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ANALYSIS OF DIMETHOATE AND DIAZINON PESTICIDE RESIDUES IN SOIL BY GAS CHROMATOGRAPHY

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Abstract

Pesticides are shown to have a great effect on soil organisms, but the effect varies with pesticide group and concentration, and is modified by soil organic carbon content and soil texture. Dimethoate and diazinon pesticide residues in soil under pesticide-treated plants were detected by use of high resolution gas chromatography equipped with phosphorus flame photometric detector (GC-PFPD). The residue concentrations in the soil were related to their physicochemical properties. Diazinon exhibited the higher concentration in the soil under pesticide-treated plants areas (0.3764 ppm, 2 h after application), while dimethoate was found at lower concentration (0.3169 ppm). All two pesticides were accumulated in soil sample during the first 24 h after application but the dimethoate concentrations rapidly decreased to 97.79 % after application for 5 days, while diazinon residues decreased to 98.51 % at the end of the 7 days monitoring period. The rate constants and half-lives of double exponential decay model and pseudo-first order equations in soil under pesticide-treated plots were determined.

Keywords: pesticides, soil, dimethoate, diazinon, phosphorus flame photometric detector

Introduction

Pesticides are extensively used in agricultural production to check or control pests, diseases weeds and other plant pathogens in an effort to reduce or eliminate yield losses and preserve high product quality. Pesticides constitute a very important group of chemical compounds that have to be controlled due to their high toxicity and their widespread use in agricultural practice for field and post-harvest protection (Ortelli *et al*., 2006). Pesticides can be as liquid sprays on the soil or crop plant, may be incorporated or injected into the soil or applied as granules or as a seed treatment. Once they have reached their target area, pesticides disappears via degradation, dispersion, volatilization or leaching into surface water and groundwater, they may be taken up by plants or soil organisms or they may stay in the soil (Mahmood *et al*., 2016).

Multimedia monitoring of contaminants such as insecticides is an essential part in investigating the entire spectrum of environmental contamination. The fate of insecticides and their transformation products (TPs) in the soil depend of interaction in the soil depend on the properties of their active ingredients and degree of interaction with the soil particles (or adsorption) (Del Prodo-Lu, 2015).

The main entry ways for pesticides in the soil are either through plants pulverization or its direct application to the soil, where the persistence in this environment is influenced by several factors such as, pesticides physicochemical properties: molecular size, water solubility, volatility, molecular structure, chemical function and acid-base nature; soil properties: soil type, moisture content, organic carbon content, pH, redox potential, microbial population; environmental conditions: climate, topography, air currents, variables related to the application of pesticides:

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concentration, frequency and mode of application; and also used of other chemicals (Muenchen *et al*., 2016).

Soil is a complex and heterogeneous environment, composed of minerals, organisms with characteristics varying according to the climate and the source material. The soil is very important for food production and maintenance of socio-economic activities, and its prevention depends on the monitoring of contamination in order to avoid drastic impacts (Boesten, 2016). Soil is the principal reservoir of environmental pesticides, this representing a source from which residues can be released to the atmosphere, ground water and living organisms (Ghabbour *et al*., 2012).

Organophosphorus pesticides are the most widely used in agriculture, home, gardens, and veterinary practice. The implications of high pesticides residue include muscle cell degeneration, which involves the respiratory muscles. Chronic exposure to organophosphate might damage the peripheral nervous system, and patient's behavioural abilities and/or personality, chronic fatigue syndrome and effects on the heart (Akan *et al*., 2013). Most organophosphorus compounds have a short residual activity e.g., diazinon, dimethoate, malathion, chlorpyrifos, fenitrothion, etc. Dimethoate and diazinon are systemic and nonsystemic organophosphate insecticides which are widely used in vegetable farms for plant protection (Ware and Whitacre, 2004).

The main aim of this study was to investigate the gas chromatographic analysis of dimethoate and diazinon pesticide residues in soil and to compare the rate constants and half-lives of both pesticides by double exponential decay model and pseudo-first order equations.

Materials and Methods

Study Area

The experiments were conducted in the vegetable farm of Alantapo Village, Hlegu Township, in Yangon Region. The experimental farm had an area of 35 x 25 ft.

Spraying of Insecticides

The commercial plant-production products used were Danadim 40 EC (dimethoate 40 %) and Dynamite 40 EC (diazinon 40 %) pesticides, applied in the trials. These products were diluted with water to obtain recommended dose (20 mL of emulsifiable concentrate per gallon) as spray tank using manual sprayer and the application required about 20 min (Ohnmar Aung *et al*., 2007). In all the treatments the operator was the same person. The spray operator walked in and out of the rows of the benches with the plants, spraying up and down from the bottom to the top of the plants and back again (Hatzilazarou *et al*., 2005).

Sample Collection, Storage and Preparation

Soil samples under pesticide-treated plants were collected according to the sampling schedule of plant on day 0 (2 h after application), day 1, day 3, day 5, day 7 and day 9 after application of pesticides. Soil sample were taken down to a depth of 10-20 cm with a stainless steel auger of 6.5 cm diameter by turning the auger once anti-clockwise before pulling it, back to surface. The soil samples were then randomly selected and bulked together to form a composite sample before being placed in clean plastic bags. The soil portion were cut off using clean steel scissors, placed into a plastic sealed bag and transported to the laboratory where they were analyzed immediately or stored at -4 °C until extraction and analysis. After thoroughly mixing of the collected soil samples, they were partially air dried under room temperature, then passed through sieves of 25 meshes to maintain a uniform particle size. The prepared soil samples were stored in plastic bag under room temperature, such a way of storage was considered to keep the used soil in normally physical conditions. The soil was subjected to the physical and chemical analysis (Seema, 1999).

Quality Assurance and Quality Control

Proper quality assurance procedures and precautions were taken to ensure the reliability of the results. The samples were carefully handled to avoid any external influences that could interfere with the integrity of the sample and hence contaminate it. All glassware were washed with detergent, rinsed with distilled water, thoroughly rinsed with analytical grade acetone and dried overnight in an oven at 150 ^{o}C. The glassware were then removed from the oven and allowed to cool down and stored in dust-free cabinets. Deionized water was used through out the study (Fosu-Mensah *et al*., 2016).

Extraction of Soil Samples

A standard laboratory procedure was used to analyze the material samples. A 50 g of each soil sample was obtained by using coning and quartering method (Black, 1965). Pesticides were extracted from soil samples with 150 mL of acetone: hexane (1:1) by soxhlet extraction apparatus for 3 h. Then, the volume extracted was measured and 5 mL of this extract was placed in a graduated test tube and reduced to 0.3 mL by using nitrogen gas stream. The concentrates were adjusted to 1 mL final volume with cyclohexane and made ready for silica cleanup step (Akan *et al*., 2013).

Cleanup of Soil Extract

The column contained a small piece of cotton wool, neutral alumina and a bed height of 5 mm of anhydrous sodium sulphate. A 1 mL of the extract was added onto column and eluted with cyclohexane until 5 mL of eluate was obtained. Then, it was evaporated just to the point of dryness with a slight nitrogen stream, after which internal standard solution (fenitrothion) was added prior to gas chromatography analysis. All extracts were kept frozen until quantification was achieved (Frimpong *et al*., 2013).

Gas Chromatographic Determination

The final extracts were analyzed by gas chromatography equipped with P-mode of flame photometric detector (PFPD). The GC conditions and the detector response were adjusted so as to match the relative retention times and response as spelt out by the analytical methods for agricultural chemicals (Syoku-An, 2006). The injection volume of the GC was 1.0 µL in a splitless mode. The retention time, peak area and peak height of the sample was compared with those of the standards for quantization. The flow rate of carrier gas, nitrogen, was set at 10 mL/ min and hydrogen and air were used for combustion. Dimethoate and diazinon extracts were determined on a gas chromatography-flame photometric detector equipped with a fused silica capillary (PE-1) column containing cross bond 100 % dimethylpolysiloxane as stationary phase (30 m length, 0.53 mm internal diameter (i.d), 1.5 µm film thickness). Temperature programming for the column temperature of 100 ^{o}C which was maintained for 3 min and raised to 250 ^{o}C at 30 ^{o}C /min and the temperature was maintained for 10 min. Both the injector and detector temperature were set at 250 ^{o}C.

The three different standard dimethoate solution (7.6764, 15.3528 and 30.7056 μg/mL) and those of diazinon solution (5.3525, 10.7050 and 21.4100 μg/mL) were mixed with internal standard (ISTD) fenitrothion (14.18 μg/mL) and subjected to gas chromatographic measurements. The peak areas whose retention times coincided with the standards were extrapolated on their corresponding calibration curve to obtain the concentration (Ohnmar Aung *et al*., 2008).

Fortified and blank samples were analyzed with soil samples from each sampling date. Quantification of pesticide residues in samples were performed by internal standard (fenitrothion) method. For recovery studies, 1mL of a standard dimethoate spiked solution (10.2352 μg/mL) and standard diazinon spiked solution (10.7050 μg/mL) in acetone was added to control and allowed to stand for 15 min before extraction, for three replications. Each peak was characterized by comparing relative retention time of those of standards.

Results and Discussion

Physicochemical Analysis of Soil

Table 1 summarizes the physicochemical properties of the soil samples collected from the experimental plots in relation to under pesticide-treated lettuce plants. The mean moisture content of the air-dried soli sample was found to be 0.93 ± 0.01 %. The mean pH recorded for the entire study was 5.30 ± 0.14, and thus it was slightly acidic. The low pH values recorded could be due to the amount of acidic cations present in the soil due to the leaching of basic cations or the presence of high level of organic matter within the soil zones. The mean total nitrogen content of soil samples from experimental plot was found to be 0.19 ± 0.17 %. The amount of nitrogen in surface soils generally ranges from 0.02 to 0.25 %. The mean organic carbon content was 0.76 ± 0.03 % and the mean humus content recorded in this study was 1.52 ± 0.12 %. Low organic matter content is due to high temperature in tropical soils and thus high decomposition rates.

Table 1 Physicochemical Analysis of Soil

Moisture content (%)	pH	Total nitrogen (%)	Organic carbon (%)	Humus (%)
0.93 ± 0.01	5.30 ± 0.14	0.19 ± 0.17	0.76 ± 0.03	1.52 ± 0.12

Preliminary Investigation of Soil Type of the Experimental Plot

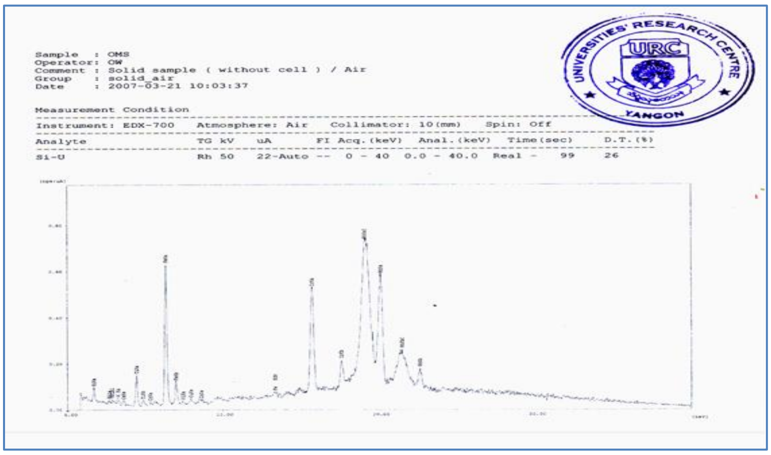
Soil is a mixture of mineral matter, organic material, air and water. The mineral portions of soil made up of particles which vary in size from stones to powder. These particles are called soil separates. Three major groups of soil separates are sand (2.00-0.050 mm), silt (0.050-0.002 mm) and clay (<0.002 mm). The soil type of experimental plot is shown in Table 2. The soil contained 46.55 % sand, 36.64 % silt, and 14.36 % clay. The texture of soil was found to be loamy soil according to textural triangle (Gee and Bouder, 1986). It was observed that soil are said to be light soil due to low clay and high sand contents.

Table 2 Preliminary Investigation of Soil Type of the Experimental Plot

Sand (%)	Silt (%)	Clay (%)	Soil type
46.55 ± 1.35	36.64 ± 1.03	14.36 ± 2.48	Loamy soil

Relative Abundance of Elements in Soil from the Experimental Plot

Semi-quantative results of soil in experimental plots are shown in Figure 1 and Table 3. The elemental contents in decreasing order were Si> Fe> Ti> K> Ca> Zr> Cr> Cu> Zn> Ni.



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Sample   : OMS
Operator: OW
Comment  : Solid sample ( without cell ) / Air
Group    : solid_air
Date     : 2007-03-21 10:03:37

Measurement Condition
--------------------------------------------------------------------------
Instrument: EDX-700   Atmosphere: Air   Collimator: 10(mm)   Spin: Off
--------------------------------------------------------------------------
Analyte              TG kV   uA      FI Acq.(keV)  Anal.(keV)  Time(sec)    D.T.(%)
--------------------------------------------------------------------------
Si-U                 Rh 50   22-Auto --  0 - 40   0.0 - 40.0  Real -   99    26
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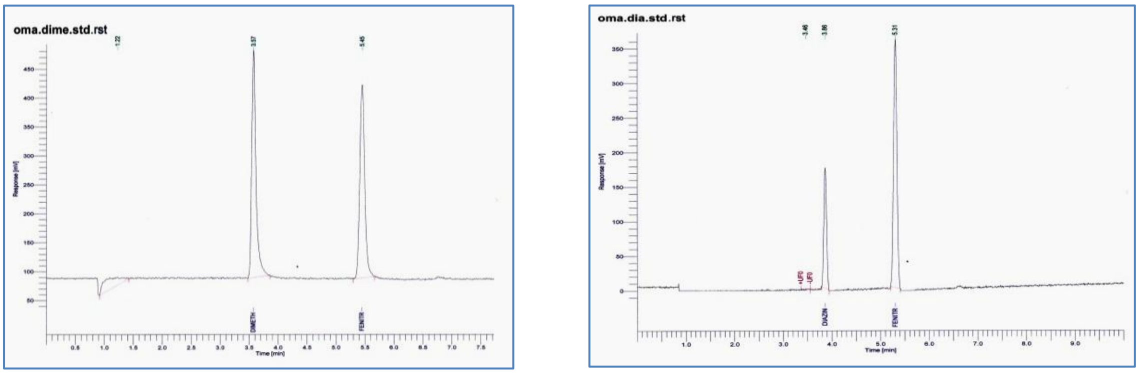
Figure 1 EDXRF spectrum of soil under study

Table 3 Relative Abundance of Elements in Soil by EDXRF

No.	Element	Relative abundance (%)	Standard deviation
1	Si	94.431	3.704
2	Fe	2.007	0.022
3	Ti	1.319	0.033
4	K	1.243	0.064
5	Ca	0.454	0.027
6	Zr	0.301	0.003
7	Cr	0.110	0.007
8	Cu	0.053	0.003
9	Zn	0.043	0.002
10	Ni	0.039	0.002

Gas Chromatograms of Standard Dimethoate and Diazinon

Figure 2 shows the gas chromatograms of standard dimethoate and diazinon. Thretention time of dimethoate and diazinon were 3.57 and 3.86 min, respectively.



(a) (b)

Figure 2 Gas chromatograms of standard (a) dimethoate and (b) diazinon

Extraction Efficiency (Recovery Percent)

Due to the diversity and complexity of soil types and interactions of their constituents with pesticides, different physicochemical properties of pesticides and low concentrations expected for those pollutants in the soil (Singh, 2014), there is a need for efficient sample extraction techniques before their determination by chromatography (Asensio-Ramos, 2009). Extraction efficiencies of insecticidal residues were commonly measured by addition of known amounts of the chemical to an untreated sample often immediately prior to the extraction procedure followed by the determination of the recovery. The efficiency of the analytical methods (the extraction and clean-up methods) was determined by recoveries of an internal standard. The efficiencies of dimethoate and diazinon were tested by using three different standard concentration levels (Table 4). In this study, good mean recovery percentages were 91.37 ± 1.16 % for dimethoate at three different standard concentration levels (7.6764 μg/mL, 15.3528 μg/mL and 30.7056 μg/mL), and 88.95 ± 1.72 % for diazinon at three different standard concentration levels (5.3525 μg/mL, 10.7050 μg/mL and 21.4100 μg/mL) in soil at 95 % confidence level respectively. On this basis, it was concluded that the good recoveries obtained for substrates spiked in the sample indicated good analytical techniques.

Table 4 Recovery Percent of Dimethoate and Diazinon Pesticide Extracts from Soil at Three Different Standard Concentration Levels

Experimental number	**Recovery (%)**					
	Dimethoate			**Diazinon**		
	Level 1	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**
1	92.36	92.49	92.04	92.30	92.90	91.01
2	89.58	88.92	88.23	86.58	86.92	87.23
3	88.82	89.41	89.28	82.32	83.41	83.28
4	93.86	93.94	93.27	91.86	92.04	92.79
5	92.87	92.98	92.49	90.67	91.43	90.49
Mean	91.50	91.55	91.06	88.55	89.34	88.96
±	±	±	±	±	±	±
SD	2.18	2.24	2.18	4.05	4.04	3.76
Mean of accuracy at level 1, 2, 3	0.23 ± 0.10			0.30 ± 0.25		
Precision	0.51			0.45		
95 % confidence interval	91.37 ± 1.16			88.95 ± 1.72		

Organophosphorus Pesticide Residues in Soil under Pesticide-Treated Lettuce Plants

Table 5 presents the mean summary results of the organophosphorus pesticide residues detected in soil samples from the study area. Two organophosphorus pesticides namely; dimethoate and diazinon were detected in the soil samples under pesticide-treated plants. Dimethoate was found in the soil at lower concentrations than diazinon. The highest concentration of dimethoate was 0.3169 ± 0.0240 ppm after 2 h application but its concentration decreased steadily within the next 5 days (120 h) (Figure 3). The concentration of dimethoate was 0.0691 ± 0.0084 ppm 3 days after the insecticide application, while its concentration of 24 h after application was 0.1988 ± 0.0193 ppm. Thus, 5 days after the application its concentration was 0.0070 ± 0.0025 ppm. The concentration of dimethoate was not detected 168 h (7 days) after insecticide application. The mean concentrations of dimethoate recorded were below the maximum residue limit (MRL) of 0.02 ppm after 120 h (5 days) of its application (WHO, Codex MRL, 2002).

Diazinon, the pesticide with the higher concentration, exhibited the mean highest concentration in soil 2 h (0 day) after application (0.3764 ± 0.0321 ppm), decreasing at the end of the 7-day monitoring period to 0.0056 ± 0.0007 ppm (Figure 3). The diazinon residue levels reached below the MRL value of 0.04 ppm after (5 days) of its application (WHO, Codex MRL, 2002).

The detection of organophosphorus pesticides in the soil samples suggests that these pesticides may have found their way into the soils via spray drift during plant spraying, wash-off from treated plants and wrong disposal of left over spray solution, sprayer wash water and used pesticide containers.

Table 5 Mean Concentrations of Dimethoate and Diazinon Residues in Soil under Pesticide-Treated Plants as a Function of Sampling Interval

Sampling interval (h)	Residues (ppm)	
	Dimethoate	**Diazinon**
0	0.3169 ± 0.0240	0.3764 ± 0.0321
24	0.1988 ± 0.0193	0.2000 ± 0.0247
72	0.0691 ± 0.0084	0.0710 ± 0.0183
120	0.0070 ± 0.0025	0.0260 ± 0.0112
168	ND	0.0056 ± 0.0007
216	ND	ND

Figure 4 shows the results of percent degradation of two organophosphorous pesticide residues in soil under pesticide-treated plants. The results obtained show that percent degradation of dimethoate was 37.27 % after 24 h (1 day) application. Similary, percent degradationn of diazinon was in 46.87 % in soil samples analyzed after 24 h. Dimethoate and dizinon residues degraded almost completely (i.e., 100 %) 120 h (5 days) and 7 days (168 h) after application respectively. The data showed that the degradation of dimethoate proceeded at a much faster rate than that of diazinon in the soil. It is because dimethoate is rapidly broken down by soil microorganisms.

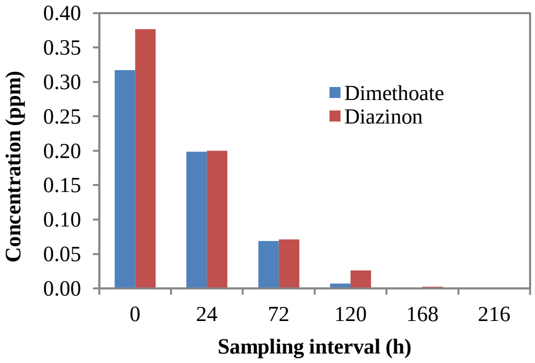


Figure 3 Concentrations of dimethoate and diazinon residues in soil as a function of sampling time interval

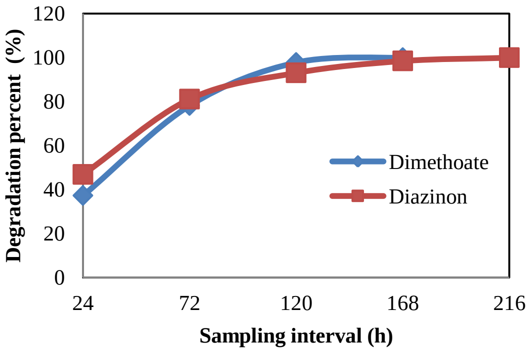


Figure 4 Percent degradation of dimethoate and diazinon residues in soil as a function of sampling time interval

Rate Constants and Half-Lives of Dimethoate and Diazinon Pesticides in Soil

In this research, the degradation of dimethoate and diazinon pesticide residues in soilwere successfully interpreted by using double exponential decay model. Non-regression model parameters of double exponential decay of dimethoate and diazinon in soil are summarized in Table 6. The dissipation curves were fitted for the individual pesticides in soil by nonlinear regression with the Sigma Plot for Windows 4.01 curve fitting package ($y = a\ e^{-bx} + c\ e^{-dx}$) with $r^2 = 0.9953$ and 0.9998 for dimethoate and diazinon-treated soil (Laab *et al.*, 2002). The data show that the calculated value from model equation (a+c) are in agreement with the measured values obtained from Gas Chromatographic analysis (GC). Rate constant and half-lives of the degradation of the pesticides were calculated by graphical method.

Table 6 Non-regression Model Parameters of Double Exponential Decay of Dimethoate and Diazinon Residues in Soil under Pesticide-Treated Lettuce Plants

$$y = a\ e^{-bx} + c\ e^{-dx}$$

Sample	Parameter						
	a (ppm)	**b (h^{-1})**	**c (ppm)**	**d (h^{-1})**	**r^2**	**(a+b) (ppm)**	**GC (ppm)**
Soil*	0.1571	0.0220	0.1644	0.0220	0.9953	0.3215	0.3169
Soil**	0.0383	1.2820	0.3381	0.0218	0.9998	0.3764	0.3764

* - under dimethoate-treated lettuce plant
** - under diazinon-treated lettuce plant

Comparison of the rate constants and half-lives of dimethoate and diazinon pesticides soil under lettuce plants were used to fit double exponential decay model and pseudo-first order equations (Table 7). Therefore, the results obtained show that the degradation rate constants and half-lives for dimethoate and diazinon pesticides in both cases were statistically not markedly different. It is because the rate constants and hence half-life do not depend on the initial concentration. The student's t test was carried out for the comparison of rate constants and half-lives between double exponential decay model and pseudo-first order equations. The calculated t-value (1.5621) is smaller than tabulated t-value (4.303) at 95 % confidence intervals (Day and Underwood, 1999). Therefore, comparison of the data obtained from double exponential decay model and pseudo-first order equations by Student's t-test indicated that no significant differences among the results.

Table 7 Comparison of the Rate Constants and Half-Lives of Dimethoate and Diazinon in Soil using Double Exponential Decay and Pseudo-First Order Equations

Pesticides	**Double exponential decay**	**Pseudo-first order**		
	k_1 (h^{-1})	**$t_{1/2}$(h)**	**k_1 (h^{-1})**	**$t_{1/2}$ (h)**
Dimethoate	0.0220	31.47	0.0216	32.08
Diazinon	0.0218	31.20	0.0212	32.67

Conclusion

The results of this study have provided an insight into the levels of organophoshorus pesticide residues (dimethoate and diazinon) contamination and dissipation in soils of pesticide treated lettuce plants. Dimethoate and diazinon pesticide concentrations were above their maximum residue limits (MRLs) for agricultural soils but reached under the MRLs after 5 days and 7 days respectively. Comparison of the degradation rate constants and half-lives of soil by using double exponential decay and pseudo-first order equations indicated that these were found to be not quite different. Based on the results of this study, routine monitoring of pesticide residues in the study area is necessary for the prevention, control and reduction of environmental pollution, so as to minimize health risks to humans. The farmers and inhabitants of the study areas should be educated on the danger of pesticides for pet control.

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