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Issue Article

Aktam Khalmanov
Samarkand State Architectural and construction institute named after Mirzo Ulugbek
Doctor of Physical and Mathematical Sciences,
Docent to department of heat-gas supply ventilation and service, Uzbekistan
a-xalmanov@umail.uz

Abdullo Nasimov
Samarkand State university named after Sharof Rashidov
Doctor of Technical Sciences, Professor to department of analytical chemistry,
Uzbekistan

Nodira Toshkuvatova
Samarkand State university named after Sharof Rashidov
PhD student to department of analytical chemistry,
Uzbekistan

STUDYING THE OBJECTS OF ENVIRONMENT PCB ANALYSIS IN NATURAL OBJECTS BY GAS CHROMATOGRAPHY WITH ECD

Abstract: *Twenty six samples for PCBs pollution in the central Region of Samarkand and Tashkent Region were investigated. Soil samples were collected from around of electricity supply of the Samarkand region and around Chirchik Transformer plant of the Tashkent region. The soil samples were extracted with chloroform mixture (1:1 v/v) for 4 hours. The final extract of 2.0 ml was analyzed for PCBs using a gas chromatograph equipped with 10 mCi 63Ni electron capture detector GC - ECD model 86/30. The retention time for the PCBs standard C=1mg/L were PCB 2,4,4', 3.683 min; PCB 2,2',5,5', 3.963min; PCB 2,2'3,4,4'5,5', 4,697 min; PCB2,2,3,4,5,5' 5.237 min; PCB 2,3'4'4'5, 5.427min; PCB 2,2'4,4'5,5' 5.67 min; and PCB 2,2'3,4,4',5' 6.290 min. Calibration curves for PCB 7 congeners were obtained, and the detection limits were estimated. We have used the standard solutions C=1mg/L and 10mg/L of PCB in isooctane for receiving calibration curves and identification for PCB 7 congeners.*

The variations of the microelements in soil around Chirchik Transformer plant were analyzed by the method of atomic absorption spectrometer "Saturn". The mean concentration (in mg/kg) of the metals were Ca (416± 19,1) >Na (222 ± 13,6)>Cu(100±3,9)>Ni (87±5,1)>Pb(57,1±2,9)>Zn (40.0 ±2.5) >Co (29.0±1,9) > Cd(21.3±1,5) > Fe (18.0±1,3)> Mn (10.0±1,2)>Cl-(0,32±0,02). The variations in the levels of the microelements were in the order Ca >Na >Cu>Ni >Pb>Zn >Co > Cd > Fe> Mn >Cl-. Very few sites were found to be contaminated with metals, but the level of metal contamination was very low. There was no significant correlation between the PCBs and any of the metals. The sources of the PCBs and metal were anthropogenic.

Key words: *the atmospheric aerosols, laser resonans-ioninazation spectroscopy, atom-ionization, cavity ring-down, polychlorinated biphenyl.*

Language: *English*

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Introduction

Development of laser equipment led to emergence of a number of methods of supersensitive detecting of the maintenance of various conditions of atoms. Among them the most perspective and developed for practical applications are the laser

resonant- ionization spectroscopy (RIS) in vacuum, atom ionization (AI) spectroscopy in a flame and cavity ring down laser absorption spectroscopy (CRLAS). Prospects of methods are defined by great opportunities for research of ultra-small concentration of atoms in various phase conditions of substance, and also for research of processes of excitation and ionization of atoms [1-3, 16-20].

Gas chromatographic method with electron capture detector was used for identification of polychlorinated biphenyls.

Global environmental pollution and adverse ecological situation in industrial regions cause need of continuous ecologic-analytical control (EAC) of air pollution, quality of water and accumulation of dangerous compounds in soil and ground deposits. According to various international agreements about 60 chemicals (priority pollutants) entered lists, their distributions providing restriction. 12 chlorinated organic compounds are called the persistent organic pollutants (POP). These are following nine organochlorine pesticides: aldrin, endrin, dieldrin, mirex, DDT, toxaphene, hexachlorobenzene, heptachlor, chlordane, industrial products: the polychlorinated biphenyls (PCB); and also products inadvertent production: the polychlorinated dibenzo-p-dioxine (PHDD) and polychlorinated dibenzofurans (PHDF) called usually dioxine [4-15].

In this regard, the adoption of the Law of the Republic of Uzbekistan "On ratification of the Stockholm Convention on Persistent Organic Pollutants (Stockholm, May 22, 2001)" (№ ZRU-535, 08.05.2019) is a clear demonstration of the state's responsible attitude to the protection of the environment, its preservation for future generations. One of the main objectives of the agreement is to protect the health of the population and the environment from the effects of persistent organic pollutants (POPs). Uzbekistan's participation in the Convention will improve the environmental situation related to POP and prevent possible negative consequences for the population, the environment, flora and fauna. PCBs are a group of chlorinated organic compounds with the general title "persistent organic pollutants" (POPs). Republic of Uzbekistan was carried on State List of Persistent Organic Pollutants (POPs). At present time certain hazardous chemicals and pesticides registered by Rotterdam Convention and Stockholm Convention came in state list [4]. Our aim is the study of PCBs pollutants around Chirchik transformer plant and electricity supply of the Samarkand region.

Objectives of the study were organization and discussion of sampling with representatives Samarkand and Chirchik State Committee for Nature Protection of the Republic of Uzbekistan. Representatives of Committee for Nature Protection of the Samarkand Region and engineer-ecologist of Chirchik Transformer plant took part in organization of sampling around of Transformer plant (Chirchik city of Tashkent region) from soil, water sediment and food-stuffs.

The Soil samples were collected from the immediate surroundings of Electricity supply of the Samarkand region in four districts (south, north, western, eastern sides) in the Central City of Samarkand. Samples of soil were taken around of Chirchik Transformer plant of Tashkent region.

Materials and methods of sampling. Soil samples were collected from four different locations in the south, north, western, eastern districts. At each sample were collected at depth of 0-30 cm. Each composite sample weighed between 400-500 g. The samples were placed in labeled polythene bags and sent to the laboratory. Samples of the soil exempt from roots, stones and any inclusions. Large units pound in a mortar. Pass through netting with openings 1-1,5 mm. At a studying of the damp soil in a parallel sample define humidity.

For the analysis 50g the air-dry soil pounded in a mortar. The soil samples were extracted with chloroform mixture (1:1 v/v) for 4 hours. A preparation from the soil is extracted by chloroform three times on 50 ml, each time stirring up a flask on 10-15 ml on the device for stirring and let's to test settle a little. Extract merge through waterless sulfate of sodium in a cup for distillation of a solution. Water extracts carefully merge in a long funnel, add three times on 30-50 ml, stirring up in a long funnel on 5-10 ml. Chloroformed extracts merge through sodium sulfate in a flask for solvent. Distillation conduct under vacuum of 10-15 mm of mercury at bath temperature no more than 40 °C. Solvent drive away to small volume (2 ml). **From average test take 10 g of a yolk and place in a conic flask on 250 ml.** In a flask add 100 ml of acetone and stir up during 1 h on the device for stirring. Then extract filter in a round-bottomed flask on 500 ml through a funnel with the fat-free cotton wool. The rest in a flask wash out in the small portions of acetone (2 x 15 ml) and merge through a funnel in the same flask. From the integrated extracts drive away acetone in the rotor evaporator at a temperature of bath of 20 - 25 °C. From the liquid phase PCB and POP which has remained in a flask extract in two portions hexane on 20 ml, stirring up each time a flask within 5 min. and dividing layers by means of a divider funnel. The integrated hexane extract transfer to a divider funnel and add carefully 20 ml of sulfuric acid, then a vitriolic layer reject and in a divider funnel flow a new portion of sulfuric acid. Operation of cleaning repeat until sulfuric acid doesn't become colorless, and at the subsequent stages hashing in a divider funnel of layers of hexane extract and sulfuric acid is necessary. The cleared hexane extract wash out the distilled water before neutral reaction of washing waters and transfer to a chemical glass for drying by waterless sulfate of sodium. The dried extract transfer to a round-bottomed flask,

sulfate of sodium wash out hexane (2 x 10 ml) and washouts unite in a round-bottomed flask. Hexane evaporates on the rotor evaporator to 2 - 3 ml, quantitatively by means of a pipette transfer to the graduated test tube and bring to a tag 4 ml (N 1 extract). Then carry out processing by spirit alkali. For this purpose 2 ml of N 1 extract place in the conic flask with a capacity of 10 ml supplied with the return water refrigerator, add 0,4 - 0,5 (four flat cakes) caustic potash, 2 ml of ethyl alcohol and mix heat within 30 min. from the moment of dissolution of caustic potash at a temperature of 55 - 58 °C and hashing on a magnetic mixer. After cooling mix quantitatively transfer to a divider funnel and add 4 ml of water for division of layers. Aqueous-alcoholic layer reject, and hexane is washed out by 2 ml of 1 percent sulfuric acid, water before neutral reaction of washing waters, dry, filtering in the graduated test tube through a funnel from 3 g previously the sodium moistened hexane sulfate. After a filtration sulfate of sodium is washed out by 2 ml hexane and wring out a glass stopper. Hexane extract evaporates to 2 ml (N 2 extract).

The sediment (50g) is centrifuged to remove excess water, wetted with 40 ml acetone, extracted with 80 ml hexane, re-wetted with 20 ml acetone, and extracted again with hexane. Between each solvent addition, the sample is shaken 20 min on a wrist-action shaker. The extraction should be repeated until at least 75% of the added solvent is recovered. PCB recoveries were 100% after 3 extractions. The final extract of 2.0 ml was analyzed for PCBs using a gas chromatograph equipped with 10 mCi 63Ni electron capture detector GC - ECD model 86/30, type Gas Chromatograph MASTER (DANI instruments S.p.A., Italy). The capillary column used was DN5 10 m × 0.15 mm id × 0.1 μm film thickness. The GC conditions were as follows: injection point temperature: 50^0C, 600^0C/min, 320^0C(2min); oven temperature programmed: 120^0C,35^0C/min, 200^0C, 30^0C/min, 300^0C, (2min). Temperature of detector was 320^0 C; carrier gas (He)-nitrogen at flow rate: 0,5 ml/min; make-up gas flow rate - 29.0 ml/min. The PCB standards were obtained from laboratory of instrumental analysis, University of Florida, USA. Suggested instrument conditions for PCB analysis are given in the table 1.

Table 1. Suggested Instrument Conditions for PCB Analysis

Name	Parameters GC Master
Column	DN 5=10mx0,1mm, 0,1μm
Oven	120^0C,35^0C/min, 200^0C, 30^0C/min, 300^0C, (2min)
PTV injector	50^0C,600^0C/min,320^0C(2min)
Carrier gas (He), flow rate	0,5mL/min
Split Ratio	1:10
ECD Detector temperature	320^0C
Signal:Min.Half Peak width	0,60 S
Digital Acq.Rate	100 Hz
Flows: Aux Type N_2	25 ml/min
Injection Volume	1μL
Total registration time	10,2 min

Results and discussion. The total runtime was 10.2 min. The retention time for the PCBs standard C=1mg/L were PCB 2,4,4', 3.683 min; PCB 2,2',5,5', 3.963min; PCB 2,2'3,4,4'5,5', 4,697 min; PCB2,2,3,4,5,5' 5.237 min; PCB 2,3'4'4'5, 5.427min; PCB 2,2'4,4'5,5' 5.67 min; and PCB 2,2'3,4,4',5' 6.290 min. The retention time for the PCBs standard C=10mg/L were PCB 2,4,4', 3.68 min; PCB 2,2',5,5', 3.96min; PCB 2,2'3,4,4'5,5', 4,7 min; PCB2,2,3,4,5,5' 5.24 min; PCB 2,3'4'4'5, 5.43min; PCB 2,2'4,4'5,5' 5.68 min; and PCB 2,2'3,4,4',5' 6.25 min. The identification of PCBs congeners in the sample was conducted by comparing the retention times of the PCBs congeners in sample to that of the PCB standards (Fig. 1). The concentrations of the individual PCBs congeners in mg/kg were calculated on dry weight basis, and the total PCBs concentration (ΣPCB) calculated by summing up the concentrations of individual PCB congeners.

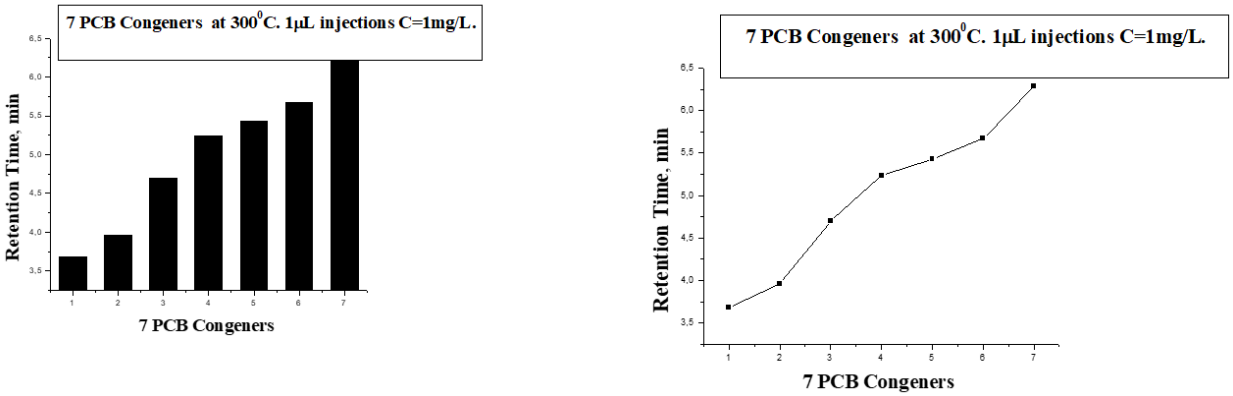

Figure 1. Dependence of retention times with PCB congeners

Theoretically, there can be 209 isomers (congeners) of PCB. 7 congeners of PCB are given in the table 2.

Chromatographic spectra of PCB 7 congeners and calibration curves are shown in Fig. 2. Calibration curves for PCB 7 congeners were obtained, linear 1-2 orders of magnitude with respect to concentration, and the detection limits were estimated. We have used the standard solutions C=1mg/L and 10mg/L of PCB in isooctane for receiving calibration curves and identification for PCB 7 congeners.

Table 2. PCB Empirical formulas, molecular weight, and number of corresponding isomers

PCB	Empirical Formula Chlorbiphenyls	Molecular Weight	Average Molecular Weight	Percent Chlorine	Number of Isomers
Chlorobiphenyl	$C_{12}H_9Cl$	188.0	188,7	19	3
2,4,4'-trichlorobiphenyl	$C_{12}H_7Cl_3$	256.0	257,6	41	28
2,2'5,5'-tetrachlorobiphenyl	$C_{12}H_6Cl_4$	289.9	292.0	49	52
2,2'3,4,4'5,5'-heptachlorobiphenyl	$C_{12}H_3Cl_7$	391.8	395.3	63	24
2,3'4,4'5,5'-pentachlorobiphenyl	$C_{12}H_5Cl_5$	323.9	326.4	54	46
2,3'4,4'5-pentachlorobiphenyl	$C_{12}H_5Cl_5$	323.9	326.4	54	46
2,2'4,4'5,5'-hexachlorobiphenyl	$C_{12}H_4Cl_6$	357.8	360.9	59	42
2,2'4,4',5'-hexachlorobiphenyl	$C_{12}H_4Cl_6$	357.8	360.9	59	42

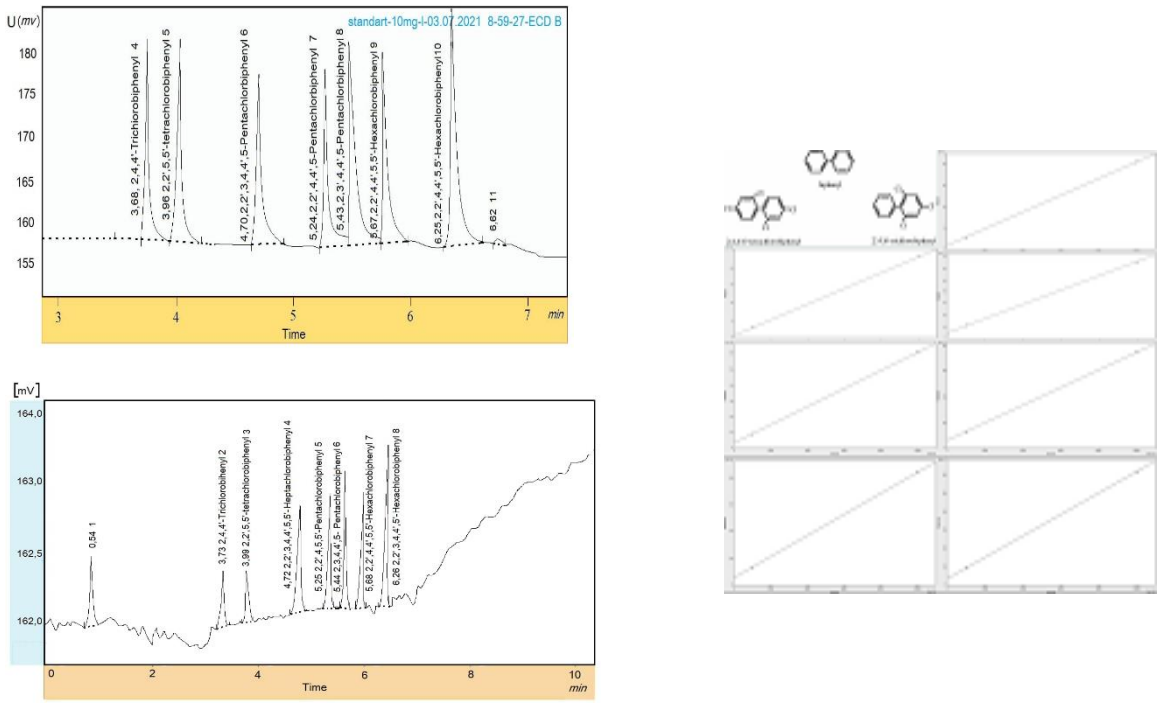

Figure 2. Chromatographic spectra of PCB (polychlorinated biphenyls)l using GC-ECD, Concentration of the standard solutions C=1mg/L and 10mg/L. Calibration curves for PCB 7 congeners.

We used PCB Congener Standard, 100 µg/mL in isooctane, 1mL/ampul.The extraction solution of the samples around of electricity supply of the Samarkand region were analyzed in Gas Chromatography of DANI MASTER GC. A standard mixture of 7 PCB congeners in isooctane was analyzed. The sampling sites are shown in figure 3.The results of determination of PCB in soil samples are given in Table 3. In the case of liquid solutions, the standard addition and calibration curve methods were used to study the effect of the matrix on the analytical signal from PCB and on the background. We have used the standard solutions C=200µg/L and 300µg/L of PCB in isooctane for studying the effect of the matrix. The results obtained show that the extraction solution from soil does not effect the PCB signal so that calibration can be performed with isooctane standard solution.

Figure 3. Map of sampling soil around of electricity supply of the Samarkand region

Table 3. Results of determination of PCB in soil samples

Analyte	Introduced, µg/mL	MATRIX 1			MATRIX 2		
		Found, µg/mL (n=6)	δ, %	S_r, %	Found, µg/mL (n=6)	δ, %	S_r, %
PCB 28	**200**	213,036	9,8	6	210,12	10,8	6,5
PCB 52	**200**	230,446	7,9	5,9	225,216	9,9	6
PCB 24	**200**	236,438	10	7,3	230,238	10,8	7,9
PCB 46	**200**	208,489	8	6,1	204,489	9,9	7,1
PCB 46	**200**	248,842	11	8,2	235,75	12	7,2
PCB 42	**200**	236,973	12	8,1	226,873	10,5	6,1
PCB 42	**200**	188,689	9	6,2	198,589	9,8	7,2

Relative error (δ) of definition of analytes and the standard deviation (S_r) in the majority cases doesn't exceed 12% and 10% respectively that it agrees with requirements to correctness and reproducibility of quantitative analysis.

Then, the content of PCB in the soil samples was determined. Detection limit of PCB in soil of the immediate surroundings of Electricity supply of the Samarkand region was much lower than the $800 \cdot 10^{-9}$ g/kg.

Samples (№1,№2,№3) of soil were taken north, south, western, eastern sides of Chirchik Transformer plant of Tashkent region in September. We have injected V=1MkL sample solution in chromatographic column. Time of all experiments is 10 minute. The sampling sites are shown in figure 4.The experimental results obtained are given in Fig.5. We have used the standard solutions C=10mg/L of PCB in isooctane for soil analysis. The results obtained were given in table 4 that the extraction solution from soil was taken south, north, eastern sides of Chirchik Transformer plant of Tashkent region.

Table 4. Results of investigations of PCB in different samples

	Reten. Time [min]	Response	Amount [mkg/l], c_{Amount}	C= c_{Amount}*V/m mg/kg	Compound Name
Sample №1					
2	4,747	59,228	9793,149	$195{,}8604 \cdot 10^{-6}$	2,2’3,4,4’5,5’-heptachlorobiphenyl
4	5,233	53,992	9021,628	$180{,}404 \cdot 10^{-6}$	2,3’4,4’5,5’-pentachlorobiphenyl
5	5,463	70,856	9569,436	$191{,}692 \cdot 10^{-6}$	2,3’4,4’5-pentachlorobiphenyl
6	5,667	45,492	6886,836	$137{,}7 \cdot 10^{-6}$	2,2’4,4’5,5’-hexachlorobiphenyl
8	6,253	58,956	4429,865	$88{,}536 \cdot 10^{-6}$	2,2’4,4’,5’-hexachlorobiphenyl
	Total		36640,84	$794{,}172 \cdot 10^{-6}$	
Sample №2					
2	4,747	90,718	14999,89	$299{,}959 \cdot 10^{-6}$	2,2’3,4,4’5,5’-heptachlorobiphenyl
3	5,240	151,29	25267,44	$505{,}314 \cdot 10^{-6}$	2,3’4,4’5,5’-pentachlorobiphenyl
	Total		40267,335	$805{,}27 \cdot 10^{-6}$	
Sample №3					
2	4,747	121,92	14684,54	$293{,}684 \cdot 10^{-6}$	2,2’3,4,4’5,5’-heptachlorobiphenyl
3	5,240	184,52	25858,9	$517{,}168 \cdot 10^{-6}$	2,3’4,4’5,5’-pentachlorobiphenyl
	Total		40543,45	$810{,}85 \cdot 10^{-6}$	

The retention time for the PCBs standard C=10mg/L were PCB 2,4,4’, 3.69 min; PCB 2,2’,5,5’, 3.97min; PCB 2,2’3,4,4’5,5’, 4,70 min; PCB2,2,3,4,5,5’ 5.24 min; PCB 2,3’4’4’5, 5.43min; PCB 2,2’4,4’5,5’ 5.68 min; and PCB 2,2’3,4,4’,5’ 6.26 min.

Figure 4. Map of sampling soil and food stuffs around Chirchik transformer plant in city Chirchik of Tashkent region

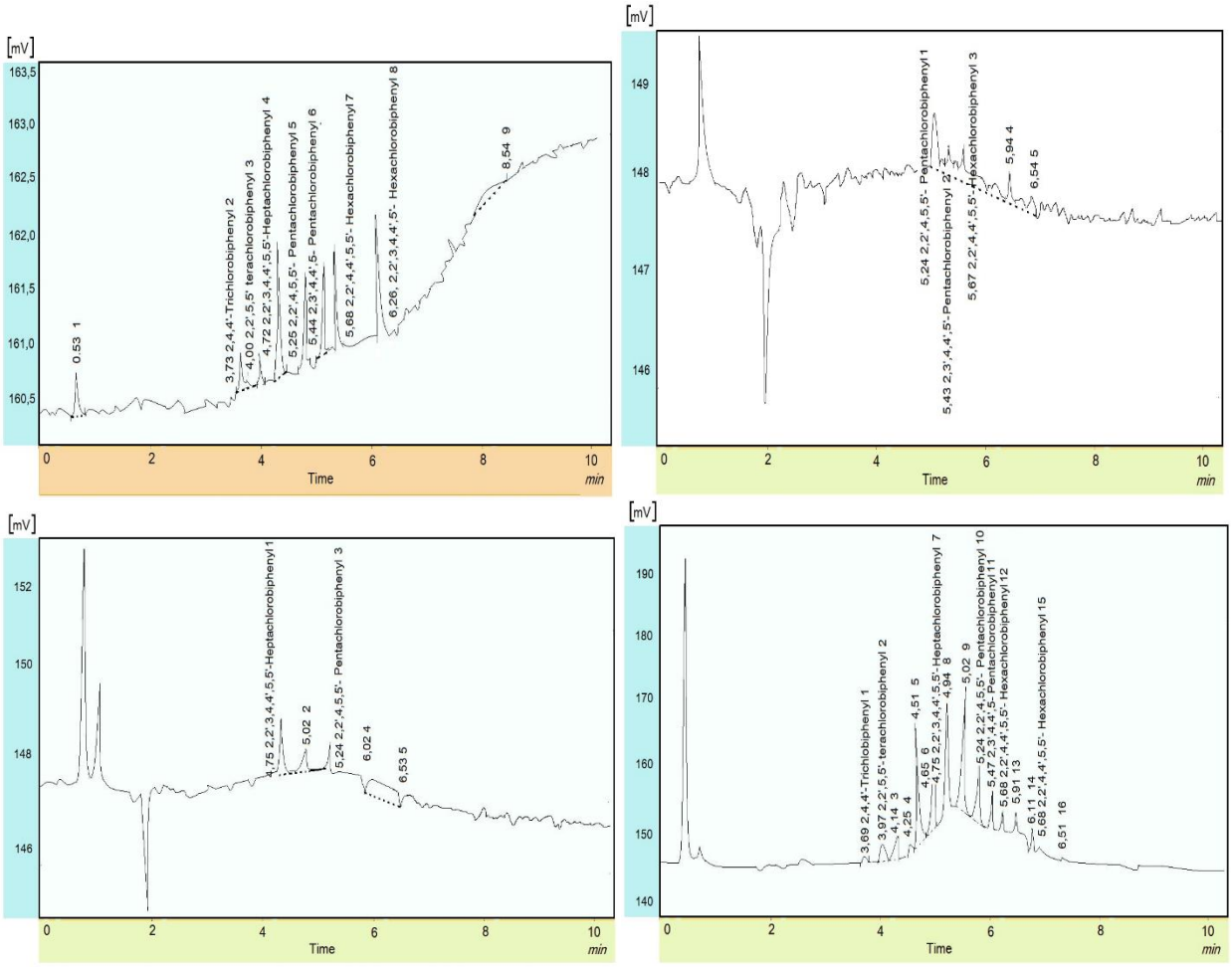

Figure 5. Chromatographic spectra of PCB (polychlorinated biphenyls) using GC-ECD, Concentration C=10mg/L; GC-ECD chromatograms of soil extract.

Samples of soil were taken north, south, western, eastern sides of Chirchik Transformer plant of Tashkent region in October. Objects of research were seaweed, leaves of a tree of a nut, corn leaves, chicken egg and cow milk. Detection limit of PCB in these objects of the immediate surroundings of Chirchik transformer plant in city Chirchik of Tashkent region was much lower than the $800 \cdot 10^{-9}$g/kg. A total of

twenty six (26) composite soil samples were collected from ten different locations. At one site three (3) composite samples were collected at depth of 0 - 10 cm, 10-20 cm and 20-30 cm. The sampling sites are shown in figure 4. The results obtained were given in table 5 that the extraction solution from soil was taken western sides (WS) of Chirchik Transformer plant of Tashkent region. Every numbered sample consists of five taken samples.

Table 5. Concentration of PCB in soils around Chirchik transformer plant in city Chirchik of Tashkent region

	Reten. Time [min]	Response	Amount [mkg/l], c_{Amount}	Amount [%]	C= c_{Amount}*V/m mg/kg	Compound Name
1	3,687	0,848	82,411	0,1	$1,648*10^{-6}$	2,4,4’-trichlorobiphenyl
2	3,970	14,096	6778,904	12	$135,578*10^{-6}$	2,2’5,5’-tetrachlorobiphenyl
7	4,747	87,560	14477,751	25,6	$289,555*10^{-6}$	2,2’3,4,4’5,5’-heptachlorobiphenyl
10	5,243	77,746	12990,738	22,9	$259,814*10^{-6}$	2,3’4,4’5,5’-pentachlorobiphenyl
11	5,470	82,600	11155,563	19,7	$223,11*10^{-6}$	2,3’4,4’5-pentachlorobiphenyl
12	5,683	67,293	10187,255	18,0	$203,745*10^{-6}$	2,2’4,4’5,5’-hexachlorobiphenyl
15	6,253	13,152	988,231	1,7	$19,7646*10^{-6}$	2,2’4,4’,5’-hexachlorobiphenyl
	Total		56660,853	100,0	$1133,214*10^{-6}$	

The results obtained were given in table 6. Obtained results show small dependence of concentrations of PCB congeners from depth of soil. The experimental results obtained are given in Fig.6. Injection volume V=1μL was soil analysis of around Chirchik transformer plant

Analysis of the microelements. The soil samples were dried at 100 °C for 48 hours in the oven. The dried samples were passed through standard screen to remove large particles. For the digestion of the soil sample, one gram of dried and homogenized soil was weighed and placed in an acid washed teflon vessel. The digestion was performed with a mixture HNO_3 and $HClO_4$ acid. The digested samples were analyzed for metals. The analytical precision and accuracy of the method was accomplished by analyzing a blank and duplicate spike samples. The Saturn AAS was used for the metal analysis.

Table 6. Variations of concentration of PCB in soils from depth around Chirchik transformer plant in city Chirchik of Tashkent region

Samle	PCB ($c*10^{-6}$ mg/kg)				Compound name
WS	0-10cm	10-20cm	20-30cm	mean	
	1,945	1,45	1,55	1,648	2,4,4’-trichlorobiphenyl
	138,678	133,478	134,578	135,578	2,2’5,5’-tetrachlorobiphenyl
	287,751	288,359	292,555	289,555	2,2’3,4,4’5,5’-heptachlorobiphenyl
	262,924	257,704	258,814	259,814	2,3’4,4’5,5’-pentachlorobiphenyl
	226,21	221,01	222,11	223,11	2,3’4,4’5-pentachlorobiphenyl
	206,845	201,645	202,745	203,745	2,2’4,4’5,5’-hexachlorobiphenyl
	18,9646	17,5646	22,7646	19,7646	2,2’4,4’,5’-hexachlorobiphenyl
Total	1143,32	1121,21	1135,12	1133,21	

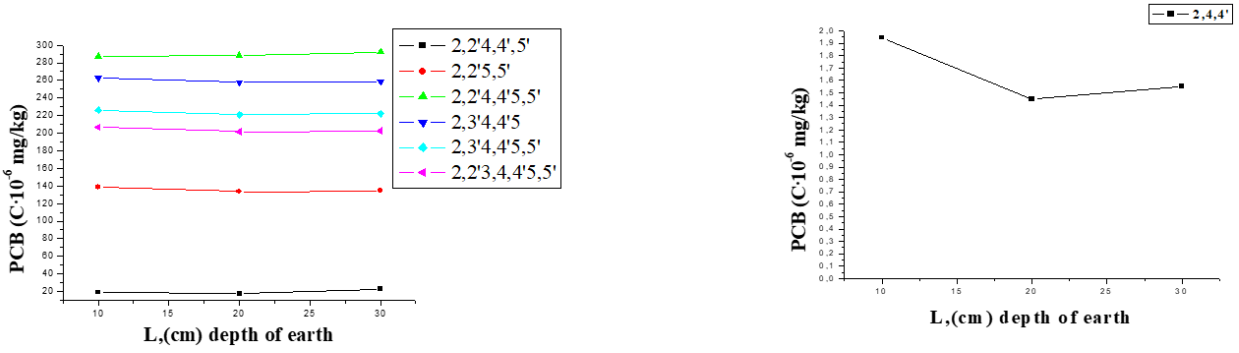

Figure 6. Dependence of concentrations of PCB congeners from depth of soil.

The mean concentration (in mg/kg) of the metals were Ca (416± 19,1) >Na (222 ± 13,6)>Cu(100±3,9)>Ni (87±5,1)>Pb(57,1±2,9)>Zn (40.0 ±2.5) >Co (29.0±1,9) > Cd(21.3±1,5) > Fe (18.0±1,3)> Mn (10.0±1,2)>Cl^-(0,32±0,02). The variations in the levels of the microelements were in the order Ca >Na >Cu>Ni >Pb>Zn >Co > Cd > Fe> Mn >Cl^-. Dependence of concentrations from micro metals and microelements is given in Fig.6.

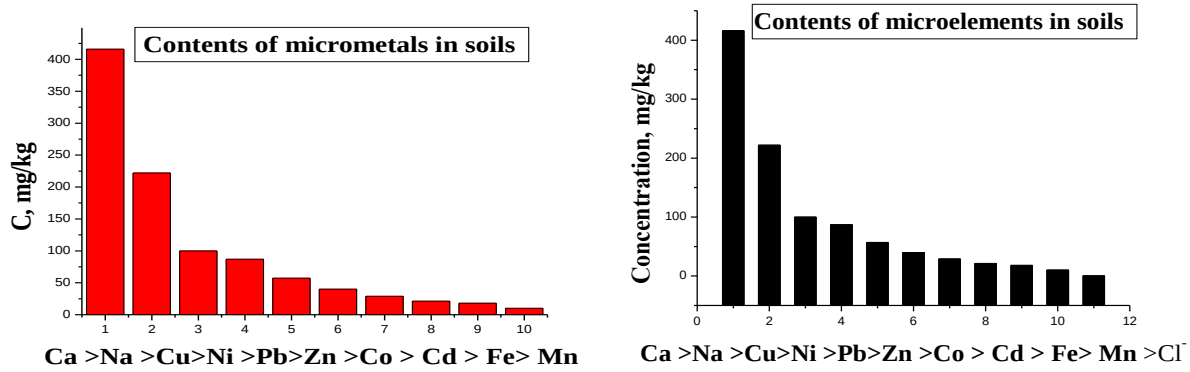

Figure 7. Dependence of concentrations from micro metals and microelements

Conclusion

Totally 26 samples of soil were organized around the territory of Chirchik transformer Plant. Experiments carried out for determination of 7 congeners of PCB in standard solutions and soil extractions. The identification of PCBs congeners in the sample was conducted by comparing the retention times of the PCBs congeners in sample of the PCB standards.

Gas chromatography-ECD and Analyzer L2000DX were used for identification of polychlorinated biphenyls in soil samples. Capillary gas Chromatography with electron capture detector is a powerful tool for the identification of individual PCB congeners in environmental samples. Calibration curves for PCB 7 congeners were obtained, linear 1-2 orders of magnitude with respect to concentration, and the detection limits were estimated.

Analysis of soil showed that low concentration of PCBs takes place in around of Chirchik transformer plant. This is not dangerous for natural objects of Chirchik city.

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