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Multiresidue Analysis of Pesticides in Soil Profile from the Loukous Valley using GC-ECD.

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ABSTRACT

A multiresidue analytical method using a gas chromatograph equipped with electron capture detection (GC/ECD) was developed for the analysis of seventeen organochlorine pesticides (17-OCPs) in four soil samples collected from the Loukous valley region. The total concentrations of 17-OCPs in all investigated samples ranged from 96.45 to 3129.81 µg/kg with an average value of 1178.41 µg/kg. Among the seventeen OCPs, cypermethrin was the most frequently occurred pesticide in the investigated area while hexachlorobenzene was the least detected pesticide. The recovery rates for 17-OCPs ranged from 70 to 129 %, with relative standard deviation RSD% values ranging from 1.5 to 25%. The results of validation parameters verified that the experimental methodology was efficient and robust. The study concluded that mitigation measures concerning pesticides application to the land should be taken to decrease OCPs residues in order to improve the quality of the Loukous valley region soil.

Keywords: pesticide, soil, Loukous valley, GC-ECD.

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INTRODUCTION

Everyone knows that the year 1914 was marked by the explosion of the First World War marked the spirits through the centuries. Moreover, this eventful year in the first locust invasion in Morocco so he used for the first time these pesticides. Since that time, the use of pesticides continues to grow reaching 21,162 tonnes in 2004. Thus, during the last two decades, Morocco was a tangible sector development pesticides. Nowadays, these quantities have covered an agricultural area, 1, 7 million hectares (knowing that the agricultural land in Morocco is 9.2 million hectares) whose application is primarily on plantations (citrus trees, fruit) in gardening (vegetables, fruits, herbs) and field crops (cereals and industrial crops). Moreover, the use of such pesticides is estimated at 1.3 kg / ha / year. (1), (étude de cas du Maroc [En ligne], [http://pesticides.e-monsite.com/pages/i-le-fruit-des-pesticides/3-etude-de-cas-du-maroc.html])

The Lukus Valley is considered the most important agriculture area in Morocco where many important crops are grown. Many agricultural insect pests and diseases attack cultivated crops in this region, so that huge quantities of pesticides were applied to control these insect pests and diseases.

Most of the agricultural activities are located in Lukus Valley and the agricultural sector is consuming huge amount of pesticides. It is expected that frequent application of pesticides to soil may cause severe contamination to the soil profile. The aim of this study is to develop an analytical method and use this method to determine the concentration levels of the pesticides and their distribution pattern in the soil profile.

METHODOLOGY

Study Area

Four surface soil samples were collected from one depth (0-20cm) from the Loukous valley region during the period of March 2013. The study area is located in the northern Moroccan region, namely: Larache, Ksar Elkbir, Tlata Drissana et Laouamra. The study area is known for intensive different agricultural practices that request frequent applications of pesticides and fertilizers. The sampling sites were chosen such that they represent the whole arable land and its different agricultural activities in the investigated area. The chosen sites are assumed to have high pesticide usage for controlling pest and diseases on vegetable crops and citrus orchards.

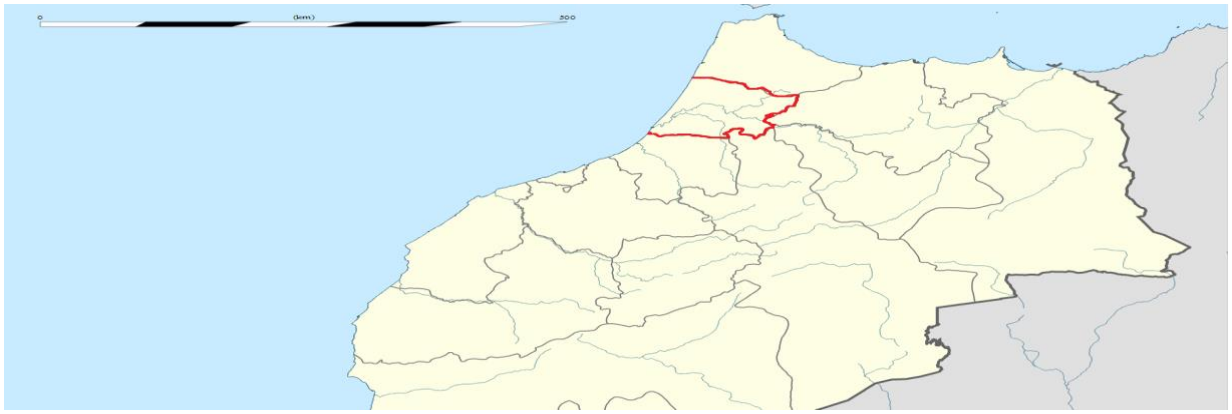

Figure 1: Study Area

Soil Sampling

The soil samples were collected from one depth: four from surface soil depth 0-20 cm. Each individual soil sample was collected randomly from an area of five square meter and pool together to get one homogenized and representative sample. Then it was separately placed in a glass jar and labeled. The samples were stored in a chilled container and transferred directly to the Laboratory. The collected samples were sieved for < 2 mm and stored at -20ºC until time of analysis. These figures exemplify the cross-section of the soil profile and the sampling technique, respectively.

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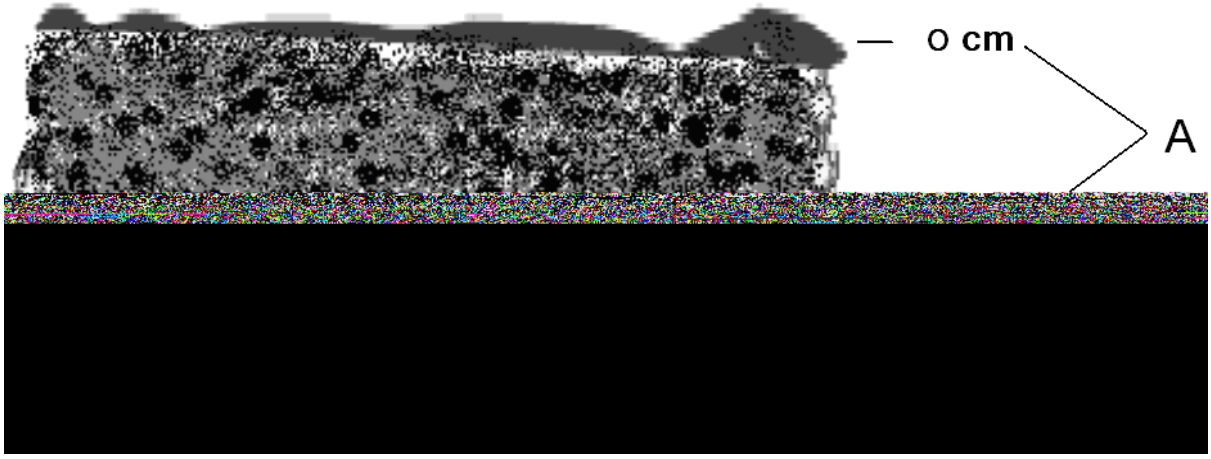


Figure 2: Soil profile and sampling depth

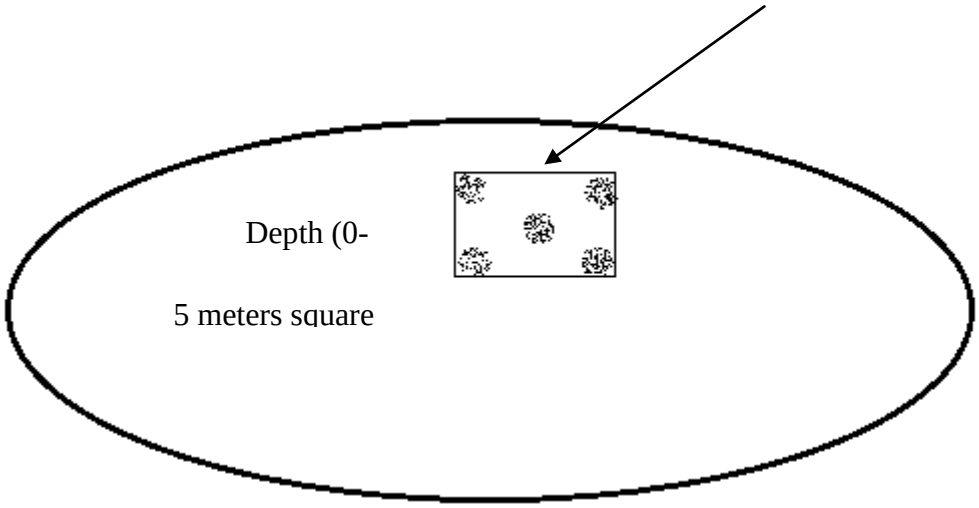


Figure 3: Sampling strategy at each site.

Soil sample characterization

Sample pre-treatment

The soil samples were sieved for < 2mm, to assure sample homogeneity. Then soil texture and total organic matter were determined. Finally, the samples were stored at -20 ºC in glass jars until time of extraction and analysis.

Moisture determination

Soil moisture content was determined by weighing 10 g of soil sample before and after drying overnight at 104ºC in the oven; the sample was cooled in the desiccators before use. The difference in the sample weight was used to determine the percentage of water in the soil sample as follows:

$$\%\ water = \frac{difference\ weight}{original\ soil\ sample} \times 100\%$$

The water amount was calculated and subtracted from soil weight in order to get the final concentration in µg/kg on dry weight basis for all samples. It was noticeable that some soil samples which

were collected few days after rainfall events contained high percentages of water and calculation based on dry weight basis would eliminate any effects of different water content.

Sample preparation

Slurry extraction

The wet homogenized soil samples containing 50 g of soil material were placed separately into 500 mL Erlenmeyer flask. Extraction was carried out with 2:1 acetone/water mixture (100mL: 50mL); soil weight was calculated for each sample according to calculated amount of water (moisture content). The mixture was shaken overnight using a horizontal shaker at shaking velocity of 220 cycle min^{-1}.

After adding 15 g of NaCl and 100 mL cyclohexane, the mixture was shaken additionally for 1 h for completing the liquid/liquid partitioning. The organic layer was decanted into 250 mL Erlenmeyer flask and dried over 15g an hydrous sodium sulfate. Then, 100 mL of the extract were evaporated using rotary evaporator and dissolved in 5 mL of (1:1, V: V) n-Hexane and ethyl acetate mixture.

Clean up methods

Sophisticated clean up procedure was developed to enhance the quality of the chromatographic analysis, because most of the co-extractants and the instrumental interfering materials were removed.

Silica gel column cleanup

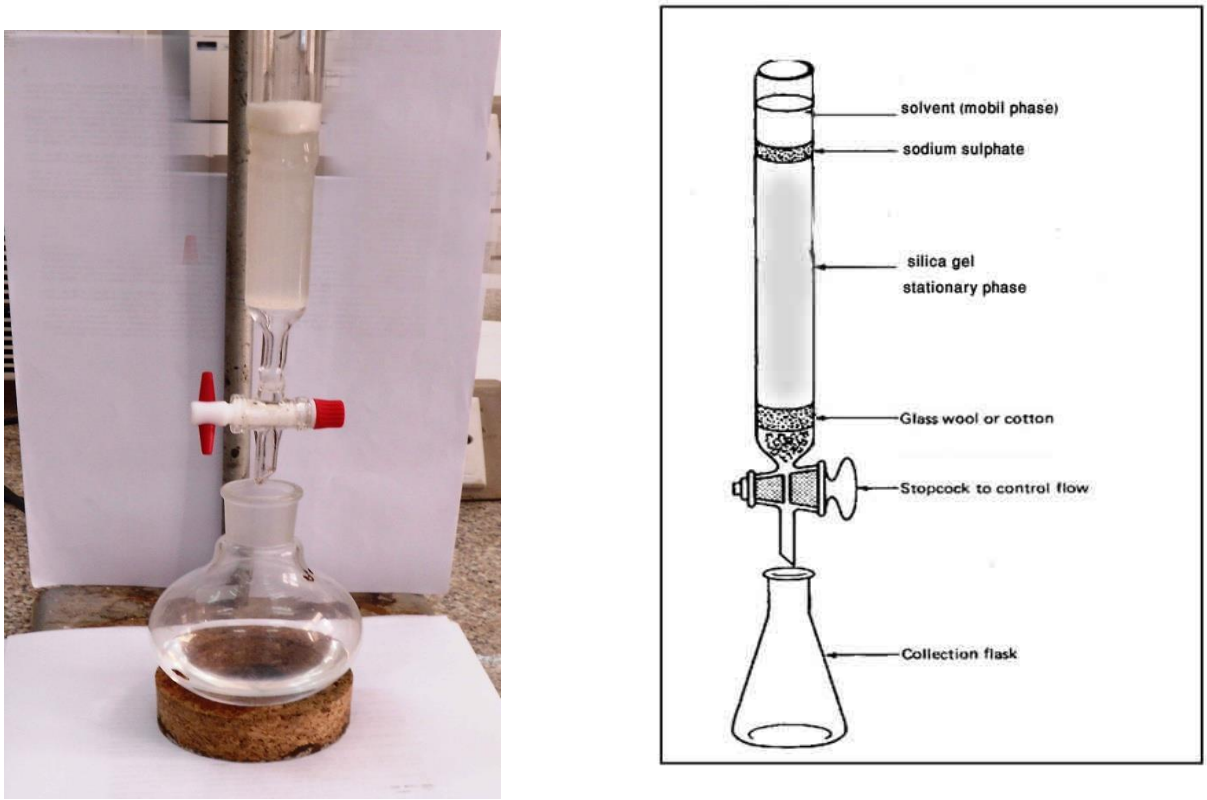
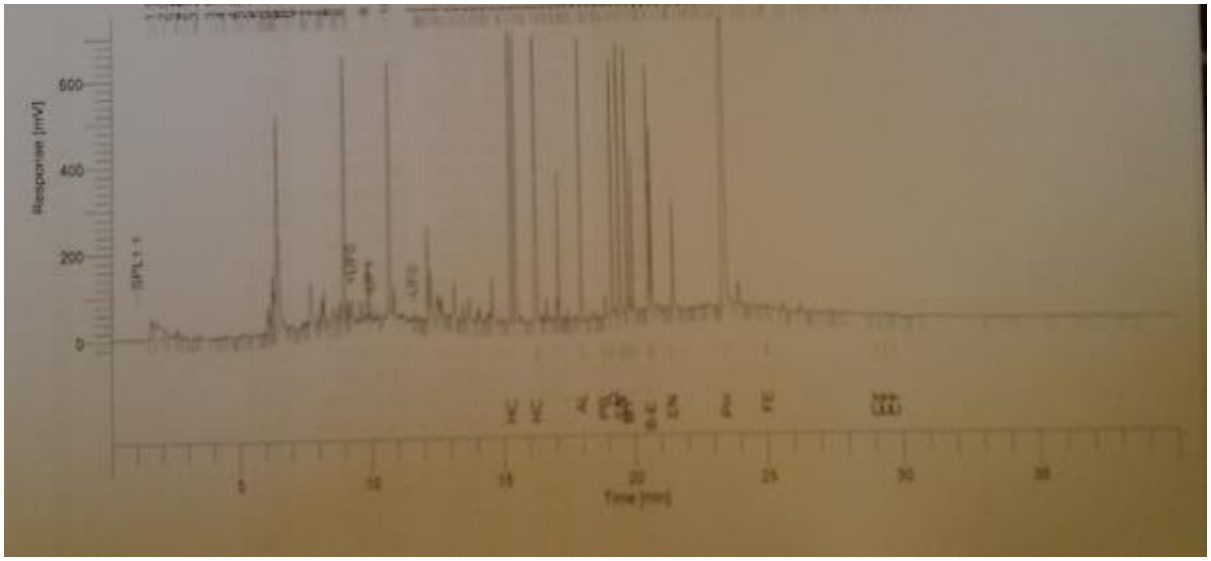


Figure 4: Column of clean up

The silica gel was activated at 220 ^{o}C overnight, then it was partly deactivated with 2 % H2O. The chromatographic column was packed with 10 g of deactivated silica gel and 1 g of oven dried anhydrous Na2SO4 on the top of the column. The non-polar fraction was transferred into the silica gel column and separated into three fractions: the first fraction was eluted with 50 mL n-hexane. The second fraction was eluted with 50 mL of n-hexane/ ethyl acetate (49:1). Then, the third fraction was eluted with 50 mL ethyl acetate. Finally, each fraction which was collected during the silica clean up procedure was rotary evaporated

and concentrated in a gentle nitrogen stream to 1 mL. 1,2,3,4- tetrachloronaphthalene (1,2,3,4-TCN) was used as internal standard for fractions 1 and 2. The first silica gel fraction was analyzed for PCBz, HCBz, and PCB congeners. However, the second and third fractions were analyzed for PAH and organochlorine compounds.



NB: as matrix we used sea sand because it does not react with pesticides that we used in the mixture

Figure 5: Chromatogram of Silica-gel on sea sand (M2)

As it's shown in the chromatogram below, we did recover only 14 pesticides from 17

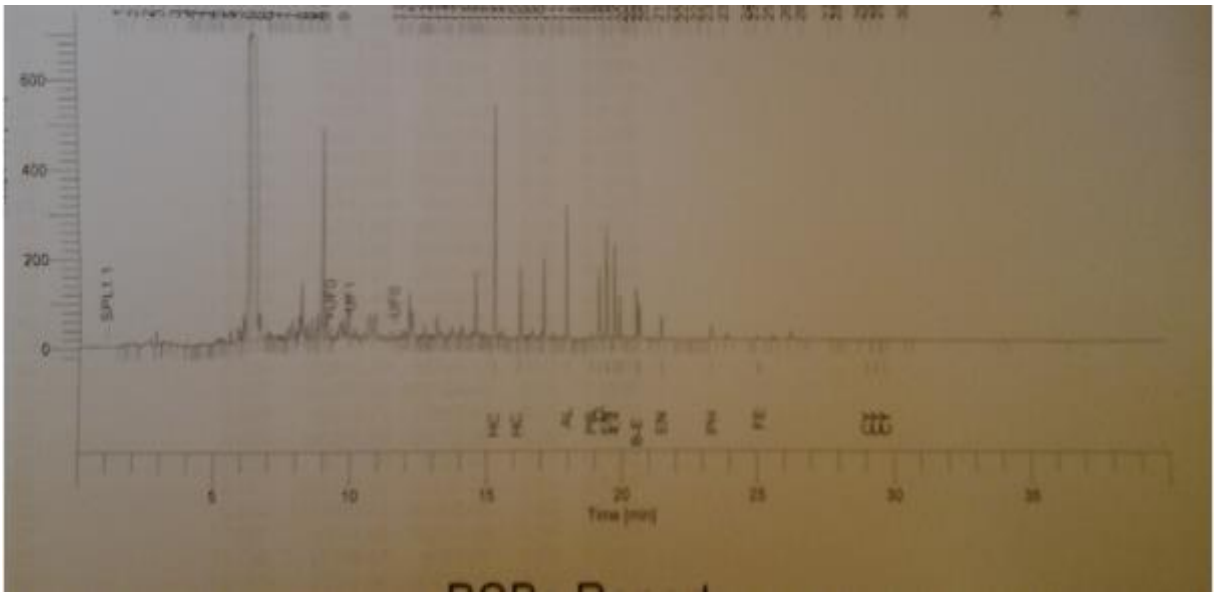


Figure 6: Chromatogram of Florisil on sea sand (M1)

Alumina column cleanup:

The alumina was activated at 220 oC overnight, then it was deactivated with 12.5 % H2O and shaken for at least 4 hours using horizontal shaker (220 cycle/min) to homogenous deactivation directly before use. 10 g of partly deactivated neutral alumina and 1 g of dried anhydrous Na2SO4 on the top of the chromatography column were used to separate non-polar from polar constituents of the sample matrix. The analyte was quantitatively transferred to the alumina column and the non-polar fraction was eluted with 60 mL n-hexane. Then it was rotary evaporated and concentrated to 1mL.

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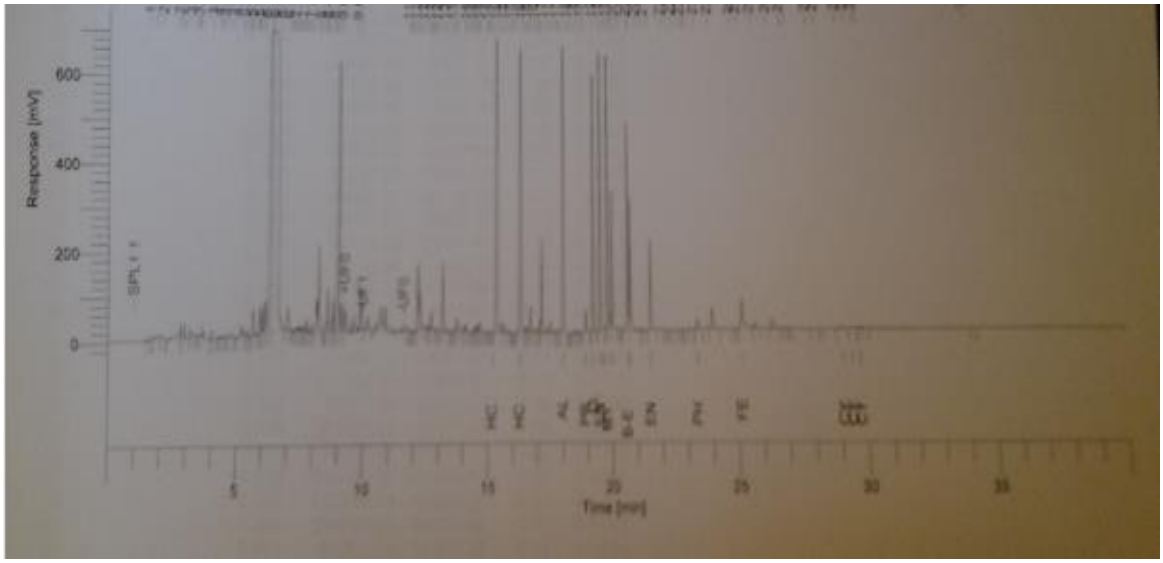

Figure 7: Chromatogram of aluminum oxide on sea sand (M3)

Chromatographic detection problem:

For many reasons, sulfur offers a number of challenges for GC analysis. The chemical reactivity of many sulfur-containing compounds can make both analysis of the compound at high temperature of concern, but also relating the compound in a sample to the level that is measured at the detector somewhat problematic.

Effect of sulfur on GC/ECD:

In GC/ECD analysis, Sulfur contamination causes a rise in the baseline of a chromatogram and may interfere with the analyses of the later eluting organochlorine compounds. Detection of the organochlorine compounds was not possible in the presence of the elemental sulfur. Sulfur is removed using tetrabutylammoniumsulfite (TBA) method (Jensen et al. 1977). The elemental sulfur is converted to the thiosulfate ion, which is water-soluble, according to the following reaction:

$$(TBA^{+})_2\ SO_3 + S(s) \rightarrow 2TBA + S_2O_3$$

Effect of sulfur on detection of electron capture detector (ECD) and sample matrix before and after its removal is shown in Figures bellow.

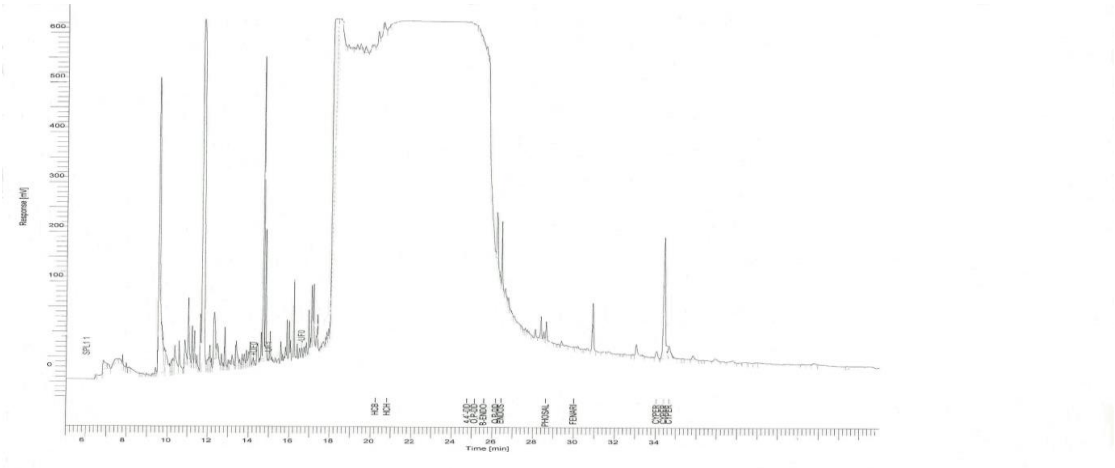

Figure 8: GC/ECD chromatogram in presence of sulfur

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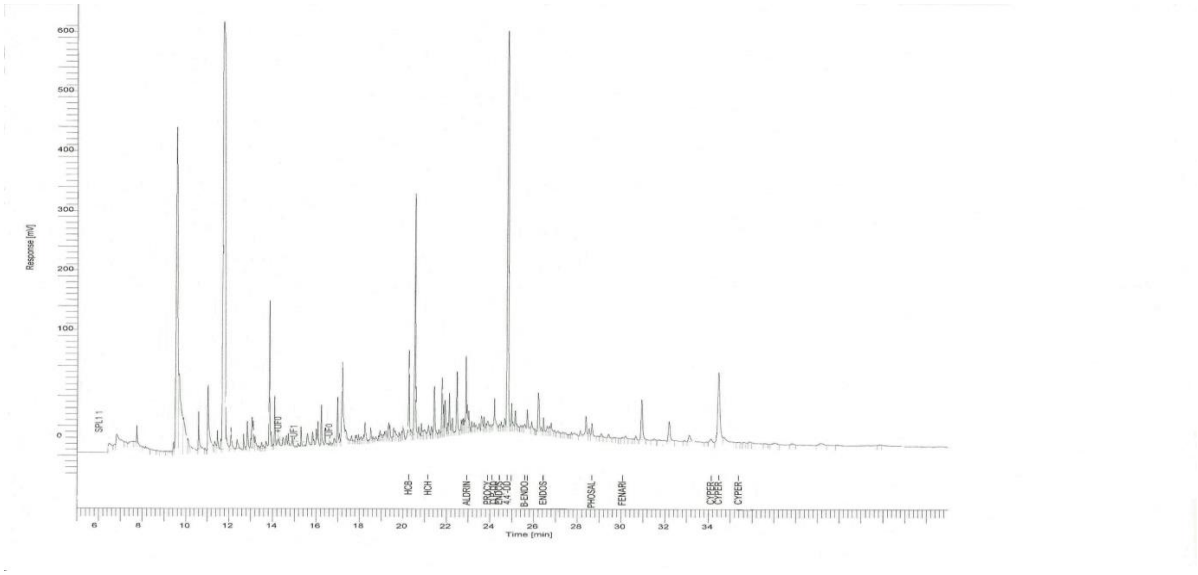

Figure 9: GC/ECD chromatogram after sulfur removal

Sulfur removal

The elemental sulfur was removed before analysis using tetrabutylammoniumsulfite (TBAS) method (Jensen et al. 1977), which converts solid sulfur from the organic phase into aqueous phase as soluble thiosulfate. Prepare 0.1 M TBAS reagent by dissolved 0.68 g TBAS and 5.0 g Na_2SO_3 in 20 mL deionized water. Then mix 1 mL of 0.1 M TBAS reagent with 1.0 mL isopropanol and few crystals of sodium sulfite, then the sample is transferred quantitatively to a test tube, the mixture was shacked for 1 min. The phase separation was carried out by adding 2.5 mL deionized water and then shaking for 1 min; few crystals of sodium sulfite were added until the appearance of a white precipitate. Finally, the clear upper n-hexane layer was collected and transferred into a GC vial.

Chromatographic sample analysis

The organochlorine pesticides analyses were carried out using a gas chromatography equipped with electron capture detector (GC/ECD).

Identification and quantification

Before analysis, relevant standards were run to check column performance, peak height, resolution, and the limits of detection (LOD). Peak identification and quantification was done using individual standard solution of each compound, identification was accomplished using relative retention time techniques while quantification was done by comparison of peak areas of samples to those of standard solutions at known concentrations and using external calibration method.

The limit of detection (LOD) and the limit of quantitation (LOQ) for organochlorine compounds in GC-ECD analysis were calculated based on two methods, statistical and empirical methods. The linearity of the calibration standards (R2 > 0.995) was taken in consideration either to accept or reject the calibration if R2 <0.995. In addition, the precision of the analytical procedures was expressed as relative standard deviation (RSD), where ±20 % as RSD was the acceptable value (González and Herrador, 2007).

Standards and calibration

Standard mixtures of OCPs with different levels (0.01, 0.1, 0.5, 1, and 2 ppm) were prepared by appropriate dilution of the stock solutions with a mixture of pesticides-grade n-hexane: acetone. These working standards were used to prepare the external calibration curves. The least square regression line method was used with best line at correlation coefficient exceeded $R^2 > 0.995$.

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Recovery rate (recovery %)

Fortification experiments were performed for all studied pesticides in order to determine the recovery rate of the analytical procedure. A 50 g of soil and sea sand samples (n=9) were spiked with: 100 µl (1 ppm) of 16 different organochlorine pesticides. Four samples (one solvent sample , one soil sample , two sea sand samples) was left unspiked and processed as a blank due to the absence of zero-soil sample (free from analytes) to check for contamination. Then the spiked as well as unspiked samples were subjected to the analytical procedure. Later on, the detected concentrations of the pesticides were obtained for the blank sample and subtracted from the spiked concentrations for the fortified samples in order to determine the recovery rates as follows:

$$Recovery = \frac{area\ of\ sample}{area\ of\ standard} \times 100\%$$

The acceptance of the recovery rate range was 80-120%.

RESULTS AND DISCUSSION

Percentage Recovery

The percentage recovery rate study was performed based on fortified soil samples with the target compounds then they were extracted using the three different analytical procedures that were explained. The aim was to select the most proper method of analysis which gives the higher recovery rate. The first method (M1) showed low recovery rates for the 16 OCPs in the range from 13% to 71% with high RSD% values ranging from 1.5 to 38 %. The second procedure (M2) showed recovery rates from 7% to 68% with RSD% values ranging from 2.7 to 59.8 %. Finally, the third procedure (M3) showed recovery rates range from 70% to 120 %, (except the recovery of Fenarimol was the highest with 129%), with acceptable RSD% values ranging from 1.5 to 25%. Therefore, the developed method (procedure M3) has shown the best recovery rates for most of the target compounds, Table 1 summarize the results. These analytical results were in comparative agreement with other recovery rates determined frequently for similar compounds worldwide (Al-Mughrabi, I. K., Qrunfleh, M.I., (2002). In general, recoveries obtained from procedure M1 were similar to the recoveries obtained from M2, their recoveries were <70%. So none of these extraction procedures was appropriate for these compounds. The best recoveries were obtained from procedure M3, because recoveries of OCPs were significantly affected by polarity of the extraction solvent. The extraction solvent for OCPs must have a high extraction capability and good chromatographic behavior. It was found that n-hexane and ethyl acetate (1:1) minimized the influence of matrix co-extractives on the response of analytes.

Table 1: The recovery rates of OCPs in fortified soil samples.

Compound	Recovery (%) (mean ± SD), RSD Procedure M1		Procedure M2		Procedure M3	
HCB	57 ± 3.0	5.4	58 ± 3.0	5.2	84 ±3.0	3.5
HCH	58 ± 6.6	11.3	53 ± 6.4	12.2	85 ± 1.5	1.8
Aldrin	62 ± 3.2	5.2	64 ± 5.0	7.9	91 ± 3.0	3.2
Procymidone	68 ± 4.1	5.9	55 ± 13	23.6	97 ± 3.5	3.6
Hexy thiazox	43 ± 6.0	14	26 ± 9.5	36.7	70 ± 7.0	10
O,P-DDE	45 ± 2.5	5.6	44 ± 4.6	10.5	76 ± 1.2	1.6
Endosulfan	46 ± 0.7	1.5	47 ± 4.6	9.7	86 ± 1.3	1.5
4,4`-DDE	71 ± 3.1	4.3	65 ± 5.5	8.5	96 ± 3.6	3.8
Myclobutanil	_____	___	67 ± 4.7	7.3	86 ± 2.5	4.2
O,P-DDT	53 ± 3.8	7.2	47 ± 4.1	8.6	80 ± 4.4	5.4
β-Endosulfan	41 ± 1.7	4.2	42 ± 7.2	17	83 ± 2.4	3
O,P-DDD	71 ± 26	37	54 ± 10.2	18.9	100 ± 5.7	5.7
Endosulfan sulfate	64 ± 7.2	11.3	68 ± 9.0	13.2	95 ± 5.1	5.3
Phosalone	50 ± 3	6	46 ± 12.5	27.2	105 ± 10.4	9.9
Fenarimol	13 ± 5	38	7 ± 4.2	59.8	129 ± 32.5	25
Cypermethrin 1	_____	___	26 ± 0.7	2.7	78 ± 15.3	19.6
Cypermethrin 2	_____	___	64 ± 18.6	29	120 ± 25.9	21.6
Cypermethrin 3	_____	___	30 ± 5.7	18.8	89 ± 29.3	3.3
Cypermethrin 4	_____	___	214 ± 0	0	82 ± 12	14.7

Procedure M1: Florisil, Procedure M2: Silica-gel, Procedure M3: Aluminum oxide

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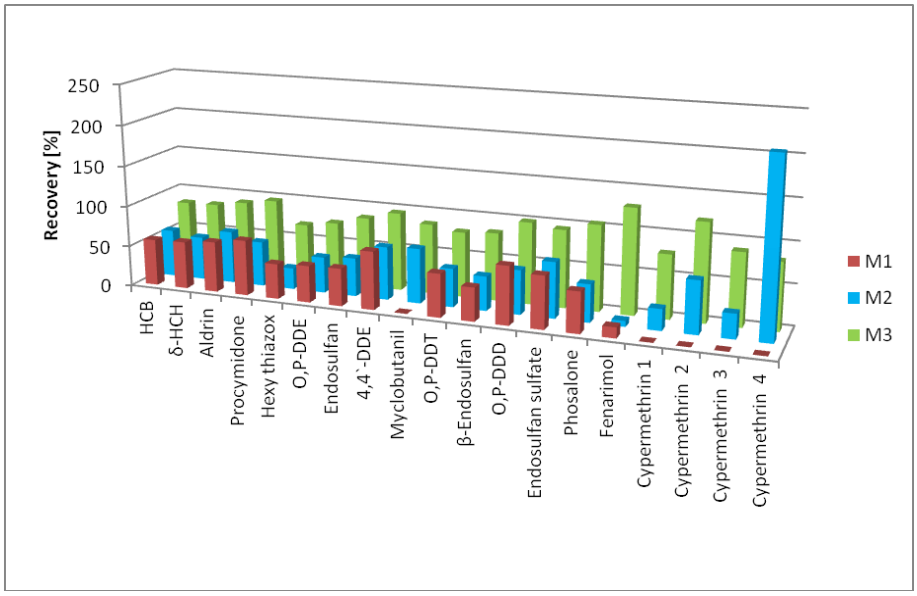

Figure 10: Recovery rates of organochlorine compounds with different method M_1, M_2 and M_3.

Chromatograms of organochlorine pesticides

The analytical procedure was developed to enhance the chromatographic separation of the pesticides. The chromatograms for standard solution of 16-OCPs and sample soil are presented in Figures 11 and 12, respectively. The retention time and name of pesticides were designated in the figure legend.

CONCLUSION

A multi-residue analytical procedure was modified for analysis of 17 different organochlorine compounds in using a gas chromatography equipped with electron capture detector (GC-ECD).

The results of this study have showed that OCPs banned in the country may still be detected in some agricultural land in northern part of Lukus Valley and its surroundings as they showed considerable concentrations in the soil and they were characterized by long half time. The concentration levels of OCPs in soil samples collected from northern region of Lukus Valley ranged from N.D to 1856.84 µg/kg. The significance of the presence of OCPs in soil is that they may enter the food chain easily and eventually affect the human health.

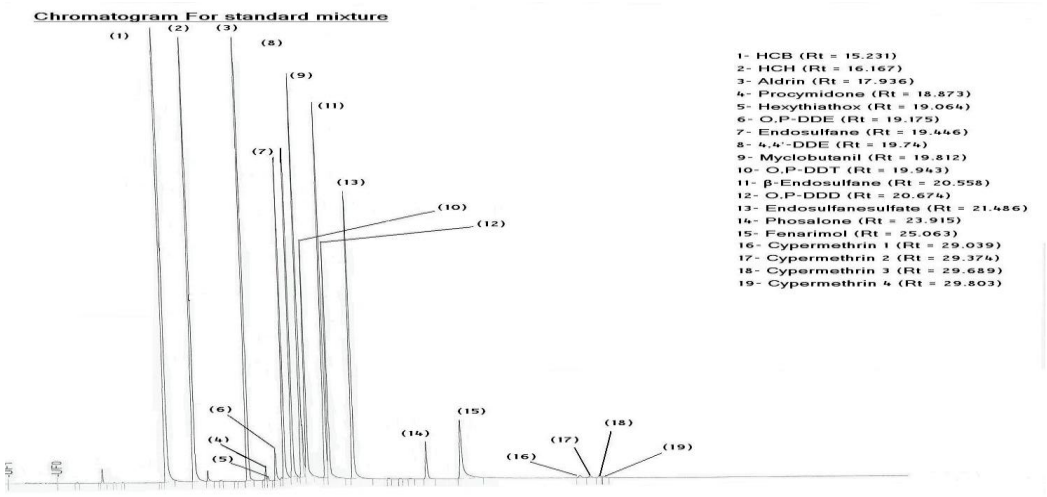

Figure 11: Chromatogram for standard solution of 16-OCPs.

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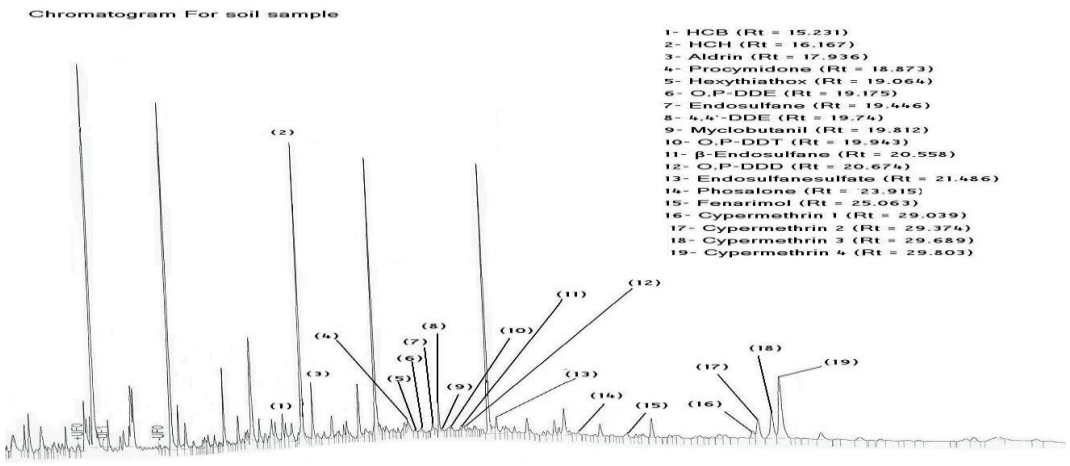

Figure 12: Chromatogram for sample soil.

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