang Farina et al. 2017). The decrease in sonication time also increases the recovery of analytes due to cellular transfer of pesticides from plant tissues to ethyl acetate through osmosis and diffusion as increase in time might cause the degradation of pesticides (Babić, Petrović & Kaštelan-Macan 1998). The main effect plot in Figure 3(b) showed that the response increases as the values of A and B decreases. The interaction plot between A and B in Figure 2(c) also signifies the highest recovery at low values of weight and time.



A B

C D

FIGURE 2. (A) Pareto chart of main standardised effects, (B) Normal plot of effects (C), Normal Probability plot, and (D) residual plot showing the interaction between variables during screening



A

B

C

FIGURE 3. Response surface plot of A and B (a) Main effect Plot for response (b), and Interaction plot for response (c)

TABLE 4. Descriptive statistics of ANOVA test for central composite design

Source	DF	Sum of Square	Mean square	F value	P value
Model	2	2.54889E+13	1.27450E+13	18.09	0.005
Linear	2	2.54889E+13	1.27450E+13	18.09	0.005
A	1	8.79567E+12	8.79567E+12	12.48	**0.017**
B	1	1.66942E+13	1.66942E+13	23.69	**0.005**
Error	5	3.52309E+12	7.04617E+11		
Total	7	2.90130E+13			

Bold Value is significant at $p < 0.05$

SEASONAL DISTRIBUTION OF PESTICIDES

VEGETABLES

The developed method was successfully applied to the detection and quantification of pesticides in leafy vegetables, such as spinach, lettuce, mint, and celery collected from local stores in Quetta Market. The residue of total pesticides concentration (aldrin, malathion and diazinon) decreased as follows; spinach > celery > lettuce > mint (Table 5). Celery and spinach are the most contaminated amongst leafy green vegetables as they are common crops due to high demand in local markets for daily cooking. Highest aldrin concentration was found in celery followed by lettuce, mint, and spinach. It is not surprising that these leafy vegetables had the greatest concentrations of pesticides because it is more susceptible to pests than other vegetables and cannot be kept for an extended period of time. Currently, it is common to find several pesticides in vegetable crops because of a lack of knowledge about pesticide used by the farmers. Since aldrin is a prohibited pesticide like HCHs, DDTs, aldrins, and chlordanes are commonly found in underdeveloped nations, including Pakistan, the presence of these harmful EDPs in vegetables is not surprising (Ali et al. 2019). The highest average concentration of pesticide was malathion (16.8 mg/kg) found in spinach. This is significantly higher than its maximum residue limit (MRL) (Table 5). These higher residues in spinach followed by celery and lettuce are higher than acceptable limits, this investigation showed that some pesticide residues in vegetables surpass safety standards, potentially posing health risks. In the case of diazinon, the highest concentration was found in celery followed by spinach and lettuce. Wastewater treatment facilities, transportation, manufacturing, and agricultural uses were the primary causes of the rise in OP levels in the environment (Kashi et al. 2021). Nine of those samples had pesticide residue levels over the maximum permissible limits (MRLs). Mint seems to be less contaminated as compared to other vegetables. This may be due to low application rates, pesticide methods, and harvest awareness and safety instructions. The high concentration of pesticides on these leafy vegetables indicates that they are owing to the large surface area. For this reason, the leafy vegetable samples crossed the MRL value.

From the discussion earlier, it is clear that humans are consuming pesticides in addition to contaminated fruits and vegetables. Pesticides have detrimental impacts on living things in the form of numerous acute and chronic ailments when they enter the food chain. It is recommended that farmers attend frequent formal training sessions regarding the application and health risks of pesticides in order to reduce the damaging effects of these chemicals on living things and the environment. Pesticides should only be used selectively on crops to eradicate harmful pests. As was done in the current study, ongoing monitoring of pesticide residues in fruits, vegetables, and crops is required to determine the results of all such preventative measures.

SOIL AND WATER

Table 6(a), 6(b), 6(c) and 6(d) shows the seasonal variation of pesticides in spring (season 1), summer (season 2), autumn (season 3), winter (season 4) of water and soil samples. In the spring season more water samples were contaminated. Aldrin concentration was highest at sampling point 1. Other than that, it was not detected at other sampling points. Its existence in both soil and water ranged from ND-5.6 mg/kg. However, its detection was found more in soil as compared to water (Table 6(a)). This might be because most environmental releases of aldrin and dieldrin are directly to the soil. They have low water solubility and a tendency to bind tightly to the soils. Aldrin was not detected in summer season. However, it was detected in autumn season ranged from ND-37.8 mg/kg in water while in soil it was not detected. This suggests that these compounds migrate downward very slowly through soils or into surface water or groundwater. Pollution from organochlorine pesticides occurs more frequently through transport and atmospheric deposition, which are responsible for their transport to distant locations. The detection of these pesticide residues in the control sites in this study could be attributed to their trans boundary properties and this suggests drift during pesticide application (Karadeniz & Yenisoy-Karakaş 2015; Osuala et al. 2024; Yang, Li & Mu 2008). Particulates from surface runoff have been blamed for the majority of surface water aldrin and dieldrin. In winter it was detected only at sampling point 2 at 2.3 mg/kg.

Diazinon was found to be more in water samples than in soil in spring season ranging from 3.1 mg/kg-8.6 mg/kg as compared to malathion (4.6 mg/kg) at sampling point 6. This suggests that diazinon is more stable in water than soil as compared to other organophosporous pesticides (OPPs) (Bokulić Petrić, Stipičević & Mešić 2023). In season 2 (summer) diazinon was detected both in water and soil samples ranged from 1.6 mg/kg-37.8 mg/kg on water and 12.4 mg/kg-37.8 mg/kg in soil samples. Whereas, malathion was not detected in both water and soil samples. This indicates the faster degradation of malathion than diazinon. This could be the higher susceptibility of malthion towards temperature causing its faster degradation than diazinon (Bokulić Petrić, Stipičević & Mešić 2023). The high percentage of diazinon pesticides in May and June confirmed this. Pesticides are typically sprayed in the spring that they stay in the soil for a while before volatilising, degrading, running off into rivers, and seeping into groundwater. This validates the elevated levels observed in the summer months of which coincide with the pesticide application time. In autumn season, diazinon and

TABLE 5. Average residues of selected vegetables in Quetta District (mg/kg)

Pesticides	Spinach	Celery	Mint	Lettue	MRLs
Malathion	16.8	12	ND	10	0.5
Aldrin	7.6	9	8.4	8.8	0.02
Diazinon	9.9	13	ND	ND	0.05

malthion was not detected in both water and soil samples. The distribution of pesticides in the area is significantly influenced by the geography and air circulation of the area (Nusrat et al. 2021). This indicated that the pesticide-related surface water pollution typically varies with the seasons. In winter season, diazinon was detected only at sampling point 4 in water only while malthion was not detected. In February, pesticide concentrations that are the lowest correspond to the conclusion of the plant-protection time. Rather, the low water flow rate may have reduced the dilution, which could account for the elevated pesticide amounts that were observed in the fall and autumn. This shows that in OPPs the most frequently detected pesticide is diazinon. Plant protection is mostly carried out in the research area from March to October, with spring time seeing the adoption of especially stringent protection measures. These findings are consistent with earlier research that found the total quantities of pesticide residues increase in the fall and summer. The low detection of malathion might be due to a low soil organic carbon partition coefficient, a very high solubility in water, and a very low persistence in sediment (Montuori et al. 2022). A shift in the kinds of compounds found during the annual cycle can be seen in addition to the quantities rising during the pesticide use period. Nevertheless, the outcomes might be connected to modifications in farming methods that lead to more sustainable management. The reduction in the detection of pesticides might be due to the government policies, advisors to farmer cooperatives, reducing back on pesticide use while also significantly increasing organic farming or cleaning containers and tools used for pesticide application in vine growers' warehouses. It is believed that soil features will have an impact on the residual amount of pesticide, as the process of adsorption by the soil's organic and inorganic components may increase the compounds persistence. Indeed, a number of studies have been published in the literature that discuss how OM and/or clay concentration affect the adsorption, decomposition, and/or persistence of certain chemicals found in soils.

Principle component analysis (PCA) is a linear dimensionality reduction technique that reduces a variety of environmental elements to a small number of principle components. However, there is fewer notable associations between the overall concentrations of pesticides and the physicochemical characteristics of soils. This is supported by principal component analysis shown in Figure 4, suggesting that different application techniques and/or mechanisms rather than soil characteristics are responsible for the residues existence in the months with the highest prevalence. Pesticide residue accumulation in the soil may depend on the route, frequency, and rate of application. While repeated application of pesticides in agricultural activities may impact the half-life of pesticides in soils, there is no uniform application that minimise pesticide losses through volatilisation and runoff. Diazinon differs from other pesticides in that it appears to be more associated with the organic content and soil pH, indicating that at high pH levels, diazinon is more persistent than other OPPs and has a stronger correlation with organic content. Biplot plot (Figure 4) shows that pesticides do not significantly correlate with soil pH 7.5 or organic content, suggesting that the adsorption of polar OPPs diminishes as pH rises (pH 7-pH 8). These findings also contradict with research by Krishna and Philip (2008) and Yang Farina et al. (2016), which found that the optimal pH range for pesticide adsorption is between pH 2 and pH 8. Here, pH is between pH 7-pH 8, therefore the retention of the diazinon in soil might be due to other reasons as OPPs bind to soil particles loosely and leave behind little soluble residue in water. Majority of them are thought to have poor soil mobility and leaching (Aggarwal et al. 2012; Roth et al. 2015). They are very persistent in the soil environment, nonetheless, according to their degradation by products.

TABLE 6. A) Season 1 pesticides concentration in soil and water samples (mg/kg)

Water sample								
Pesticides	1	2	3	4	5	6	7	8
Aldrin	5.6	ND	ND	ND	ND	ND	ND	ND
Diazinon	7.5	ND	ND	ND	8.6	7.9	3.11	ND
Malathion	Not	ND	ND	ND	ND	4.6	ND	ND
Soil samples								
Pesticides	1	2	3	4	5	6	7	8
Aldrin	1.4	1.1	1.3	ND	1.5	ND	ND	ND
Diazinon	ND	ND	ND	ND	ND	ND	ND	ND
Malathion	ND	ND	ND	ND	4.8	ND	ND	4.8

TABLE 6. B) Season 2 pesticides concentration in soil and water samples (mg/kg)

Water sample								
Pesticides	1	2	3	4	5	6	7	8
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND
Diazinon	ND	ND	1.6	ND	9.2	ND	13.4	37.8
Malathion	ND	ND	ND	ND	ND	ND	ND	ND
Soil samples								
Pesticides	1	2	3	4	5	6	7	8
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND
Diazinon	ND	37.8	4.9	ND	12.4	ND	25.6	ND
Malathion	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 6. C) Season 3 pesticides concentration in soil and water samples (mg/kg)

Water sample								
Pesticides	1	2	3	4	5	6	7	8
Aldrin	ND	37.8	4.9	ND	12.4	ND	25.6	ND
Diazinon	ND	ND	ND	ND	ND	ND	Not	ND
Malathion	ND	ND	ND	ND	ND	ND	Not	ND
Soil samples								
Pesticides	1	2	3	4	5	6	7	8
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND
Diazinon	ND	ND	ND	ND	ND	ND	ND	ND
Malathion	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 6. D) Season 4 pesticides concentration in soil and water samples (mg/kg)

Water sample								
Pesticides	1	2	3	4	5	6	7	8
Aldrin	ND	2.3	ND	ND	ND	ND	ND	ND
Diazinon	ND	ND	ND	8.3	ND	ND	ND	ND
Malathion	ND	ND	ND	ND	ND	ND	ND	ND
Soil samples								
Pesticides	1	2	3	4	5	6	7	8
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND
Diazinon	ND	ND	ND	ND	ND	ND	ND	ND
Malathion	ND	ND	ND	ND	ND	ND	ND	ND

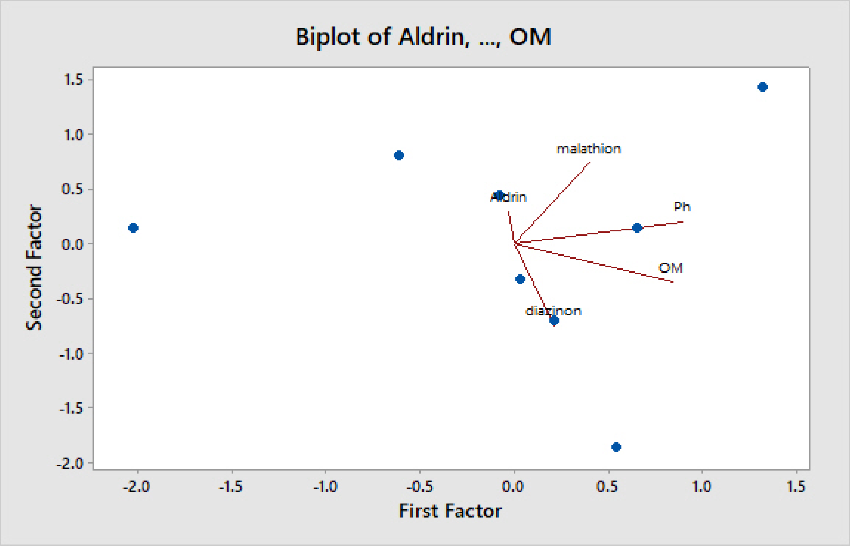


FIGURE 4. Biplot showing interaction of organic content and pH with pesticides concentration

CONCLUSION

The optimised method was successfully applied to real sample analysis for pesticide extraction. The results indicate that the multivariate approach is successful in terms of method development with few experiments run. The sample weight and sonication time had a significant effect on the extraction of pesticides. The sonication time is greatly reduced, thus making the sample preparation procedure easy as compared to univariate studies. The average recovery achieved is ranged from 85%-93.2%, with RSD 11% (n = 3). The obtained LODs were lower than the MRLs of EU Directives. With the decrease in sonication time and sample weight, the proposed method offers a similar recovery of around 85% and lower LOD as compared to other extraction methods and at the same time, the proposed ultrasonication method does not need any cleanup step. Seasonal distribution of pesticides showed that there is no significant correlation of pesticides concentration with pH and organic contents of soil.

Finally, the discussion earlier suggests that the city consumers are exposed to lower doses of pesticides that have the potential to cause chronic illnesses. Additionally, consumers of vegetables may be simultaneously exposed to hundreds of pollutants from the same or distinct pesticide groups, raising concerns about food safety. Aldrin has the ability to bioaccumulate and magnify many times in food chains over time, the high prevalence of pesticides contamination is concerning. As a result, future food safety regulations should focus on identifying and removing pesticides contamination sources from vegetables and other food commodities grown in Pakistan's various agroclimatic zones, given that the farmer is not adhering to good agricultural practices (GAP) or suggested waiting periods. It is also stated that farmers are not informed of how to apply pesticides safely. Fruit and vegetable residue levels can be lowered by using pesticides in the right amounts and limiting their use right before crop harvest or during transportation.

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