Determination of Pesticide Residue in Water and Khat (*Catha edulis*) Leaves Using GC-ECD

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Abstract : In this work the concentration of some selected pesticides such as aldrin, dialdrin, BHC, diazinon, DDT, 4,4-DDE and heptachlor were investigated in water and khat samples that were collected from five different sites. The pesticides were extracted using liquid-liquid and solid phase extraction technique and analyzed by GC-ECD. The recovery test, linearity cure and extraction efficiency were tested by using standard pesticide samples. The highest concentration of pesticide obtained in water samples is diazinon in WS_2 (0.0698mg/L), and the lowest concentration is heptachlor in WS_3 (0.0006mg/L). The highest and lowest concentrations of pesticides obtained in Khat samples are diazinon in KS_1 (0.0323mg/L) and KS_3 (0.0001mg/L) respectively. The concentration of diazinon and four isomers of BHC detected in each water sample are higher than the recommended limits set by WHO which shows contamination of the lake water. Diazinon and DDT levels in KS_1 (0.0323mg/L), KS_2 (0.0293mg/L) and KS1 (0.0134mg/L), KS_2 (0.0173mg/L) respectively are above the maximum residue level.

Key words: *concentration, DDT, diazinon, extraction, heptachlor*

1. Introduction

The use of chemicals in modern agriculture has significantly increased productivity. But it has also significantly increased the concentration of pesticides in food and in our environment, with associated negative effects on human health. Annually there are dozens of million cases of pesticide poisonings worldwide [1]

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In recent times, the extent of the use of pesticides, and their mode of application including their abuse especially in agriculture have been of much concern to environmental scientists [2]

Organochlorine (OCs) and organophosphorus (OPs) pesticides are still being used unofficially in large quantities in many parts of developing countries because of their effectiveness as pesticides and their relatively low cost [3].

Pesticides are mainly sprayed on agricultural fields such as cereal crops and vegetables. Especially farmers in Eastern Hararge Zone use different types of pesticides for crop and Khat cultivation in different seasons.

Organochlorine pesticides are very persistent, bioaccumulative and toxic, and they can easily find their way into an adjacent water course such as the lakes via soil run-off and leaching.

The aim of this study is to determine the concentration levels of some selected pesticides in water and khat leaves.

The determination of pesticide residues in khat and water can give indication of the extent of aquatic contamination and accumulation characteristics of these compounds in the khat that help in understanding the behavior and fate of these persistent chemicals. This work tries to provide baseline information on levels of pesticide residues including aldrin, dialdrin, BHC, diazinon, DDT, 4,4-DDE and heptachlor in water (Adele lake) and khat leaves (Harmaya Wereda) Eastern Hararge Zone, Ethiopia.

2. Materials and Methods

2.1 Description of the Study Area

Lake Adele (300 ha) is found in Haramaya Wereda, East Harerghe Zone. The lake is 26 km west of the city of Harar. It is located at latitude of 9° 25’ 33” N and longitude of 41° 57’ 03” E. The lake is separated by a 15-km-wide strip of cultivated land from Lake Haramaya. The Lake is surrounded by small hills and derives its water directly from rainfall and from several small streams that drain catchments to the west and north; floods from adjacent watersheds also occur [4].

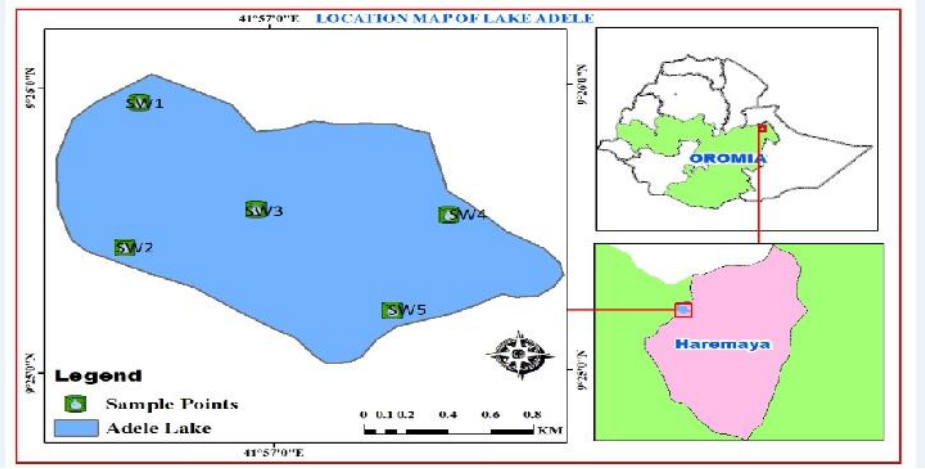

Fig.1 Location of the study area

2.2 Materials and Apparatus

The apparatus and equipments used were; GC-ECD (Agilent 7890A), Analytical balance (OHAUS, made in Switzerland), Deionizer (ELGACAN, Germany), Separatory funnel Round, bottom flask, Rotary evaporator, Centrifuge (K_2 series, CENTERION SCIENTIFIC LTD, made in west Sussex.U.K), Volumetric flask, Pipettes and Beakers.

2.3 Chemicals and Reagents

Standard pesticides with the corresponding purity: a-BHC (99.46 %, CF:$C_6H_6Cl_6$, MW:290.83g/mol), DDT (99.6%, CF: $C_{14}H_9Cl_5$, MW:354.48g/mol), 4,4-DDE (99%,CF: $C_{14}H_8Cl_4$, MW: 318.02g/mol), Aldrin (98.1%, CF:$C_{12}H_8Cl_6$,MW:364.90g/mol), Diazinon (96.5 %), CF: $C_{12}H_{21}N_2O_3PS$, MW: 304.34g/mol) and Heptachlor (98.7%,CF: $C_{10}Cl_7$, MW: 373.32g/mol) were purchased from Sigma–Aldrich, (Germany). Chemicals used were: Analytical grade acetone (99.5%), ethyl acetate (99.5%) and n-hexane (95%) obtained from BDH limited Poole (BDH AnalaR), dichloromethane, anhydrous sodium sulfate, silica gel, sodium chloride. All the solvents and reagents used were of analytical grade.

2.4 Method Validation

All sampling, extraction and analysis were done in replicates to verify the detected pesticide residue. Recovery tests were carried out using the reference pesticide standards to determine

performance of the methodology. Quantification of pesticides residues were carried out using high purity pesticide reference standards. Field blanks and method blanks were also incorporated to check contamination during sampling, transportation and laboratory preparation procedures.

2.5 Sampling, Extraction and Cleanup of Pesticides in Water

Water samples were collected on October 2018 from five different locations using plastic bottles, which were previously cleaned with detergent and rinsed with water followed by ethylacetate and then dried for 24 hours. The bottles were also rinsed with sampling water before sampling. The water samples were immediately transported to Haramaya University laboratory in an ice chest with ice and stored at 4^{o}C in a refrigerator until extraction and analysis. The samples were transported to Animal Products Veterinary Drugs and Feed Quality Assessment Center laboratory, Addis Ababa for further analysis.

The extraction was performed using US EPA Method 3510 (US-EPA, 2004)[5], for aqueous matrix for the analysis of semi-volatile and non-volatile organics and the cleanups of the extracts were done by using the US EPA Method 3620B (US-EPA, 1989)[6].

A 500 mL from each of the aqueous sample was measured and transferred into a 1000 mL separatory funnel. The aqueous sample was extracted three times with portions 100 mL of 1:1 (v/v) ethylacetate/dichloromethane mixture. The separatory funnel was clapped for 30 min to allow phase separation. The combined organic phases were collected into a 500 mL beaker with the aqueous phase discarded. The combined organic layer then dried off any aqueous substance with 20 g of anhydrous sodium sulfate and allowed to settle. The organic content then decanted into a 300 mL round bottom flask and the content evaporated to dryness using the rotary evaporator at 40 °C. The pesticide in the rotary flask then was dissolved and collected with 2 mL of ethyl acetate and transferred into a 2 mL vial ready for the cleanup.

For the cleanup, a 10 g portion of deactivated silica gel was weighed and transferred into a 10 mm i.d. glass chromatographic column followed by the addition of 3 g of anhydrous sodium sulfate. A 10 mL of the 1:1 (v/v) ethylacetate/dichloromethane mixture was used to wet and rinse the column. The extract residue in 2 mL ethyl acetate then transferred into the column and the extract vial rinsed (three times) with 2 mL ethylacetate and added to the column. The column

then eluted with 80 mL portion of ethylacetate/dichloromethane at a rate of 5 mL/min into a conical flask as fraction one. The column was eluted again with 50 mL portion of ethylacetate/dichloromethane for the second elution and added to the first extract. All the fractions of each sample was concentrated to dryness using a rotary evaporator at 40 °C. Each residue was then dissolved and collected in 2 mL ethylacetate for GC-ECD analysis.

2.6 Collection and Extraction of Pesticides from Khat Leaves

Khat samples were collected from five different farms each in October 2018 from Haramaya Werede (9°24′N42°01′E) Eastern Harerghe where the lake water is used for agriculture. The collected khat samples were packed with polyethylene bags until the next work proceeded. Then, the leaves of khat (consumable part) was separated and washed respectively to clean unwanted materials. The cleaned leaves dried under shade to manage photooxidation.

Pesticide extraction and clean up from khat samples was performed following the procedure described by Huang et al. 2007. The collected leaf samples were dried under shade and powdered. A 1 g of the powdered sample was mixed with 5.0mL water and 1.0 g sodium chloride. The mixture was sonicated for 3 min and kept at room temperature for 30 min. Then the mixture was extracted three times with 4.0 mL each of the extraction solvent [acetone : ethyl acetate : n-hexane (1:2:1 v/v/v)] for 3 min followed by centrifugation at 2,500 rpm for 2 min, and the organic phase from the extractions was combined and dried with 1.0 g of anhydrous sodium sulfate. The solution was then filtered and reduced to about 5mL at 45^0C. A 5 mL of the concentrated residual extract was introduced into the Solid Phase Extraction (SPE) column, which was preconditioned with 6.0mL of the extraction solvent. The retained pesticides on the column were eluted with 6.0mL of acetone–ethyl acetate mixture (1:2 v/v). The eluent was collected and evaporated to dryness in vacuum. Finally, the residue was re-dissolved in 0.5 mL ethyl acetate and used for GC–ECD analysis.

3. Result and Discussion

3.1 Concentration of Pesticides in Water Samples

Five surface-water and khat as well as quality control samples were analyzed using GC-ECD for the determination of pesticide concentration. Performance of the GC-ECD method was assessed by examining data for chemical surrogates and spiked samples.

Mean recoveries of each analyte, including surrogates, should ideally be 100 percent; mean recoveries substantially different from 100 percent may indicate inaccurate spike (or surrogate) solution concentrations, degradation, extraction efficiencies less than 100 percent, sample contamination, or analytical interference [8].

The recovery analysis was done for each selected pesticides to check the efficiency of the method. The linearity curves of area verses concentration of the standard pesticides were $\geq$ 0.995, which indicates strong correlation. The result of the recovery test indicates that most of them are in the range of good recovery (70-120%), except dialdrin.

Table 1: The Concentration level of pesticides in water samples

Pesticides	Mean concentrations of pesticides (mg/L)					% Recovery	Correlation (R^2)
	WS_1	WS_2	WS_3	WS_4	WS_5		
α-BHC	0.0377	0.0231	0.0419	0.0213	0.0101	83.43	0.9976
Diazinon	0.0346	0.0698	0.0369	0.0512	0.0173	74.00	0.9975
γ-BHC	0.0097	0.0060	ND	0.0051	ND	75.34	0.9975
β-BHC	ND	0.0223	0.0125	0.0193	0.0017	79.24	0.9986
δ-BHC	ND	0.0022	ND	0.0050	ND	73.30	0.997
Heptachlore	ND	0.0031	0.0006	0.0043	ND	100.12	0.9976
Aldrin	0.0304	0.0040	0.026314	0.0239	ND	77.32	0.9964
Dialdrin	ND	ND	ND	0.0010	ND	66.47	0.9966
DDT	0.0020	0.0051	0.0083	0.0073	0.0019	80.18	0.9957
4,4-DDE	0.0290	0.0215	0.0253	0.0244	0.0154	77.20	0.9958

Note: WS_1.....WS_5 = water sampling sites, ND = non detected

The highest concentration of pesticide obtained in water sample is diazinon in WS_2 (0.0698), and the lowest concentration is Heptachlor in WS_3 (0.0006).

Alpha-BHC, diazinon, DDT and 4,4-DDE were detected in all the water samples. But the other pesticides were undetected at list in one water sample. For example, Dialdrin was detected in 20% of the sample. Delta-BHC was detected in 40% of the samples, gama-BHC and Heptachlor were detected in 60% of the samples, beta-BHC and Aldrin were detected in 80% of the samples.

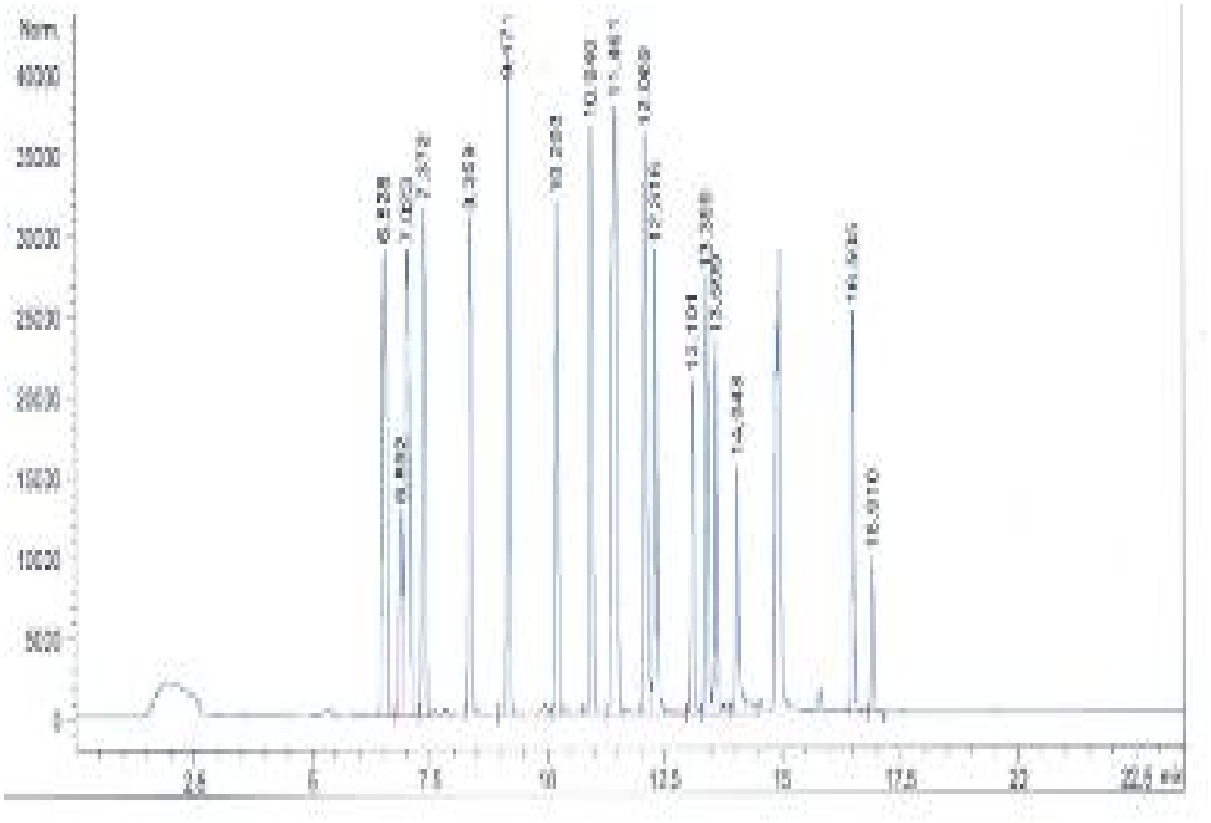

Fig.2 The chromatogram of the standard pesticides

Diazinon is one of the organophosphorous insecticides used to control insects. It is the highest concentration of all pesticides detected in water sample. Its recommended concentration limit set by WHO is 0.001mg/L. Therefore, the concentration of diazinon detected in each sample is higher than the recommended limit. This indicates that the farmers around the lake use diazinon in large amount.

Heptachlor is applied as a soil treatment, as a seed treatment (maize, small grains and sorghum) or directly to foliage. It is used to control ants, cutworms, maggots, termites, thrips, weevils, wireworms and many other insect pests in both cultivated and uncultivated soils. Heptachlor also controls household insects and pests of humans and domestic animals [9]. The value obtained in the water sample is less than the value set by WHO (0.004mg/L).

Aldrin and dieldrin are highly effective insecticides for soil-dwelling pests and for the protection of wooden structures against termites and wood borers. Dieldrin has also been used against insects of public health importance [10]. Aldrin and dieldrin are the common names of two

structurally similar compounds that are used as insecticides. The only difference between the structures of aldrin and dieldrin is the presence of an epoxied ring, in dialdrin, at the site of one of the carbon-carbon double bonds in aldrin. Aldrin detected in four sampling sites, on the contrary, dialdrin detected only in one sampling site (WS_1), and this value is less than the values of aldrin detected in all the sampling sites. This indicates that dialdrin is unstable in water due to the reactive group epoxies.

Hexachlorocyclohexane (HCH), also known as benzene hexachloride (BHC), exists in different chemical forms (isomers). The different isomers are named according to the position of the hydrogen atoms in the structure of the chemical. Benzene hexchloride (BHC) is used as an insecticide on fruit, vegetables, crops, and animals. The four isomers (α-BHC, β-BHC, γ-BHC and δ-BHC) were assessed in the water sample and they were detected in five, four, three and two sampling sites respectively. The concentration level of the four isomers is in the order of α-BHC > β-BHC > γ-BHC > δ-BHC. The WHO recommended value of BHC is 0.001mg/L, all the four isomers are above the given limit.

DDT is a non-systemic contact insecticide with a broad spectrum of activity. It was banned in several countries in the early 1970s because of ecological considerations, and many other countries have more recently restricted or banned its use except when it is needed for the protection of human health. DDT is still used in some countries for the control of vectors that transmit yellow fever, sleeping sickness, typhus, malaria and other insect-transmitted diseases [9]. DDT and its derivative 4,4-DDE were detected in all the water samples. This indicates that even if DDT is banned in the country, the farmers around the lake water use it as an insecticide. The value obtained are in comparable with the values that were investigated by Essmang et al. 2009 at Ghana but slightly greater than the values obtained by Farshid et al. 2012 at Iran [11]. DDE was undetected in water on the work done by Upadhi F. and Wokoma O. 2012 at Nigeria[12]

3.2 Concentration of Pesticide Residue on Khat Leaves

A summary of the pesticide residues found in khat samples from five different farms of Haramaya werede Eastern Hareregе is shown in 2. Some level of a-BHC was detected in KS_1, KS_2 and KS_3. In KS_1 and KS_2 its level are higher which implies its excess use in this farms than

the rest. The highest concentration of α-BHC is detected in water sample compared with that of khat leaves.

Diazinon was detected almost in all the khat samples except KS_5 even if it is below the quantification level in some samples (the maximum residue level (MRL) under EU-regulation set for vegetables and fruits is 10μg/kg). Diazinon level in KS_1 and KS_2 is higher or above MRL which indicates it is frequently used in these farms than the others. Diazinon in Ethiopia is registered for the control of pests that attack vegetables and fruits and some concentrations of residue observed in khat samples with higher amount indicate its excessive use in that farms. The concentration of diazinon is slightly greater in water sample than is khat leaves.

Currently the use of aldrin in Ethiopia is restricted. However, its presence in samples (KS_1, KS_2 and KS_4 below MRL) could be from historical application and could be attributed to the persistence of organochlorine pesticides in the environment. The concentration of aldrin in khat leaves are less than that of water sample obtained in this work.

Table 2: Observed pesticides residue level in khat samples collected from different farms

S/N	Pesticides	Mean concentration (μg/Kg)					Spiked (μg/Kg)	Spiked reading (μg/kg)	Recovery (%)
		KS_1	KS_2	KS_3	KS_4	KS_5			
1	a-BHC	13.8	13.9	3.4	ND	ND	20.0	14.46	72.30
2	Diazon	32.3	29.3	0.1	0.3	ND	20.0	15.73	78.70
3	Aldrin	2.4	3.2	ND	2.0	ND	20.0	12.30	61.51
4	Heptachlore	8.5	4.9	ND	ND	ND	20.0	14.80	74.21
5	Dialdrin	ND	ND	ND	ND	ND	20.0	14.04	70.17
6	4,4-DDE	ND	ND	ND	ND	ND	20.0	17.2	85.99
7	DDT	13.4	17.3	7.8	ND	3.4	20.0	15.40	76.23

Note: KS_1….KS_5 = Khat sampling sites

Heptachlor also detected in KS_1 and KS_2, however it is below the quantification level. The concentration of heptachlor is higher in khat leaves than in water samples. The average total DDT in KS_1, KS_2, KS_3, and KS_5 is (13.4, 17.3, 7.8, and 3.4 g/kg) respectively and its amount was not detected in KS_4. The total DDT in KS_1 and KS_2 from these samples was higher than the EU set MRLs for total DDT in edible foodstuffs, citrus fruits, vegetables and sugar plants (10 μg/kg). The highest amount of total DDT observed in two of the khat samples (KS_1 and KS_2 μg/Kg) were about 3/2 times the EU MRL for foodstuff. Giving the lipophilic nature of

organochlorine compounds, foodstuffs having less water and greater lipid contents have greater potential to accumulate the organochlorine pesticides. Because khat has high water and low lipid contents, the obtained results indicate that very high levels of DDT have been used than the other farms. The high level of DDT in the khat samples instead of DDE suggest that DDT is currently in use for control of pests in the study regions. If the accumulation were the result of historical use, the level of DDE would have been much higher than DDT as DDT is metabolized to DDE over time. However, the level of DDE was not detected in all samples totally. The half-life of DDT in soil and in plant material is about 2,000 days [12]. The range for total DDT residue level in khat samples is 3.4–17.3 μg/Kg . This range of total DDT residue level indicates the difference in the level of DDT usage by farmers within each farm and suggests that there is lack of regulation or information on the use of these pesticides. The detection of high residue level of organochlorine pesticides in khat samples collected from different farms shows that such pesticides are still in use. Currently, DDT is not permitted for agricultural use in Ethiopia. However, because of its easy accessible to the farmers from the depots meant for malarial control, it is illegally used on khat farms.

Several reviews on the adverse health effects of chewing khat documented negative health impact of the active compounds in khat and in some cases in association with smoking (commonly used in khat chewing sessions) but did not account the role that may be contributed by pesticides. Organochlorines have been shown to cause abnormalities in the reproduction and immune systems of birds and marine mammals and several abnormalities caused by organophosphorous have been reported in many organisms [14]. The incidence of oral and oesophageal cancer among khat chewers may also be in part attributed to the carcinogenic effects of organochlorine pesticides [15].

4. Conclusion

The result of the recovery test indicates that most of them are in the range of good recovery (70-120%). Alpha-BHC, diazinon, DDT and 4,4-DDE were detected in all the water samples. However, the other pesticides were undetected at list in one water sample. The highest concentration of pesticide obtained in water sample is diazinon in WS_2 (0.069845), and the lowest concentration is heptachlor in WS_3 (0.000594). The concentration of diazinon, adrin,

dialdrin, DDE and α-BHC were higher in water samples than is khat leaves. However, the concentration of heptachlor and DDT were higher in khat leaves.

The concentration of all the pesticides detected, except heptachlor, in each water, samples were higher than the recommended limit set by WHO. This shows that even if some of the pesticides including DDT are banned to use in the country, farmers are still using them for different purpose and the lake water is highly contaminated by these pesticides. The value of DDT and DDE obtained in this work are in agreement with the values that were investigated by other researchers in Ghana but slightly greater than the values obtained at Iran. The value of diazinon obtained is higher than the value that was obtained in Nigeria.

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Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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