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MULTIVARIANTE ANALYZE USED FOR INTERPRETATION OF ORGANOCHLORINATED POLLUTANT LEVELS IN SOIL SAMPLES OF KOSOVO

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ABSTRAKT

Soil contamination is one of most important factors influencing the quality of agricultural products. Usage of heavy farm equipment, the land drainage, an excessive application of agrochemicals, emissions originating from mining, metallurgical, and chemical and coal power plants and transport, all generate a number of undesired substances (nitric and sulphur oxides, PAHs, heavy metals, pesticides), which after deposition in soil may influence crop quality. Thus, input of these contaminants into the environment should be carefully monitored. This paper presents the data obtained for organochlorinated pesticides, their residues, PCB and chlorobenzenes in the soil samples of agricultural areas. Levels of organochlorinated pesticides, PCB and chlorobenzenes contamination were evaluated in agriculture areas that are in use in soil samples. 11 soil samples were taken in agricultural areas, near Peja, Kosovo in March 2014. Representative soil samples were collected from 0-30 cm top layer of the soil. In the analytical method we combined ultrasonic bath extraction and a Florisil column for samples clean-up. The analysis of the organochlorinated pesticides in soil samples was performed by gas chromatography technique using electron capture detector (GC/ECD). Optima-5 (low/mid polarity, 5% phenyl methyl siloxane 60 m x 0.33 mm x 0.25µm film) capillary column was used for isolation and determination of organochlorinated pesticides. Interpretation of data were performed using cluster analyze models. Relatively low concentrations of organochlorinated pesticides and their metabolites were found in the studied samples. Two were the main groups, DDTs and HCHs. The presence of organochlorinated pesticides residues is probably resulting of their previous uses for agricultural purposes. PCB 52 was the main congener found in soil samples (the main grup of cluster) and hexachlorobenzene was the main compound for chlorinated benzene compounds.

Keywords:Organochlorinated pesticides, PCB marker, Chlorobenzenes, Soil samples, Gas chromatography, Cluster analyze

1. Introduction

Soil contamination is one of most important factors influencing the quality of agricultural products. Usage of heavy farm equipment, the land drainage, an excessive application of agrochemicals, emissions originating from mining, metallurgical, and chemical and coal power plants and transport, all generate a number of undesired substances (nitric and sulphur oxides, PAHs, heavy metals, pesticides), which after deposition in soil may influence crop quality. Thus, input of these contaminants into the environment should be carefully monitored. Levels of organochlorinated pesticides contamination were evaluated in agriculture areas that are in use (Nuro&Marku, 2011). Representative soil samples were collected from 0-30 cm top layer of the soil. This study could be a first step for monitoring of organic pollutants in agricultural areas of Kosovo.

The concentrations and toxicity of organic compounds present in such complicated mixtures range very widely and depend also on possible interactions (synergies) among chemicals. The development of instrumental analysis techniques and the lowering of the detection limit have made it possible to identify new organic compounds that are present in the soil in very low concentrations. The list of the most commonly studied soil pollutants has been expanded to include polychlorinated biphenyls (PCBs), aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo dioxins (PCDDs), polychlorinated dibenzo furans (PCDFs) and polychlorinated naphtalenes (PCNs). The continuous introduction of these

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persistent compounds into the environment has resulted in their accumulation. Runoff is the movement of pesticides in water over a sloping surface. The amount of pesticide runoff depends on: the slope, the texture of the soil, the soil moisture content, rainfall, and the type of pesticide used. Leaching occurs downward, upward, or sideways. Many chromatographic methods were developed last years for detecting all possible and known organic pollutants in soil samples (Di Muccio, 1996; Erikson, 2001). Determination of halogenated pollutants is based mainly in capillary GC/ECD and GC/MS methods. LOD for chlorinated pesticides and PCBs in soil samples is in ppb levels. EPA and EN norms suggested these methods as routine and confirmatory analysis for responsible laboratory.

Different software work with three different procedures that can be used to cluster data: hierarchical cluster analysis, k-means cluster, and two-step cluster. If you have a large data file (even 1,000 cases is large for clustering) or a mixture of continuous and categorical variables, you should use the two-step procedure. If you have a small data set and want to easily examine solutions with increasing numbers of clusters, you may want to use hierarchical clustering. If you know how many clusters you want and you have a moderately sized data set, you can use k-means clustering. There are numerous ways in which clusters can be formed. Hierarchical clustering is one of the most straightforward methods. It can be either agglomerative or divisive. Agglomerative hierarchical clustering begins with every case being a cluster unto-itself. At successive steps, similar clusters are merged. The algorithm ends with everybody in one jolly, but useless, cluster. Divisive clustering starts with everybody in one cluster and end up with everyone in individual clusters. Obviously, neither the first step nor the last step is a worthwhile solution with either method. In agglomerative clustering, once a cluster is formed, it cannot be split; it can only be combined with other clusters. Agglomerative hierarchical clustering doesn’t let cases separate from clusters that they’ve joined. There is no right or wrong answer as to how many clusters you need. It depends on what you’re going to do with them. To find a good cluster solution, you must look at the characteristics of the clusters at successive steps and decide when you have an interpretable solution or a solution that has a reasonable number of fairly homogeneous clusters (Arabie et al, 1996; Ball, and Hall, 1997).

2. Material and Methods

2.1 Preparing of adsorbents for determination of organochlorinated pesticides and PCBs in soil samples

Silicagel, Florisil and sodium sulphate anhydrous were activated for 12 hours on 250^{o}C on oven. After that Silica gel were treated with 45% with concentrated sulphuric acid and Florisil with 5% with distilled water.

2.2 Preparation of glassware for GC analyze

Preparation of glassware’s for determination of organochlorinated pesticides and polychlorinated biphenyls in nano levels is very important for getting real values. All glassware’s were treated with sulphuric acid in rate 1:4 in volume for 6 hours. Washing firstly with distilled water and after that with organic solvents (hexane:dichloromethane). All glassware’s were dry in thermostat for 6 hours in 150^{o}C (Beltran et al, 2000; Muir &Sverko, 2006).

2.3 Sampling of soil samples and their pretreatment

Levels of organochlorinated pesticides and PCBs were evaluated in agriculture areas that are in use. 10 soil samples were taken in agricultural areas, near Peja, Kosovo. Representative soil samples were collected from 0-30 cm top layer of the soil. Sampling stations of soil samples was shown in Table 1. Soil samples were air dried. A representative sub-sample of each sample was taken for determination of humidity and another sub-sample for determination of organochlorinated pesticides and PCBs with gas chromatographic analyze.

Table 1. Sampling stations of soil samples analyzed in Institute of Agricultural and Food, Peja, Kosovo

Sample	Station	Agricultural area	Deep of soil	Humidity	Dry weight

			samples	(%)	(g)
T1	Memushe	sere - tranguj	0-30 cm	2.9	13.3
T2	Peje	pemishte -molle	0-30 cm	6.53	13.65
T3	Memushe	sere - domate	0-30 cm	6.98	15.48
T4	Peje (Qyshk)	sere	0-30 cm	13.15	15.1
T5	Nepervisht	sere - tranguj	0-30 cm	4.05	13.22
T6	Nepervisht	sere - domate	0-30 cm	8.32	14.73
T7	Nepervisht	sere - tranguj	0-30 cm	10.7	17.54
T8	Gjakove	pemishte	0-30 cm	12	12.2
T9	Peje (Leshan)	sere	0-30 cm	24.85	16.81
T10	Peje (Leshan)	sere	0-30 cm	18.2	14.5

2.4 Soil sample treatment for GC analyze

A fresh sub-sample from 10-20 g of soil samples were extracted by ultrasonic bath assisted extraction with 50 ml hexane/dichloromethane 3/1, (v/v) (Fluka, Germany, pesticide grade). The extract was purified by shaking with 2 gr sodium sulphate and 2g silica gel, impregnated previously with 45% sulfuric acid. A further clean-up of this extract was performed in a open glass column packed with Florisil (particle size 0.063±0.2 μm; Merck, Darmstadt,Germany), deactivated with 5% water. The organochlorine compounds were eluted with 7 ml of hexane/dichloromethane 4/1(v/v) (Spectroscopy grade; Fluka, Germany). The extract was concentrated to 1 ml in nitrogen concentrator and after that analyzed by GC-ECD (Beltran et al, 2000; Muir &Sverko, 2006).

2.5 Gas chromatography analyze of soil samples

Gas chromatographic analyses were performed with a DANI 1000 Gas chromatograph equipped with a ^{63}Ni Electron Capture Detector and a split/splitless injector. The column used was an Optima-5 (low/mid polarity, 5% phenyl methyl siloxane 60 m x 0.33 mm x 0.25μm film). The split/splitless injector and detector temperatures were set at 300^{0}C and 320^{0}C, respectively. Carrier gas was helium at 2 ml/min and make-up gas was nitrogen at 25 ml/min flow. The initial oven temperature was kept at 110^{0}C for 4 minutes, than increased to 200^{0}C at 20^{0}C/min, and then increased to 280^{0}C at 40C/min. The temperature was finally increased to 320^{0}C, at 10^{0}C/min, than held for 7 minutes. Injection volume was 1μl and injections were done in splitless mode. Organochlorine pesticide and PCB quantification was performed by external standard method (Nuro&Marku, 2011).

3. Results and Discussion

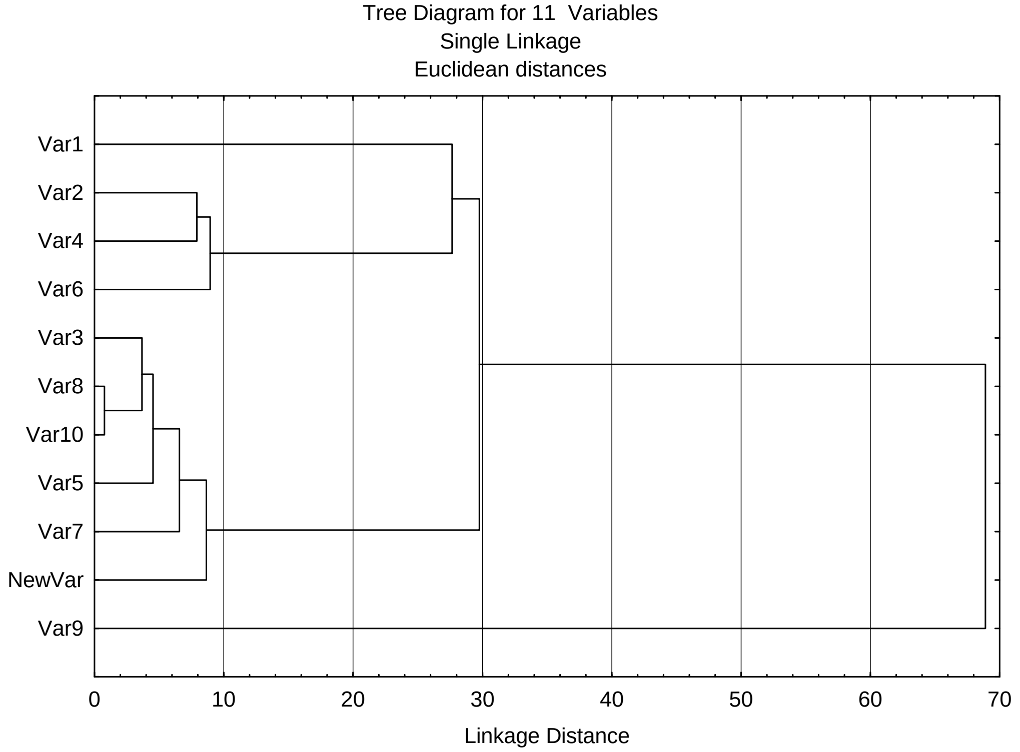
10 soil samples of agricultural areas near Peja, Kosovo were chosen for determination of organochlorinated pesticides and PCBs. Extraction, clean-up and concentration procedures were realized in laboratory of Agricultural Institute in Peja, conform protocols for determination of PCBs and organochlorinated pesticides for soil samples.

Data of organochlorinated pollutants in soil samples from Kosova was shown in table 1. Total of organochlorinated pesticides was shown in Figure 1. Maximum level was found for sample T5 and T8 with 0.019 mg/kg soil sample. Minimum level was for T1 sample with 0.0055 mg/kg. Distribution and profile of organochlorinated pesticides were shown respectively in Figure 2 and Figure 3. Almost for all studied samples have the same distribution of organochlorinated pesticides. The most dedected pesticides were: 24’-DDT, 44’-DDD, HCB, Isodrine, Heptachlor, alfa-Klordan. Note that for all samples were found in higher levels metabolites and not organochlorinated pesticides. This fact suggests that found concentrations could be because of before use of organochlorinated pesticides in agricultural areas and their degradation. All found pesticides concentrations were lower than Maximal allowed values (MAV) in the soil samples according Administrative Project instruction in Allowing Norms of

Hazardous Substances and Harmful Presence in Soil, Prishtinw, Kosovo, 2011.
Total of PCBs for analyzed soil samples was show in Figure 4. Maximum level was for sample T3 with 0.0025 mg/kg soil sample. In 6 soil samples PCBs were not detected. PCB levels were lower than Maximal allowed values (MAV) in the soil samples according Administrative Project instruction in Allowing Norms of Hazardous Substances and Harmful Presence in Soil, Prishtinw, Kosovo, 2011. Distribution of PCBs was shown in Figure 5 and their profile in Figure 6. PCB 52 and PCB 138 were most detected biphenyls. PCB 52 presence could be because of atmospheric factors. PCB 138 presence could be because of its physic-chemic properties.

Tabela 2.Tëdhënat e pesticideveklororganikenëmostrat e tokës

Pesticidi	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Hekzaklorbutadien	0.1	0.01	0.01	0	0.01	0.02	0.01	0	0.01	0.01
Teknazen	0.22	0.09	0.04	0.06	0.14	0.04	0.03	0.05	0.02	0.06
a-HCH	0.96	0.01	0.05	0.02	0.02	0.03	0.02	0.1	0.01	0.02
HCB	6.88	0	0.09	0	0.03	0	0.05	0	0.02	0.04
b-HCH	2.31	0	0.19	0.08	0.71	0.17	0	0	0	0.14
Lindan	0.87	0.19	0.61	0.09	0.05	0.07	0.01	0.22	0.07	0
Kvintozen	0.72	0.12	0.19	0	0	0.02	0	0.32	0.05	0.16
d-HCH	0.76	0.16	0.06	0.11	0.46	0.16	0.24	0.08	0.03	0.07
e-HCH	1.52	0.06	0.06	0	0.28	0	0	0.04	0	0.04
Heptaklor	0.85	0.11	0.03	0.09	0.08	0.1	0.11	0.04	0	0.07
Aldrin	4.73	0.72	0.19	0.06	0.36	0.17	0.09	0.15	0.16	0.13
Izodrin	0.98	0.3	0.25	0.09	0.1	0.21	0.15	0.06	0.09	0.26
Oksiklordan	10.72	0.56	0.41	0.09	0.72	0.74	0.48	0.32	0	0.95
Heptaklorepoksidcis	36.88	57.64	5.02	61.12	6.16	69.91	11.82	2.12	13.03	1.88
Heptaklorepoksid trans	0	0.03	0.04	0	0	0.09	0	0.03	0	0.03
Klordan trans	0.53	0	0.02	0	0.34	0	0.08	0	0.01	0
2,4'-DDE	0.82	0.02	0	0	0.39	0.05	0.08	0	0	0
Endosulfanalfa	0.67	0	0.15	0	0.43	0	0.13	0	0	0
Klordancis	0.88	0.08	0.22	0	0.43	0.18	0.22	0	0.03	0
Dieldrin	4.1	0.56	1.85	0.03	0.86	0.49	0.56	0	0.08	0
4.4'-DDE	1.54	0.66	0.22	0.04	2.73	0	0.92	0.09	0.26	0.09
2.4-DDD	1.8	0.38	0.08	0.09	1.04	0.51	0.48	0.09	0	0.09
Endrin	1.09	0.07	0.05	0	0.84	0.07	0.04	0	0	0
Endosulfan beta	1.04	0	0.02	0	0.74	0	0.13	0	0.04	0
4.4-DDD	0	0	0	0	0	0	0	0	0	0
2.4-DDT	1.61	0.52	0.17	0	0	0.2	0	0	0	0
Klordekon	1.99	0	0	0	0.5	0.23	0.44	0	0	0
Endosulfansulfat	0.21	0	0.1	0	0.71	0	1.28	0	0	0
4,4'-DDT	0.43	0.78	0.25	0.06	2.9	0	1.06	0.13	0.24	0.13
Dikofol	18.34	6.95	1.09	0	0.37	1.37	1.38	0.55	7.34	0.55
Metoksiklor	1.44	0.1	0	0	0.6	0.29	0	0.12	0	0.12
Mirex	0.69	0.4	0.66	0.46	0.68	0.31	0.13	1.64	69.57	1.64



Euclidean distances (Spreadsheet1)

Variable	Var1	Var2	Var3	Var4	Var5	Var6	Var7	Var8	Var9	Var10	NewVar
Var1	0.0	27.7	38.9	33.9	38.3	39.6	33.6	41.0	76.3	41.0	29.8
Var2	27.7	0.0	53.0	7.9	52.0	13.5	45.2	55.9	82.3	55.2	34.9
Var3	38.9	53.0	0.0	56.1	4.5	64.9	7.2	3.7	69.7	3.9	14.9
Var4	33.9	7.9	56.1	0.0	55.2	9.0	45.4	59.0	84.6	59.3	43.0
Var5	38.3	52.0	4.5	55.2	0.0	63.9	6.6	6.1	69.7	6.3	13.0
Var6	39.6	13.5	64.9	9.0	63.9	0.0	58.1	67.0	89.8	68.1	51.8
Var7	33.6	45.2	7.2	45.4	6.6	58.1	0.0	10.1	69.7	10.3	8.7
Var8	41.0	55.9	3.7	59.0	6.1	67.0	10.1	0.0	69.1	0.8	17.6
Var9	76.3	82.3	69.7	84.6	69.7	89.8	69.7	69.1	0.0	69.2	68.9
Var10	41.0	55.2	3.9	59.3	6.3	60.1	10.3	0.8	69.2	0.0	17.8
NewVar	29.8	34.9	14.9	43.0	13.0	51.8	8.7	17.6	68.9	17.8	0.0

Variable	Mean	Std Dev
Var1	3.302500	7.14023
Var2	2.203750	10.18893
Var3	0.378750	0.92666
Var4	1.952013	10.79711
Var5	0.708750	1.20002
Var6	2.357188	12.33013
Var7	0.623123	2.07097
Var8	0.192188	0.46331
Var9	2.016623	12.44951
Var10	0.202500	0.45123
NewVar	0.967500	3.39940

Spreadsheet1

	1 Var1	2 Var2	3 Var3	4 Var4	5 Var5	6 Var6	7 Var7	8 Var8	9 Var9	10 Var10	11 NewVar
Var1	0.000000	27.78117	38.93676	33.94466	38.27818	39.61107	33.57058	41.80526	76.25238	41.84180	29.75809
Var2	27.76117	0.000000	52.97184	7.91936	52.02817	13.54150	45.18863	55.51867	82.31974	55.15729	34.93477
Var3	38.93676	52.97184	0.000000	56.14638	4.53517	64.91298	7.19141	3.67506	69.88219	3.92043	14.83570
Var4	33.94166	7.91936	56.14638	0.000000	55.16232	8.95785	45.36425	59.01626	84.57541	59.26133	43.04578
Var5	38.27616	52.02817	4.53517	55.16232	0.000000	63.90885	6.57273	6.12377	69.71902	6.26570	12.99002
Var6	39.61107	13.54150	64.91298	8.95785	63.90885	0.000000	58.12130	67.81511	89.82936	68.05303	51.75982
Var7	33.57058	45.18863	7.19141	45.36425	6.57273	58.12130	0.000000	10.06219	69.73280	10.25801	8.66002
Var8	41.80526	55.51867	3.67506	59.01626	6.12377	67.81511	10.06219	0.000000	69.13686	0.77311	17.63554
Var9	76.25238	82.31974	69.88219	84.57541	69.71902	89.82936	69.73280	69.13686	0.000000	69.18066	68.90621
Var10	41.84180	55.15750	3.92043	59.26133	6.26570	68.05303	10.25801	0.77311	69.18066	0.000000	17.81547
NewVar	29.76005	34.93477	14.83570	43.04578	12.99002	51.75982	8.66002	17.63554	68.90621	17.81547	0.000000
Means	3.30250	2.20375	0.37875	1.95261	0.70875	2.35719	0.62313	0.19219	2.01563	0.20250	0.96750
Std.Dev.	7.14023	10.18893	0.92666	10.79711	1.20002	12.33013	2.07097	0.46331	12.44951	0.45123	3.39940
No.Cases	32.00000										
Matrix	3.00000										

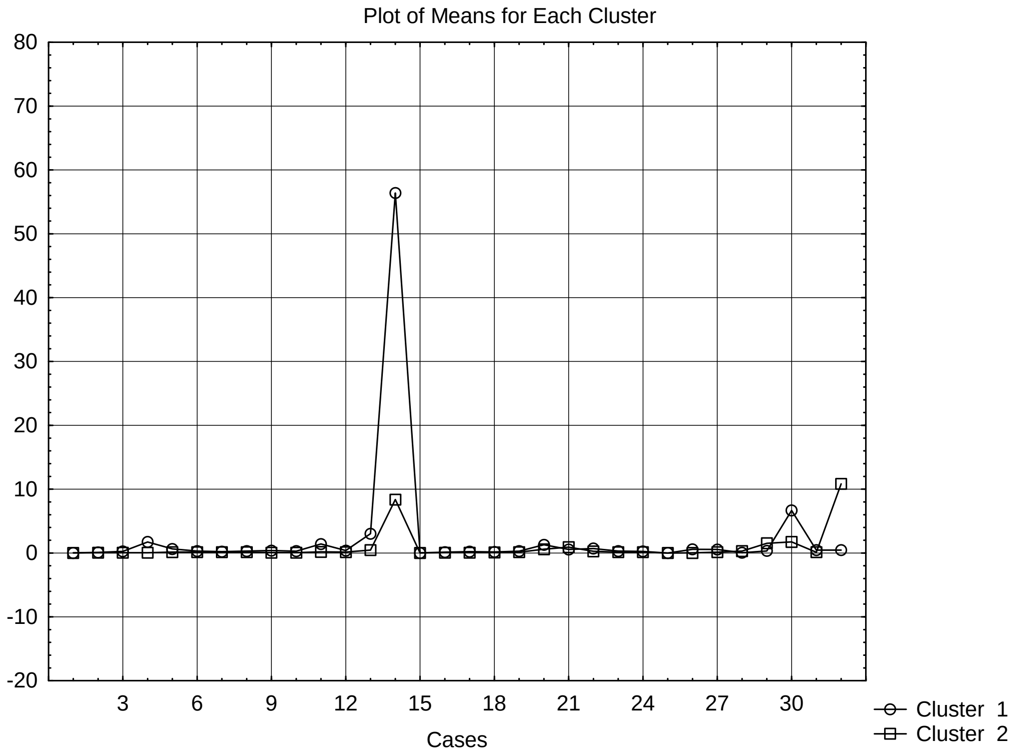
Case ID	Cluster Means (Spreadsheet1)	
	Cluster No. 1	Cluster No. 2
C_1	0.03250	0.00714
C_2	0.10250	0.04857
C_3	0.25500	0.04143
C_4	1.72000	0.04000
C_5	0.64000	0.14857
C_6	0.30500	0.14857
C_7	0.21500	0.10857
C_8	0.29750	0.13714
C_9	0.39500	0.06000
C_10	0.28750	0.05857
C_11	1.42000	0.17714
C_12	0.39500	0.15000
C_13	3.02750	0.45857
C_14	56.38750	8.37000

Cluster Number	Euclidean Distances between Clusters (Spreadsheet1 Distances below diagonal Squared distances above diagonal	
	No. 1	No. 2
No. 1	0.000000	76.62121
No. 2	8.753354	0.00000

Case ID	Cluster Means (Spreadsheet1)	
	Cluster No. 1	Cluster No. 2
C_1	0.03250	0.00714
C_2	0.10250	0.04857
C_3	0.25500	0.04143
C_4	1.72000	0.04000
C_5	0.64000	0.14857
C_6	0.30500	0.14857
C_7	0.21500	0.10857
C_8	0.29750	0.13714
C_9	0.39500	0.06000
C_10	0.28750	0.05857
C_11	1.42000	0.17714
C_12	0.39500	0.15000
C_13	3.02750	0.45857
C_14	56.38750	8.37000

Cluster Number	Euclidean Distances between Clusters (Spreadsheet1 Distances below diagonal Squared distances above diagonal	
	No. 1	No. 2
No. 1	0.000000	76.62121
No. 2	8.753354	0.00000

Case ID	Analysis of Variance (Spreadsheet1)				
	Between SS	df	Within SS	df	F
C_1	0.002	1	0.006	9	2.29519
C_2	0.007	1	0.032	9	2.09776
C_3	0.116	1	0.670	9	1.56059
C_4	7.184	1	35.506	9	1.82108
C_5	0.615	1	4.138	9	1.33693
C_6	0.062	1	0.714	9	0.78537
C_7	0.029	1	0.434	9	0.59793
C_8	0.065	1	0.441	9	1.33697
C_9	0.286	1	1.750	9	1.46921
C_10	0.133	1	0.430	9	2.78981
C_11	3.932	1	14.903	9	2.37453
C_12	0.153	1	0.515	9	2.67065
C_13	16.798	1	79.683	9	1.89734
C_14	5869.004	1	822.109	9	64.25063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80
70
60
50
40
30
20
10
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6
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12
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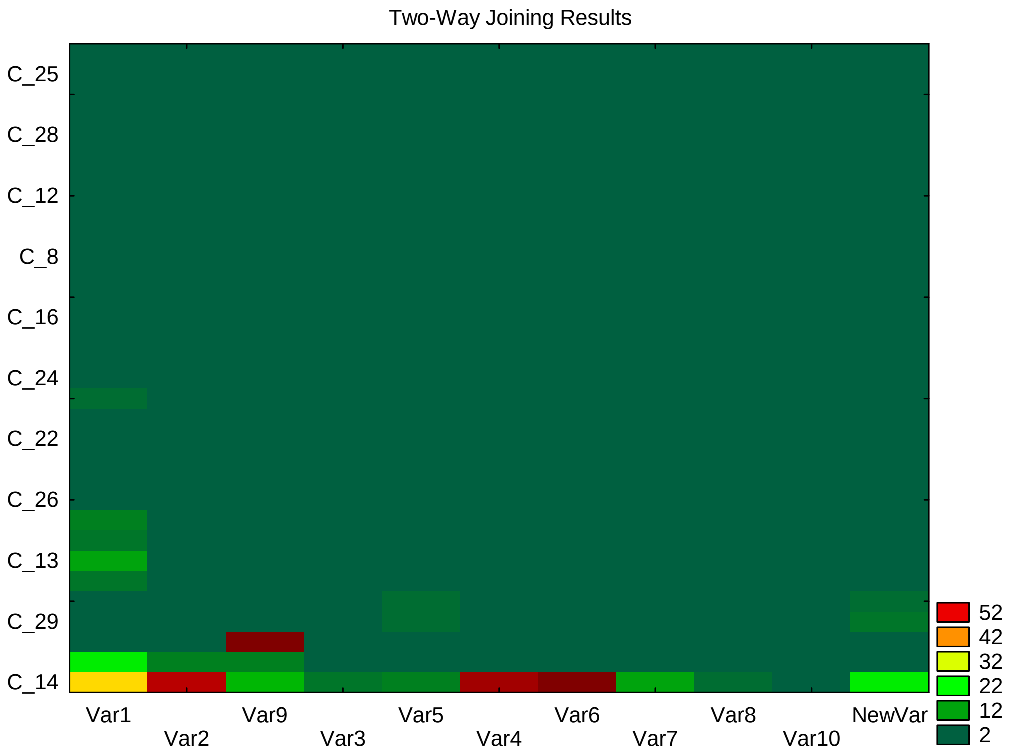
30
Cases
Cluster 1
Cluster 2


Two-Way Joining Results
C_25
C_28
C_12
C_8
C_16
C_24
C_22
C_26
C_13
C_29
C_14
Var1
Var2
Var9
Var3
Var5
Var4
Var6
Var7
Var8
Var10
NewVar
52
42
32
22
12
2

4. Conclusions(in two columns)

Determination of organochlorinated pesticides and PCBs in soil samples were realized based on EU protocols in laboratory of Agricultural Institute located in Peja, Kosovo. Capillary GC/ECD were used for their qualitative and quantitative analyze.

All studied samples have the same distribution of organochlorinated pesticides. The most detected pesticides were: 24’-DDT, 44’-DDD, HCB, Isodrine, Heptachlor, alfa-Klordan. Note that for all samples were found in higher levels metabolites of organochlorinated pesticides (not organochlorinated pesticides). This fact suggests that found concentrations could be because of before use of organochlorinated pesticides in agricultural areas and their degradation. All found pesticides concentrations were lower than Maximal allowed values (MAV) in the soil samples according Administrative Project instruction in Allowing Norms of Hazardous Substances and Harmful Presence in Soil, Prishtina, Kosovo, 2011.

PCBs were not detected for the main part of samples. PCB 52 and PCB 138 were most detected biphenyls. PCB 52 presence could be because of atmospheric factors. PCB 138 presence could be because of its physic-chemic properties. PCB levels were lower than Maximal allowed values (MAV) in the soil samples according Administrative Project instruction in Allowing Norms of Hazardous Substances and Harmful Presence in Soil, Prishtinw, Kosovo, 2011.

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