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Saphan Ochieng Anode
Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Justus Onguso
Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Gabriel Magoma
Pan-Africa University of Science and Technology, Nairobi, Kenya

Correspondence
Saphan Ochieng Anode
Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Qualitative and quantitative analysis of pesticide residues in flower farms soils around lake Naivasha basin, Kenya

Saphan Ochieng Anode, Justus Onguso and Gabriel Magoma

Abstract
Lake Naivasha basin is the highest producer of flower in Kenya. The area also has higher pesticide application to increase quality and quantity of flower mainly for export. Even though these chemicals may be effective in achieving this goal, the incidence of xenobiotic pesticide residues on the environment, some of which leach into Lake Naivasha, have been of serious concerns. The objective of this study was to assess the level of flower farm soils contamination by organochloride and organophosphate pesticides in major flower farms around Lake Naivasha basin. One hundred soil samples were collected from randomly selected greenhouses within main flower farms. The analytical methods included solvent extraction of pesticide residues and their subsequent identification and quantification using High Pressure Liquid Chromatography (HPLC). Ten different organochloride and ten different organophosphate pesticides were analyzed. The analytical results showed that aldrin, dieldrin and endosulfan, endrin, lindane, dimethoate, malathion, parathion, chlorpyrifos and pirimofos residues were detected in various concentrations. Methyl parathion had the highest mean concentrations of 87.84 ± 3.86µg/g of soil samples while Endrin, lindane, chlorpyrifos and pirimofos were not detected in some greenhouses. Routine monitoring of pesticide residues in these study areas is necessary for the prevention, control and reduction of environmental pollution and health risks.

Keywords: flower farms; lake naivasha; organochlorides; organophosphates; xenobiotics

1. Introduction
The accumulation of recalcitrant xenobiotic compounds is due to continuous efflux from population, agricultural and industrial inputs that have created a serious impact on the pristine nature of our aquatic and terrestrial environment [1]. Apart from this, these compounds are mostly carcinogenic, posing health hazards which persist over a long period of time. Both natural and anthropogenic activities result in accumulation of wide ranges of toxic xenobiotic compounds in the environment, causing a global concern [2]. The main concern with xenobiotic compounds is the toxicity threat they pose to public health. Some xenobiotic compounds especially organophosphates, phenols, biphenyl compounds and phthalates act as endocrine disruptors and inhibition of acetyl choline esterase (AchE) enzymes [3]. Once 80 % of the enzyme is inactivated, usually within four days of exposure, potentially lethal symptoms can be observed, including neck muscle weakness, diarrhea and respiratory depression [4]. Lake Naivasha and its environs have experienced increased levels of pesticide application due to the rapid expansion in floriculture farming. The flower farms around the lake currently occupy 4,000 hectares [5]. This sector is linked to intensive irrigation and pesticide use. These pesticides have significant effect since over 98% of sprayed insecticides and 95% of herbicides reach destination other than the target namely: air, water, bottom sediments and food [6].
Furthermore, poor cultivation methods have made it easier for alluvial and loamy soils found in the Lake's surrounding to be carried by erosion to the Lake. Some flower farms had extended their boundaries right down to the water bodies [7].
Further, accelerating drifting of pesticides due to the soils' fine texture, high water retaining ability and high levels organic content [5]. This makes it easier for pesticide residues to be carried into the Lake without any treatment through surface run off.
Farms far away are not spared as their waste water eventually end up into the Lake since once these pesticides had been applied in the fields they are transported to the Lake by surface runoff, rivers and streams [8, 9] In addition, wind and rain also carried pesticides away from their point source, causing contamination of surface waters [10].

The objective of this study was therefore to determine the types of organochloride and organophosphate pesticide residues found in flower farm soil around the Lake Naivasha basin and the quantitative residue level of such pesticides.

2. Materials and Methods

2.1 Sampling sites

Lake Naivasha is a freshwater lake in Kenya, outside the town of Naivasha in Nakuru County, approximately 100 km North West direction from Nairobi to Nakuru. It is part of the Great Rift Valley. The lake is located at latitude 0:5S, longitude 36:2E and Altitude 2100 meters above sea level in the Rift valley province. The length of the shoreline is 68,000 m. The catchment area is 2,378 km2 and the population is approximately 200,000 inhabitants. The study area is located within the UTM zone 37, lying between the co-ordinates X_{min}: 19243512.12 Y_{min} 99040335.35 and X_{max}: 218173.83, Y_{max} 9929234.30 [9]. The most significant activity in Lake Naivasha, albeit for large scale farmers, is the extensive irrigated greenhouse floriculture and horticulture industry. Livestock ranching and private game sanctuaries and conservation areas also exist in the catchment [6]. To meet the market demands for quality flowers, fruits and vegetables in Europe, the horticultural farmers use large volumes of pesticides [9].

2.2 Sample Collection

Soil samples were collected from randomly selected five greenhouses from each five flower farms namely Crescent flower farm, Elsamere flower farm, Karuturi flower farm, Malewa flower farm and Sewage flower farm around Lake Naivasha basin. Systemic random sampling method was used to collect the samples. Four sampling points for each greenhouse within the farms were randomly selected i.e. two points within the greenhouses and two water drainage points around the greenhouses. A soil core was dug using hoe and scooped using a spade down to the depth of 5-10 cm (for assessment of adsorption depth) from the four different locations from each greenhouses and approximately 200 g of the scooped core taken. The cores from each greenhouse were thoroughly mixed to give a composite sample of 100g. The soil sub-samples were then stored in clean plastic bags awaiting further analysis.

2.3 Standard Chemical Reagents

In the preparation of the stock solution, 0.25g of the organochloride pesticides standards (aldrin, dieldrin, endosulfan, endrin and lindane) and organophosphate pesticides standards (dimethoate, malathion, parathion, chlorpyrifos and pirimofos) were weighed into 250 ml volumetric flasks and dissolved to the mark with methanol to make standard stock solution of 1000 ppm. Six different concentrations of each pesticide (10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm) were prepared from the stock solutions by serial dilutions using the formula $C_1V_1 = C_2V_2$ and stored in refrigerator at 4°C.

2.4 Extraction of Pesticide Residues from Farm soil samples

The soil samples were extracted in triplicate using a soil-packed bulb column. 5g of each sample was ground into fine particles with clean porcelain mortar and pestle, weighed into a glass jar, and fortified at this step, before adding 10g of granular anhydrated sodium sulfate (Na_2SO_4) to absorb moisture. The sample mixtures were manually shaken for 30 seconds, placed on a roller for 30 seconds, and then allowed to stand for 20 minutes to provide time for the sodium sulfate to adsorb any residual moisture from the soil. The sample mixtures were then transferred to a 250ml bulb column and the sample jar was triple rinsed with small amounts of 5ml of hexane and transferred to the bulb column. The soil content was extracted with acetone: hexane (1:1 v/v, 250ml) and the eluate collected and concentrated to 100ml using a rotary evaporator. The soil extract was then subjected to additional cleanup. The concentrated soil eluate was washed by liquid-liquid partitioning with saturated sodium sulfate (25ml) and distilled, deionized water (300ml) in a separator funnel (500ml). After shaking, the aqueous layer was drained into a beaker and the non-aqueous hexane was transferred to a separator funnel (250ml). The aqueous layer was returned to the 500ml separator funnel and re-extracted with 15% dichloromethane in hexane (40ml). The organic layer was combined in the 250ml separator funnel and gently washed with distilled water (100ml) for about 30 seconds. After discarding the aqueous layer, the organic layer was filtered through sodium sulfate, evaporated to near dryness on a rotary evaporator, then the sides of the flask rinsed with hexane (20ml), and evaporated to about 1ml. The extraction procedure was done in triplicates for each of the greenhouse soil samples. The sample extract was quantitatively transferred to a centrifuge tube, concentrated on a nitrogen evaporator to 0.5ml and made ready for silica clean up step.

2.5 Clean-up of Soil Extracts

The silica gel clean-up process for the soil extracts were carried out by the methods described by Frimpong *et al*., (2013) [11]. 1g of silica gel that was previously activated at 110˚C for 8 hours was carefully packed into 10mls polypropylene cartridge column and 5mls acetonitrile solution was used to condition the cartridge. The concentrated extracts were then loaded onto the column and 50mls pear shaped flask was placed under the column to collect the eluate. 10mls acetonitrile was then used to eluate the column and the total filtrate collected, concentrated to dryness using rotary evaporator set at 37˚C. The residues were re-dissolved in 1ml methanol and diluted to 2.0ml final volume in hexane prior to High Performance Liquid Chromatography (HPLC) analysis.

2.6 Method Validation

The procedure and method was validated by use of matrix spikes and the reference sample (soil not spiked with pesticide standard). The matrix blanks and laboratory recovery samples were extracted at the same time with the actual samples. The standard pesticides were added as 30% of the average concentration of pesticide residues in each of the farm samples as spiked samples. 10 g of activated anhydrous sodium sulphate was placed in the thimble and extraction procedures followed as for soil samples. Each spiked sample was homogenized for even distribution of pesticide residues and stored in deep freezer overnight to attain equilibrium. Recovery samples were also extracted following the procedures as for the field samples. Recovery was done in triplicate for each of the farm soil samples. The identified retention times, were confirmed by running the samples on the two different columns used above with different stationary phases. The concentration of the analyte was determined for spiked portion, *F*, and un-spiked portions, *I*, and the percent recovery, *%R*, was calculated $\%R = \frac{F - I}{A} \times 100$

Where *A* is the concentration of analyte added to the spiked portion [12].

2.7 Quantitative and qualitative analysis of pesticide residues in soil samples

High Performance Liquid Chromatography (HPLC) machine with the following description was used: A LC 10ADVP Shimadzu liquid chromatograph with SPD-10AVP Shimadzu UV-VIS detector High Performance Liquid Chromatography (HPLC) with UV-VIS detectors, equipped with model 410Varian auto sampler, and a three system 210 pump; all coordinated by a galaxy workstation software. The separation was done on Luna C18, (5 µm, 250 x 4.6 mm) stainless steel column at Room temperature operation. The mobile phase was methanoic acid: acetonitrile (90:10, v/v) at a flow rate of 0.4mls/min. UV detection was realized at 257 nm, and the injection volume was fixed at 5µL for partial loop filling. The total run time was 15min.

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's were rigorously washed with detergent, rinsed with distilled water, thoroughly rinsed with analytical grade acetone and dried overnight in an oven at 150 °C. The glassware's were then removed from the oven and allowed to cool down and stored in dust-free cabinets. Deionized water was used throughout the study. The concentration of the standard and peak area was used to draw a calibration curve that was used to determine the concentration of the residues.

In quantitative analysis, known concentration of each pesticide standard was prepared by serial dilution using the formula $C_1V_1 = C_2V_2$ to obtain the final concentration of 1ppm. Thereafter 5µL of each pesticide standard was injected into HPLC and the absorbance measured. The measurements were then used to calibrate the HPLC instrument so that the concentration of the soil samples could be read directly from the HPLC instrument. This gave the concentration of the pesticide residues in each soil samples analyzed. 5µL of each pesticide extracts were then injected into HPLC and the absorbance measured. The measurements were done in triplicates for each greenhouse soil samples. The average concentration of each pesticide residues in each green house was then tabulated and thereafter the mean concentration of each pesticide residues on every flower farm calculated.

2.8 Data handling

Laboratory analytical results were subjected to analysis of variance (ANOVA) using SAS software, version 9.2, 2nd edition of 2010. Separation of the means was performed using Duncan significance test ($p < 0.05$).

3. Results and Discussions

3.1 Soil texture and properties

Soil contents of clay and organic carbon are the main factors that regulate pesticide retention capacity in the soil, with higher retention the more clay and organic matter soil contents. Table 1 below shows the mean of soil texture and properties of soil samples from greenhouses in the five flower farms around Lake Naivasha basin. All soil samples had clay content varying between 38-58%. Loamy content of the soil varied between 42-58% while sand varied between 12-18%. These findings corresponds to Arusei (2002) [13] that classified soils in these areas as dark brown to pale brown soils, defined further by the Kenyan Soil Survey (KSS), as loam to clay loam. The total organic carbon (TOC) varied between 2.73-5.50%. Moisture content ranged from 45.56-56.66% in the flower farm soil samples.

Table 1: Soil texture and properties of flower farm soils around Lake Naivasha basin

Soil texture			Flower	Farms		
Properties	**Crescent**	**Elsamere**	**Karuturi**	**Malewa**	**Sewage**	**Range**
pH	6.78	6.32	6.63	6.22	6.72	6.22-6.78
%Sand	10	18	24	14	12	12-18
%Loam	55	42	48	58	56	42-58
%Clay	44	54	55	38	58	38-58
%TOC	2.73	3.55	5.50	3.03	2.93	2.73-5.50
% Moisture	57.27	55.57	45.56	58.65	46.62	45.56-58.65
Mn (ppm)	54.5	43.7	34.55	56.66	55.40	34.55-56.66
Cu (ppm)	1.05	2.15	2.41	1.53	1.96	1.05-2.41
Fe (ppm)	2.20	6.55	8.55	5.92	3.75	2.20-6.55
Zn (ppm)	2.05	2.65	1.75	1.64	4.55	1.64-4.55
Texture Grade	CL	CL	SCL	SCL	CL	--

C = Clay, L = Loam, S = Sand

3.1 Extraction of Pesticide residue from Flower Farm Soil and method validation

The viability of the SPE method used to extract pesticide residues in the flower farm soil samples was validated using percent recovery (%R) and Method Detection Limit (MDL).

The % R method was used to determine whether a systematic shift occurs in the analytical signal of an analyte due to matrix effects while the MDL is the smallest measurable concentration of analyte that is statistically different from the blank. From the stock solutions 30% of the mean pesticide concentrations of the standard solution were used to spike the soil samples and 20µL of the spiked and unspiked samples injected into the HPLC system at suitable conditions. Table1 shows the mean %R and MDL of the spiked and unspiked samples.

Table 2: Percent recoveries (%R) and Limit of Detection Method (MDL)

Pesticide Compound	Standard Conc(µg/g)	Sample Conc(µg/g)	Spiked sample	Recovered Conc.	Recovered (% ± SD)	MDL(µg/g)
Aldrin	4.42	14.72	19.14	14.55	76.11±1.35	0.0045
Dieldrin	3.47	11.57	15.04	14.46	96.42±2.91	0.0035
Endosulfan	3.08	10.27	13.35	11.32	84.81±0.88	0.0024
Endrin	1.5	3.5	5	1.55	31.00±0.24	0.0044
Lindane	1.5	3.5	5	0.85	17.00±0.45	0.0055
Dimethoate	11.98	39.94	51.92	44.53	85.77±3.58	0.0016
Malathion	20.31	67.63	87.94	80.91	92.03±0.99	0.0012
Parathion	24.44	81.33	105.77	96.12	90.88±0.49	0.0015
Chlorpyrifos	2.5	5	7.5	4.55	60.67±0.85	0.0055
Pirimofos	1.5	3.5	5	2.15	43.00±0.75	0.0015

n = 3

Dieldrin pesticide residues recorded the highest % recovery at 96% probably due to its lowest aqueous solubility and higher solubility in organic solvents used that were evaporated to give the solid phase extracted product. The mean overall %R of 87.67 was apparently higher enough to consider the SPE extraction method used as reliable.

3.2 Qualitative Analysis of Pesticide Residues Extracts

The overall detection frequency found organophosphates pesticide residues at higher concentration than organochloride pesticides in all the five farms. The detection level of all the residues showed positive Pearson correlation coefficient ($P<0.05$) for all the ten pesticide residues detected in all the five flower farms. The general unknown screening carried out on the remaining peaks offers several options for automatic identification of the found peaks: database search, elemental composition determination based on isotopic pattern matching, spectral library search, and internet search. Organic components in the soil samples went through the identification process. Database and spectral library searches were carried out using built-in resources. Internet search was carried out using a selection of databases listed in the ChemSpider® online search portal.

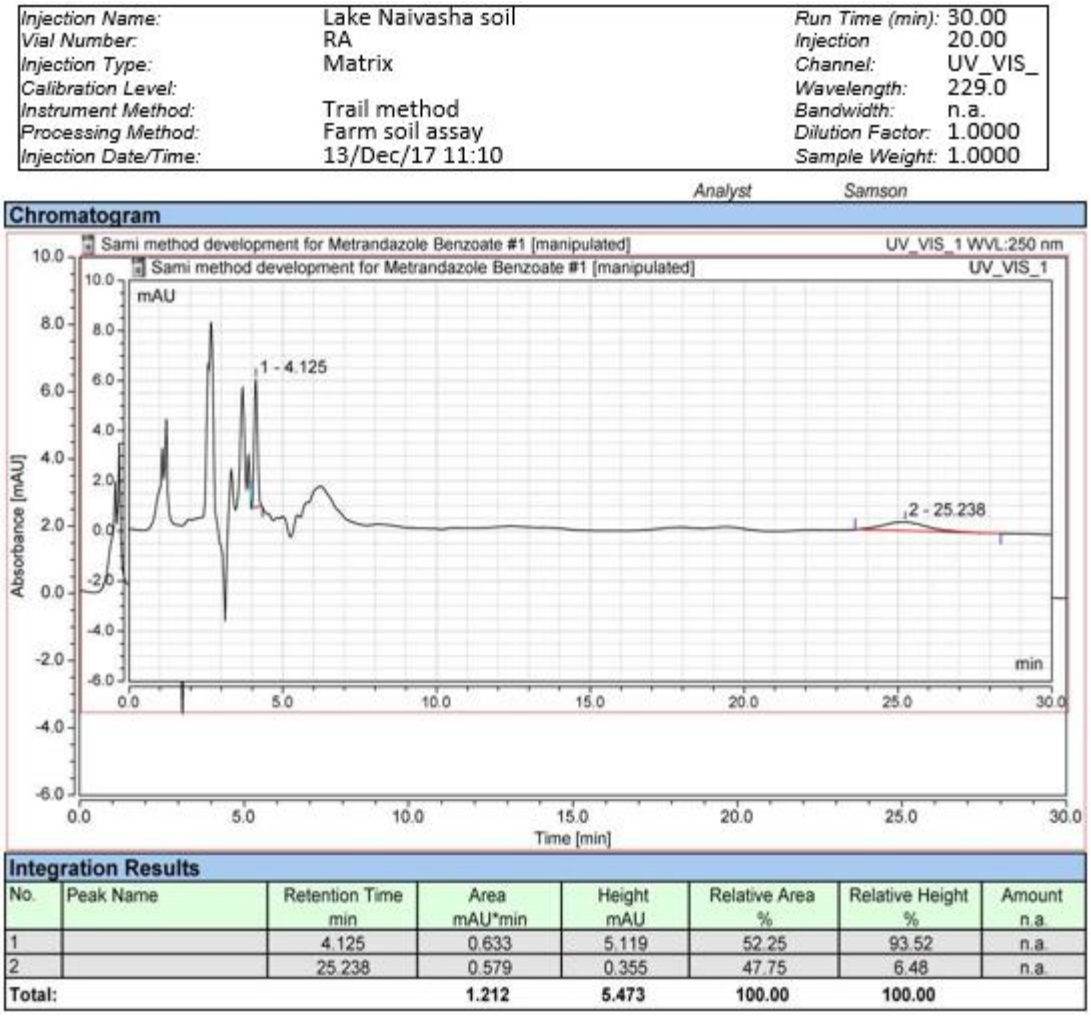

Fig 1: Retention times diagram of pesticide residue extract from flower farm soil samples around Lake Naivasha. Retention times 1.22, 3.05, 4.19, 4.25, 6.87, 7.56 and 27.54 minutes are dieldrin, aldrin, endosulfan, dimethoate, malathion, parathion and an unknown compound respectively

3.3 Quantitative analysis of flower farm soil pesticide extracts

The concentration of each pesticide residues in every greenhouse in all the five flower farms was analyzed quantitatively using HPLC. The calibration of the HPLC was done by measuring the absorbance of known concentration of standard solution of each pesticide residues. The concentration of each pesticide sample was then read directly from the HPLC instrument after calibration. Of the 10 different organochloride and 10 different organophosphate pesticides analyzed, only aldrin, dieldrin, endosulfan, endrin, dimethoate, malathion and parathion and chlorpyrifos were found in all the greenhouses in the five flower farms samples and in some cases were above the Food and Agriculture Organization (FAO) Manual of Joint Pesticide Residues (MJPR), 2015 Maximum Residue Level (MRL) for flower farm soils [14]. Lindane and pirimofos were not detected in some flower farms. Methyl parathion pesticide recorded the highest mean concentration in all the farm soil samples at 81.32±5.28µg/g of the soil samples while organochloride endosulfan recorded the lowest concentration in all the farm soil samples with the lowest concentration of 10.27±2.16µg/g. The differences in the concentrations of these pesticide residues in the soil samples may be due to ban in the use of most organochloride pesticides in most countries including Kenya, as well as the differences in their degradation rates of pesticide residues in the environment [13].

Table 3: Mean ± SD concentration (µg/g) of pesticide residues in flower farm soil samples around Lake Naivasha

Pesticide residues	Crescent	Elsamere	Flower farms Karuturi	Malewa	Sewage	FAO 2015 RV
Aldrin	15.31 ± 0.47^{d}	15.18 ± 0.92^{d}	13.54 ± 1.24^{d}	13.60 ± 1.23^{d}	15.97 ± 1.06^{d}	13.00 - 15.00
Dieldrin	13.27 ± 1.35^{e}	12.21± 1.81^{e}	10.99 ± 1.61^{e}	9.70 ± 1.34^{e}	11.68 ± 0.82^{e}	9.00 – 12.00
Endosulfan	10.31 ± 0.95^{f}	10.50 ± 1.20^{e}	8.27 ± 1.03^{f}	8.82 ± 1.86^{e}	13.45 ± 0.69^{e}	8.00 – 10.00
Endrin	1.40± 1.10^{f}	3.36± 0.95^{f}	1.10± 1.05^{f}	0.07± 0.10^{f}	3.17± 0.45^{f}	5.00 – 7.00
Lindane	0.42±0.66^{f}	2.15±0.45^{f}	DL	DL	DL	5.00 – 7.00
Dimethoate	37.30 ± 1.67^{c}	41.24 ± 2.09^{c}	44.29 ± 3.94^{c}	39.14 ± 3.34^{c}	37.70 ± 2.51^{c}	40.00 – 42.00
Malathion	66.80 ± 2.54^{b}	70.22 ± 3.61^{b}	69.88 ± 3.42^{b}	67.96 ± 7.24^{b}	63.28 ± 3.83^{b}	65.00 – 70.00
Parathion	82.26 ± 3.74^{a}	87.84 ± 3.86^{a}	79.50 ± 0.89^{a}	77.38 ± 4.89^{a}	79.64 ± 4.92^{a}	75.00 – 80.00
Chlorpyrifos	4.76± 1.95^{f}	3.87± 0.55^{f}	1.23± 0.66^{f}	1.74± 0.56^{f}	2.85± 0.91^{f}	3.00 – 5.00
Pirimofos	DL	1.76± 0.23^{f}	1.85± 0.68^{f}	0.24± 0.10^{f}	0.25± 0.12^{f}	5.00 – 6.00

Within rows, mean ± SD with different letters are statistically significant (P≤0.005)

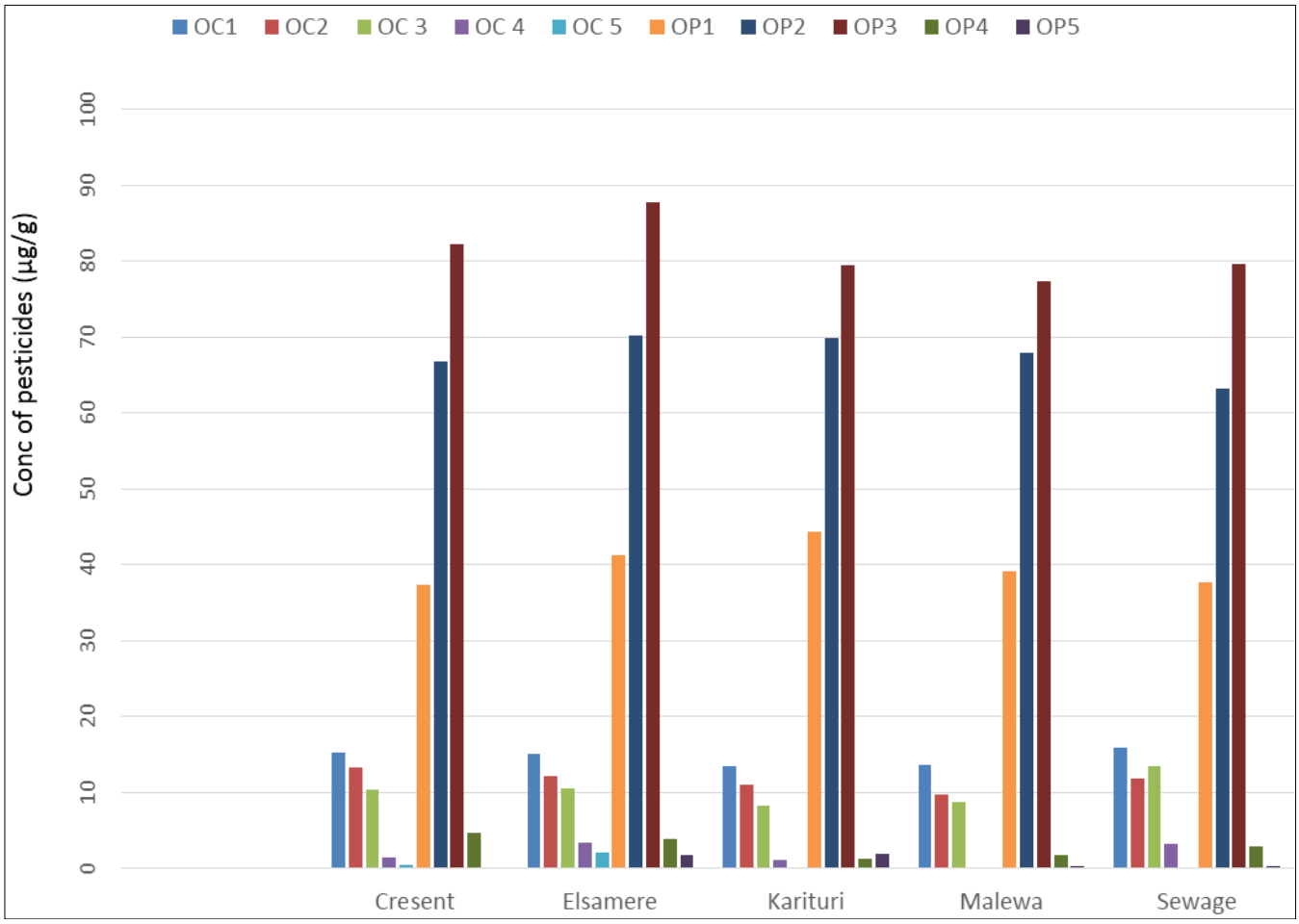

Fig 2: Mean Concentration of pesticide residues in µg/g of soil samples in five flower farms around Lake Naivasha. OC1 = Aldrin; OC2 = Dieldrin; OC3 = Endosulfan; OC4 = Endrin; OC5 = Lindane; OP1 = Dimethoate; OP2=Malathion; OP3 = Methyl parathion; OP4 = Chlorpyrifos; OP5 = Pirimofos

In all the flower farm soil samples, Methyl parathion had the highest mean concentration followed by malathion then dimethoate. Aldrin organochloride pesticide residue recorded the highest organochloride pesticide concentration while endosulfan had the lowest concentration in all the greenhouses soil samples in all the five flower farms.

3.4 Pesticide residues concentration in flower farm greenhouses

3.4.1 Pesticide residues concentration in Crescent flower farm greenhouses

Table 4.1 below shows the mean ± SD of pesticide residues in the five greenhouses in Crescent flower farm. Statistical analysis of the data of the concentration of the ten detected xenobiotic pesticides in the flower farm showed that some of the greenhouses had pesticide residue concentration slightly above the FAO JMPR 2015 reference values. These were aldrin in greenhouse 1, 2 and 5 (15.31 ± 0.47, 15.18 ± 0.92 and 15.97 ± 1.06 respectively); dieldrin in greenhouses 1 and 2 (13.27 ± 1.35 and 12.21 ± 1.81 respectively); endosulfan in greenhouses 1 and 2 (10.31 ± 0.95 and 10.50 ± 1.20 respectively); dimethoate in greenhouse 3 (44.29 ± 3.94); malathion in greenhouse 2 (70.22 ± 3.61) and methyl parathion in greenhouses 1 and 2 (82.26 ± 3.74 and 84.84 ± 3.86 respectively). Endrin was detected only in greenhouses 3 and 5, while lindane was detected only in greenhouse 1 and 2. Chlorpyrifos was not detected only in greenhouse 4, while Pirimofos was detected only in greenhouse 2 and 3. There were no significant differences ($P \leq 0.05$) in the concentration of all the detected pesticide residues in all the five greenhouse soil samples in Elsamere flower farm.

Table 4.1: Mean concentration ± SD (µg/g) of pesticide residues in Crescent flower farm greenhouses.

Pesticide	Greenhouse 1	Greenhouse 2	Greenhouse 3	Greenhouse 4	Greenhouse 5	FAO 2015 RV
Aldrin	15.31 ± 0.47^{d}	15.18 ± 0.92^{d}	13.54 ± 1.24^{d}	13.60 ± 1.23^{d}	15.97 ± 1.06^{d}	13.00 - 15.00
Dieldrin	13.27 ± 1.35^{e}	12.21 ± 1.81^{e}	10.99 ± 1.61^{e}	9.70 ± 1.34^{e}	11.68 ± 0.82^{e}	9.00 – 12.00
Endosulfan	10.31 ± 0.95^{f}	10.50 ± 1.20^{e}	8.27 ± 1.03^{f}	8.82 ± 1.86^{e}	9.45 ± 0.69^{e}	8.00 – 10.00
Endrin	DL	DL	2.07 ± 1.03^{f}	DL	3.12 ± 0.33^{e}	5.00 – 7.00
Lindane	0.31 ± 0.56^{f}	1.40 ± 1.10^{f}	DL	DL	DL	5.00 – 7.00
Dimethoate	37.30 ± 1.67^{c}	41.24 ± 2.09^{c}	44.29 ± 3.94^{c}	39.14 ± 3.34^{c}	37.70 ± 2.51^{c}	40.00 – 42.00
Malathion	66.80 ± 2.54^{b}	70.22 ± 3.61^{b}	69.88 ± 3.42^{b}	67.96 ± 2.14^{b}	63.28 ± 3.83^{b}	65.00 – 70.00
Parathion	82.26 ± 3.74^{a}	84.84 ± 3.86^{a}	79.50 ± 0.89^{a}	77.38 ± 4.89^{a}	79.64 ± 4.92^{a}	75.00 – 80.00
Chlorpyrifos	4.31 ± 0.65^{f}	2.50 ± 0.20^{f}	0.27 ± 0.03^{f}	DL	1.5 ± 0.69^{f}	3.00 – 5.00
Pirimofos	DL	0.20 ± 1.20^{f}	2.33 ± 1.03^{f}	DL	DL	5.00 – 6.00

Mean values within the rows followed by the same alphabet letters are not significantly different ($p < 0.05$) while using ANOVA and test of Duncan. DL = below instrument detection limit (0.001 µg/g). Notes: all values in table are Mean ± Standard deviation of three replicates. RV (reference values) data indicate Maximum Residue Limit (MRL) for soils in which flowers are grown [µg/g soil] (*FAO JMPR Manual 2015*). Figures in red colour are mean concentration above FAO RV permissible concentrations.

3.4.2 Pesticide residues concentration in Elsamere flower farm greenhouses

Statistical analysis of the data of the concentration of the ten detected xenobiotic pesticides in Elsamere flower farm showed that most of the greenhouses had pesticide residue concentration below the FAO JMPR 2015 reference value apart from aldrin pesticide residue in greenhouse 1 (16.41 ± 0.57µg/g), dieldrin in greenhouse 1 (12.11 ± 1.03µg/g), dimethoate in greenhouse 3 (43.45 ± 1.04µg/g), malathion at greenhouse 3 (70.88 ± 1.42µg/g) and methyl parathion in greenhouse 1 (85.26 ± 2.74µg/g), greenhouse 2 (87.88 ± 2.86µg/g), greenhouse 3 (81.50 ± 0.85µg/g) and in greenhouse 4 (80.38 ± 2.87µg/g). Endrin was detected only in greenhouse 3; Lindane was detected only in greenhouse 1 and 2; Chlorpyrifos was detected only in greenhouse 2; while Pirimofos was detected in greenhouse 2, 3 and 5. There were no significant differences ($P \leq 0.05$) in the concentration of all the detected pesticide residues in all the five greenhouse soil samples in Elsamere flower farm. The results are shown in table 4.2 below.

Table 4.2: Mean concentration ± SD (µg/g) of pesticide residues in Elsamere flower farm greenhouses.

Pesticide	Greenhouse 1	Greenhouse 2	Greenhouse 3	Greenhouse 4	Greenhouse 5	FAO 2015 RV
Aldrin	16.41 ± 0.57^{d}	14.18 ± 1.52^{d}	14.24 ± 0.24^{d}	13.15 ± 1.03^{d}	14.97 ± 1.06^{d}	13.00 - 15.00
Dieldrin	12.11 ± 1.03^{e}	11.54 ± 0.61^{e}	10.07 ± 0.61^{e}	10.90 ± 0.34^{e}	12.75 ± 0.62^{e}	9.00 – 12.00
Endosulfan	9.44 ± 0.25^{f}	9.20 ± 0.20^{e}	8.77 ± 0.03^{f}	8.52 ± 0.76^{f}	9.85 ± 0.77^{f}	8.00 – 10.00
Endrin	DL	DL	0.17 ± 0.03^{f}	DL	DL	5.00 – 7.00
Lindane	0.51 ± 0.46^{f}	0.80 ± 0.60^{f}	DL	DL	DL	5.00 – 7.00
Dimethoate	39.30 ± 0.67^{c}	38.14 ± 1.19^{c}	43.45 ± 1.04^{c}	39.25 ± 0.54^{c}	38.70 ± 1.41^{c}	40.00 – 42.00
Malathion	69.80 ± 1.54^{b}	69.42 ± 0.74^{b}	70.88 ± 1.42^{b}	65.96 ± 2.24^{b}	68.68 ± 2.83^{b}	65.00 – 70.00
Parathion	85.26 ± 2.74^{a}	87.88 ± 2.86^{a}	81.50 ± 0.85^{a}	80.38 ± 2.87^{a}	77.64 ± 3.05^{a}	75.00 – 80.00
Chlorpyrifos	DL	2.20 ± 0.20^{e}	DL	DL	DL	3.00 – 5.00
Pirimofos	DL	3.45 ± 1.20^{e}	1.85 ± 0.03^{f}	DL	2.85 ± 0.53^{f}	5.00 – 6.00

Mean values within the rows followed by the same alphabet letters are not significantly different ($p < 0.05$) while using ANOVA and test of Duncan. DL = below instrument detection limit (0.001 µg/g). Notes: all values in table are Mean ± Standard deviation of three replicates. RV (reference values) data indicate Maximum Residue Limit (MRL) for soils in which flowers are grown [µg/g soil] (*FAO JMPR Manual 2015*). Figures in red colour are mean concentration above FAO RV permissible concentrations.

3.4.3 Pesticide residues concentration in Karuturi flower farm greenhouses

Table 4.3 below shows the mean ± SD of pesticide residues in the five greenhouses in Karuturi flower farm. Most greenhouses in Karuturi flower farm had higher concentration of pesticide residues above FAO JMPR 2015 MRL permissible reference values. Aldrin pesticide residue in greenhouses 1, 2 and 5 (17.36 ± 1.45µg/g, 15.31 ± 0.47µg/g, 15.22 ± 0.82µg/g and 15.55 ± 2.06µg/g respectively) was slightly above the FAO JMPR 2015 permissible MRL of 13.00 – 15.00µg/g of soil. Dieldrin residues in greenhouses 2, 3 and 5 (15.31 ± 0.47^{d}, 15.31 ± 0.47^{d} 15.31 ± 0.47^{d} respectively) were also slightly above the permissible FAO 2015 JMPR MRL values of 9.00 – 12.00µg/g of soil in flower growing farms. Endosulfan in greenhouses 2 and 5 (10.20 ± 2.20µg/g and 13.45 ± 0.69µg/g respectively); dimethoate in greenhouse 3 and 5 (43.29 ± 3.04µg/g and 42.70 ± 1.51µg/g respectively); and malathion in greenhouses 1, 3 and 4 (71.80 ± 0.54µg/g, 70.68 ± 2.42µg/g and 72.66 ± 1.24µg/g) were also slightly above the FAO 2015 JMPR MRL for flower farm growing soil. Methyl parathion in greenhouse 4 (79.38 ± 2.59µg/g), was below the FAO MJPR 2015 MRL value of flower farm soil while other greenhouses recorded methyl parathion values above FAO MJPR 2015 MRL for flower farm soil. Endrin was detected only in greenhouses 2, 3 and 5, while lindane was detected only in greenhouse 2. Chlorpyrifos was detected only in greenhouses 1 and 5, while pirimofos was detected in greenhouses 2 and 3. There were no significant differences (P≤ 0.05) in the concentration of all the detected pesticide residues in all the five greenhouse soil samples in Karuturi flower farm.

Table 4.3: Mean concentration ± SD (µg/g) of pesticide residues in Karuturi flower farm greenhouses

Pesticide	Greenhouse 1	Greenhouse 2	Greenhouse 3	Greenhouse 4	Greenhouse 5	FAO 2015 RV
Aldrin	17.36 ± 1.45^{d}	15.22 ± 0.82^{d}	14.74 ± 1.34^{d}	13.76 ± 1.63^{d}	15.55 ± 2.06^{d}	13.00 - 15.00
Dieldrin	11.27 ± 1.45^{e}	14.21± 1.81^{e}	12.99 ± 1.81^{e}	10.70 ± 0.34^{e}	12.68 ± 0.82^{e}	9.00 – 12.00
Endosulfan	9.31 ± 0.75^{f}	10.20 ± 2.20^{e}	9.33 ± 2.03^{f}	8.82 ± 1.86^{f}	13.45 ± 0.69^{e}	8.00 – 10.00
Endrin	DL	4.33 ± 1.03^{f}	2.00 ± 0.03^{f}	DL	3.21 ± 0.45^{e}	5.00 – 7.00
Lindane	DL	2.00 ± 1.10^{f}	DL	DL	DL	5.00 – 7.00
Dimethoate	40.30 ± 2.67^{c}	41.54 ± 2.11^{c}	43.29 ± 3.04^{c}	38.14 ± 3.84^{c}	42.70 ± 1.51^{c}	40.00 – 42.00
Malathion	71.80 ± 0.54^{b}	68.22 ± 0.61^{b}	70.68 ± 2.42^{b}	72.66 ± 1.24^{b}	68.28 ± 2.05^{b}	65.00 – 70.00
Parathion	85.26 ± 2.74^{a}	80.64 ± 2.86^{a}	87.50 ± 1.89^{a}	79.38 ± 2.59^{a}	82.64 ± 2.02^{a}	75.00 – 80.00
Chlorpyrifos	1.31 ± 0.95^{f}	DL	DL	DL	3.45 ± 0.69^{e}	3.00 – 5.00
Pirimofos	DL	3.20 ± 1.20^{e}	5.33 ± 1.03^{f}	DL	DL	5.00 – 6.00

Mean values within the rows followed by the same alphabet letters are not significantly different (p < 0.05) while using ANOVA and test of Duncan. DL = below instrument detection limit (0.001 µg/g). Notes: all values in table are Mean ± Standard deviation of three replicates. RV (reference values) data indicate Maximum Residue Limit (MRL) for soils in which flowers are grown [µg/g soil] (*FAO JMPR Manual 2015*). Figures in red colour are mean concentration above FAO RV permissible concentrations.

3.4.4 Pesticide residues concentration in Malewa flower farm greenhouses

Endrin, Lindane and pirimofos pesticide residues were not detected in all the five greenhouse soil samples in Malewa flower farm. Chlorpyrifos was detected only in greenhouse 2, 3 and 4. Most of the greenhouses in Malewa flower farms recorded pesticide residue concentration below FAO JMPR 2015 MRL permissible values. The concentration of aldrin in greenhouse 3 and 5 (15.54 ± 2.24µg/g and 15.05 ± 2.05µg/g respectively) was slightly above 15.00µg/g permissible level for flower growing soil. The concentration of dieldrin and endosulfan and malathion pesticide residues in all the five greenhouses were below their respective FAO JMPR 2015 MRL for flower farm soil. Only greenhouse 3 recorded the mean concentration (44.29 ± 2.06 µg/g) of dimethoate above FAO JMPR 2015 MRL of 42.00µg/g of flower farm soil. The mean concentration of methyl parathion in greenhouse 1 and 5 (82.26 ± 3.74 and 85.64 ± 3.86µg/g respectively) was above the FAO JMPR 2015 MRL permissible value of 80.00µg/g for flower farm soil. The results are summarized in table 4.4 below.

Table 4.4: Mean concentration ± SD (µg/g) of pesticide residues in Malewa flower farm greenhouses.

Pesticide	Greenhouse 1	Greenhouse 2	Greenhouse 3	Greenhouse 4	Greenhouse 5	FAO 2015 RV
Aldrin	14.85 ± 2.17^{d}	13.18 ± 0.52^{d}	15.54 ± 2.24^{d}	14.60 ± 2.23^{d}	15.05 ± 2.06^{d}	13.00 - 15.00
Dieldrin	11.55 ± 1.05^{e}	11.11± 1.01^{e}	10.55 ± 1.01^{e}	10.70 ± 1.34^{e}	11.55 ± 1.82^{e}	9.00 – 12.00
Endosulfan	9.31 ± 0.75^{f}	9.50 ± 1.20^{f}	9.17 ± 1.03^{f}	8.12 ± 2.86^{f}	8.45 ± 0.79^{f}	8.00 – 10.00
Endrin	DL	DL	DL	DL	DL	5.00 – 7.00
Lindane	DL	DL	DL	DL	DL	5.00 – 7.00
Dimethoate	37.30 ± 1.67^{c}	41.24 ± 2.09^{c}	44.29 ± 3.94^{c}	39.14 ± 3.34^{c}	37.70 ± 2.51^{c}	40.00 – 42.00
Malathion	66.80 ± 2.54^{b}	69.25 ± 3.61^{b}	69.88 ± 3.42^{b}	67.96 ± 7.24^{b}	63.28 ± 3.83^{b}	65.00 – 70.00
Parathion	82.26 ± 3.74^{a}	77.54 ± 3.86^{a}	79.50 ± 0.89^{a}	77.38 ± 4.89^{a}	85.64 ± 4.92^{a}	75.00 – 80.00
Chlorpyrifos	DL	1.50 ± 0.20^{f}	0.27 ± 0.03^{f}	2.82 ± 0.86^{f}	DL	3.00 – 5.00
Pirimofos	DL	DL	DL	DL	DL	5.00 – 6.00

Mean values within the rows followed by the same alphabet letters are not significantly different (p < 0.05) while using ANOVA and test of Duncan. DL = below instrument detection limit (0.001 µg/g). Notes: all values in table are Mean ± Standard deviation of three replicates. RV (reference values) data indicate Maximum Residue Limit (MRL) for soils in which flowers are grown [µg/g soil] (*FAO JMPR Manual 2015*). Figures in red colour are mean concentration above FAO RV permissible concentrations.

3.4.5 Pesticide residues concentration in Sewage flower farm greenhouses

The mean concentration of pesticide residues in the five greenhouses in Sewage flower farm are shown in table 4.5 below. Most of the greenhouses in Sewage flower farm had mean concentration of pesticide residues above respective FAO JMPR 2015 MRL permissible values for flower farm soils. The concentration of aldrin in greenhouse 1, 2 and 5 (15.42 ± 0.47µg/g, 15.09 ± 0.52µg/g and 15.07 ± 1.16µg/g respectively) were above the FAO JMPR 2015 MRL value of 15.00µg/g of flower farm soil. Greenhouses 1 and 2 recorded dieldrin concentration of 12.27 ± 1.35µg/g and 12.51 ± 1.08µg/g respectively which surpassed FAO JMPR 2015 MRL value of 12.00µg/g of flower farm soils. Only greenhouse 5 recorded mean concentration of endosulfan (11.45 ± 0.59µg/g) slightly above FAO JMPR 2015 MRL value of 10.00µg/g for flower farm soils. The concentration of Endrin in all the five greenhouses were below the FAO JMPR 2015 MRL value for flower farms, while lindane was not detected in all the five greenhouses in Sewage flower farm. Only greenhouse 3 recorded the concentration of dimethoate of 44.29 ± 3.94µg/g above FAO JMPR 2015 MRL of 42.00µg/g for flower farms, while malathion concentration of 71.32 ± 3.61µg/g in greenhouse 2 was the only residue concentration in the farm that was above FAO JMPR 2015 MRL of 70.00µg/g for flower farm soils. The FAO JMPR 2015 MRL value for methyl parathion of 80.00µg/g for flower farms was surpassed in greenhouses 1 and 2 (82.26 ± 3.74µg/g and 86.24 ± 3.86µg/g respectively). The concentration of chlorpyrifos in greenhouses 1, 2, 4 and 5 (7.31 ± 0.95µg/g, 5.50 ± 1.20µg/g, 6.75 ± 1.56µg/g and 7.15 ± 0.99µg/g respectively) was above FAO JMPR 2015 MRL permissible value of 5.00µg/g for chlorpyrifos in flower farm soils. Pirimofos was only detected in greenhouse 4.

Table 4.5: Mean concentration ± SD (µg/g) of pesticide residues in Sewage flower farm greenhouses.

Pesticide	Greenhouse 1	Greenhouse 2	Greenhouse 3	Greenhouse 4	Greenhouse 5	FAO 2015 RV
Aldrin	15.41 ± 0.47^{d}	15.09 ± 0.52^{d}	13.00 ± 0.24^{d}	14.60 ± 0.23^{d}	15.07 ± 1.16^{d}	13.00 - 15.00
Dieldrin	12.27 ± 1.35^{e}	12.51± 1.08^{e}	10.99 ± 1.61^{e}	11.70 ± 1.34^{e}	11.68 ± 0.82^{e}	9.00 – 12.00
Endosulfan	8.31 ± 0.95^{f}	9.50 ± 1.80^{e}	9.27 ± 1.75^{f}	9.82 ± 1.86^{e}	11.45 ± 0.59^{e}	8.00 – 10.00
Endrin	1.40 ± 1.10^{e}	2.40 ± 0.10^{e}	0.07 ± 1.03^{f}	0.70 ± 0.10^{e}	DL	5.00 – 7.00
Lindane	DL	DL	DL	DL	DL	5.00 – 7.00
Dimethoate	37.30 ± 1.67^{c}	41.24 ± 2.09^{c}	44.29 ± 3.94^{c}	39.14 ± 3.34^{c}	37.70 ± 2.51^{c}	40.00 – 42.00
Malathion	66.80 ± 2.54^{b}	71.32 ± 3.61^{b}	69.88 ± 3.42^{b}	67.96 ± 7.24^{b}	63.28 ± 3.83^{b}	65.00 – 70.00
Parathion	82.26 ± 3.74^{a}	86.24 ± 3.86^{a}	79.50 ± 0.89^{a}	77.38 ± 4.89^{a}	79.64 ± 4.92^{a}	75.00 – 80.00
Chlorpyrifos	7.31 ± 0.95^{f}	5.50 ± 1.20^{f}	4.27 ± 1.13^{f}	6.75 ± 1.56^{f}	7.15 ± 0.99^{f}	3.00 – 5.00
Pirimofos	DL	DL	DL	1.40 ± 1.10^{e}	DL	5.00 – 6.00

Mean values within the rows followed by the same alphabet letters are not significantly different ($p < 0.05$) while using ANOVA and test of Duncan. DL = below instrument detection limit (0.001 µg/g). Notes: all values in table are Mean ± Standard deviation of three replicates. RV (reference values) data indicate Maximum Residue Limit (MRL) for soils in which flowers are grown [µg/g soil] (*FAO JMPR Manual 2015*). Figures in red colour are mean concentration above FAO RV permissible concentrations.

4. Recommendations and Conclusion

4.1 Recommendations

The results of this study have provided an insight into the levels of agrochemical pesticide residues contamination in flower farm soils around lake Naivasha basin. From the study, aldrin, dieldrin and endosulfan organochlorides and dimethoate, malathion and methyl parathion organophosphates were detected in all the greenhouses soil samples within the five flower farms analyzed. Endrin, lindane, chlorpyrifos and pirimofos were not detected in some greenhouses. There were no significant differences ($P \leq 0.05$) in the mean concentrations of pesticide residues in all the greenhouse soil samples in the five flower farms. However, there was generally higher mean concentration of organophosphates compared to organochlorides probably due to global ban on most organochloride organic compounds. In some greenhouses, the concentration of the pesticide residues were above the FAO JMPR 2015 MRL permissible level for flower farm soils.

The occurrence of xenobiotic pesticide compounds in the flower farm soil samples from these flower farms may result in pollution of the ecosystem and food chain. Apart from the potential danger these pesticide residues may pose to soil organisms, there is also the possibility of translocation of these residues from the soil into the ground water basins and other water bodies such as lake Naivasha water which is source of fresh water to humans and both terrestrial and aquatic organisms. These xenobiotic pesticide residues are also absorbed through the roots of food crops planted around these flower farms posing health risks to consumers of these produce. In addition, water bodies are likely to be contaminated with these pesticide residues through runoff and leaching from soils in these farms exceeding the recommended MRL values for pesticide concentration in the flower farm soils.

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.

Farmer sensitization on safe pesticide use should be intensified to reduce the levels of pesticide residues in soils and in drinking water sources as poor agricultural practices such as improper disposal of empty pesticides containers were observed in the study area.

4.2 Conclusion

This study documents lists of organochloride and organophosphate pesticides that are commonly used in five major flower farms around Lake Naivasha basin. The results reveals that organophosphate pesticides are used in higher concentration than organochloride probably due to global ban of most organochloride in many countries including Kenya. From the results of this study and other studies conducted within Lake Naivasha basin [7, 8, 9, 13, 14, 15,16], it would be important to monitor and ascertain the residue levels of organophosphates, banned or restricted organochloride pesticides in soil and aquatic environment along the other drainage basins in Lake Naivasha. It would also be important

to monitor pesticide residues in food crops grown around the lake basin.

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