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Macrovipera Lebetina Obtusa Venom as a Biological Indicator of Environmental Pollution

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ABSTRACT

In this research, the effect of environmental pollution factors on Caucasian Macrovipera Lebetina Obtusa venom was studied. The readings of radionuclide activity of the snake venom captured in the neighborhood of Baku and in Absheron Peninsula of Azerbaijan were taken at Camberra spectrometer. An unessential composition of Ra ^{228}, Cs ^{137}, K^{40} and Ra^{226} radioactive elements was detected in the samples. It was revealed that biogeocenosis pollution by radioactive substances could be considered as a new abiotic factor of living organisms' habitation area effecting on biosynthesis products of snake venom gland. İt was established by method of atom-absorbing spectrometry that the maintenance of heavy metals in venom of snake changes depending on degree of impurity of district of dwelling and corresponds: Pb (49.13-134.9), Zn (360.8-863.6), Cd (1.6-1.9) mg/kg. Natural radioactive elements- isotopes Ra^{226}, Ra ^{228} and K^{40} in investigated samples has been revealed. Insignificant activity of isotope Ra ^{228} and Ra^{226} in investigated samples was defined, minimum concentration of radionuclides was 0.174 Bk/g and 2.48 Bk/g. Minimum radiating activity of isotope Ra^{226} was in limits 0.539663Bk/g. The minimum radiating activity of isotope K^{40} was 1.44382Bk/g and activity of other isotopes was insignificant.

KEYWORDS: *Macrovipera lebetina obtusa, venom, environment, heavy metals, radionuclides*

1.INTRODUCTION

In connection with intensive studying of snake venoms, the variability of pharmacological activity, physicochemical and biophysical parameters of Transcaucasian viper venom (Vipera lebetina obtusa) under the influence of ecological factors represents considerable interest from ecological -physiological positions .In references the review has been resulted in studying of physical and chemical properties of solutions of venom of the Central Asian snakes. At the analysis at the temperature range 303-308K deviations of these parameters on viscosity and density isotherms have been noted [7].Water solutions of venom of snakes are unstable and lose toxicity in some days after mixing. Snake venoms (Echis carinatus, Vipera lebetina obtusa, Naja naja) in the physiological solution containing 50 % of glycerin, at storage in a refrigerator within 6 months do not reduce toxicity. Venom of a cobra, at storage on a cold in the soldered ampoule has kept toxicity more than 20 years. Snake venoms are thermostabilite and in the sour environment maintain heating to 120°C without loss of activity [14]. Snake venoms activation under the influence of physical factors, for example, at influence of an ultra-violet irradiation and X-rays. So, in 7 days after a venom irradiation of Filipina cobras by γ radiation (^{60}Co) in doses 0.25, 0.5, 1 mrad , toxicity of snake venom made 83, 66 and 43 %, accordingly from not irradiated venom [27].

The considerable variation in the maintenance of sulfur proteases and metal proteases is marked in snake venoms [28, 12, 15, 14]. Venom of Vipera lebetina is applied as a source of reception of commercial preparations FGN, phosphorus distresses and oxygen dases L-amino acids, and also as a diagnostic preparation at illnesses of curtailing system of blood [9, 21]. Considerable influence on toxicity and quantity of allocated venom is rendered various biological, ecological factors, and also chemical agents and some physical factors. The basic component of venom responsible for its toxic properties are biologically active components of the aluminous nature and including various enzymes [18,26,11,16, 20,24]. Venom of vipera lebetina renders anti-inflammatory and analogizing action. It is considered possible to use of venoms of Vipera libetina, Echis carinatus and cobras in otolaryngology [13,17, 28, 6, 10].

Snake venoms are a great value for medicine and biology[1,4,5,27,8,19]. In venom of rattlesnakes Grotalus durissus are found out ions of some metals: Na, Ca, K, Mg, Zn, Fe, Cu, Co, Mn which activate a number of enzymes of venom or temporarily break their action [22, 23]. Elementary structure of venom of Vipera lebetina obtusa caught of the various areas of Azerbaijan polluted by technogenic emissions of the industrial enterprises has been studied by the atom -absorption spectrophotometer method. In venom of Vipera lebetina obtusa the maintenance of heavy metals: Cr, Pb, Cd, Ni and Zn [25,26] have been defined Researches on revealing of influence of γ -radiation on toxicity and on pharmacological properties of venom of Vipera lebetina obtusa have been carried out [2,3]. Studying of influence of ecological factors (abiotic and biotic factors and including heavy metals, radiation) on venom of snake, definition of concentration of heavy metals in venom of Macrovipera lebetina obtusa, studying of influence of heavy metals and radiation on biochemical indicators and venom spectral characteristics, on toxicity of venom Macrovipera lebetina obtusa, and also selection of conditions of radiating sterilization of snake venom - an actual problem of biochemistry, toxicology, medicine and biology. These

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problems still are not perfect, demand deep theoretical and practical analyses and are still actual.

2. MATERIALS AND THE METHODS OF STUDY

We have conducted summer field researches in vicinities of Baku, and also areas of Azerbaijan. During the expedition catching of viperas has been spent with a capture of venom, soil and vegetation tests. A part of venom of snake has been subjected to the analysis of heavy metals by a method of atom-absorbtion spectrometry (AAS-300 Perkin Elmer, USA), also on the maintenance of radionuclides on installation "Camberra" γ-spectrometer with a plenary Ge-detector (experiment time-24 hour). Irradiation of venom vipera small dozes scale of radiation (γ -radiation D=0.75kGy/sec) carried out on K-25 to isotope installation with application ^{60}Co.

The expedition has been conducted in the area of the snake venom selection and the soil, water and plant samples have been taken in the neighborhood of Absheron peninsula of Azerbaijan. Research objective - studying of influence of ecological factors, including heavy metals, ionizing radiation on biochemical characteristics of venom of Macrovipera lebetina obtusa.As a result of researches chemical compound changes, and also influence of heavy metals, ionizing on albuminous structure, fermentative. Activity and other biochemical indicators of venom of Macrovipera lebetina obtusa will be established. Conditions of radiation sterilization of snake venom will be picked up. Detection of heavy metals in snake venom was carried out at direct influence of solution trichloroacetic acid on venom. After precipitation of fibers in snake venom by trichloroacetic acid, the content of heavy metals in samples of snake venom was performed. It was necessary to consider the fact of overestimate of the given experimental measurements at direct detection of the content of the above-stated metals in samples of snake Venom. The technique of study of viper venom by atomic absorption spectrometry consists in the following. An exact amount of snake Venom in quantity of 20 mg was placed in centrifuge tube, 10 ml of solution HCl (1:1) was added and further a solution left in the thermostat at 40^0 C at 1 hour. After that 2 ml of 20 % solutions CCl_3COOH was added, with the subsequent keeping during of 1st hour at room temperature and centrifuged during 10 minutes at 1500 rpm. Fe, Cr, Cu, Cd was detected in the filtrate. It is necessary to consider that fact that standard solutions should contain 5 % trichloroacetic acid. Thus, we pick up optimum conditions for detecting Fe, Cr, Cu, Cd, Zn from trichloroacetic acid filtrate. For qualitative determination of concentration of investigated metals in bioobjects we constructed the graduated diagrams of determination of standard metals in coordinate's A-C. Under the graduated diagrams in coordinate's A-C concentration of detected elements was determined. Construction of graduated diagrams for detection of standard metals. For construction of graduated diagrams working standard solutions were entered serially into an air-acetylene flame of a burner, beginning from a solution with the minimum content of a detected element not less than four concentration, including the concentration close, to that which is expected in an analyzed solution. Each measurement repeated twice (not less than 2 times), at diagram construction average value was taken.

3. RESULTS AND THEIR DISCUSSION

We have conducted summer field researches in vicinities of Baku, and also areas of Azerbaijan: Gobustan, Shamakhi, Kurdamir and Sabirabad. During the expedition catching of viperas has been spent with a capture of venom, soil and vegetation tests. Snakes after milking have been released in the nature, venom is placed in exicators, dried up for analysis carrying out on the maintenance of heavy metals by a method of atom-absorbtion spectrometry. A part of venom of snake has been subjected to the analysis on the maintenance of radionuclides on installation "Camberra" γ-spectrometer with a plenary Ge-detector. Data by definition of the maintenance of heavy metals in investigated samples is presented in tables 1-11.

Table 1. The data content of heavy metals in investigated samples

Territory	**Baku**				
Samples	Concentration, mg/kg (M±m) standard deviation				
	Cr	Pb	Cd	Ni	Zn
Plant	132,3±3.080	23,0±0.154	2,08±0.010	40,47±0.362	701,7±2.14
Soil	82,9±0.60	6,0±2.094	0,63±0.042	36,0±0.765	53.41±6.418
Venom					

Table 2. The data content of heavy metals in investigated samples

Territory	**Baku - Airport**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	130,4±2.528	23,0±1.379	2,06±0.064	40,30±0.307	701,39±0.303
Soil	80,7±4.842	5,9±0.154	0,59±0.007	36,11±0.275	53,00±0.098
Venom		133,9±14.464	1,9±0.949		665,0±3.989

Table 3. The data content of heavy metals in investigated samples

Territory	**District Shamakhi**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	137.0±1.316	22,7±4.695	2,05±0.063	40,33±0.1685	701,4±0.077
Soil	83,0±0.459	5,3±0.073	0,62±0.004	28.0±0.658	53,0±0.055
Venom		134.9±0.056	1,76±0.245		644±0.099

Table 4. The data content of heavy metals in investigated samples

Territory	**District Gobustan - Childag**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	131.0±1.300	23,0±4.480	2,05±0.058	39,99±0.1600	700,4±0.091
Soil	89,9±0.438	5,5±0.079	0,70±0.001	35,15±0.542	52,7±0.049
Venom		133.9±0.033	1,9±0.200		666,9±0.0034

Table 5. The data content of heavy metals in investigated samples

Territory	**District Gobustan Maraza**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	130.0±1.200	20,9±3.480	1,99±0.038	39,36±0.500	700,3±0.090
Soil	80,2±0.356	4,9±0.030	0,53±0.025	35,15±0.678	52,20±0.071
Venom		133.7±0.029	1.6±0.177		663,7±0.027

Table 6. The data content of heavy metals in investigated samples

Territory	**District Sabirabad Village Karatugay**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	66,5±1.290	4,9±0.090	1,0±3.480	40,4±0.670	280,1±0.040
Soil	100,4±0.556	7,3±0.027	0,6±0.030	45,6±0.798	98,0±0.088
Venom	87,0±0.049	87,0±0.030			669,0±0.076

Table 7. The data content of heavy metals in investigated samples

Territory	**District Sabirabad Village Shihsalahli**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	87,0±0,990	4,9±0.487	0,5±0,589	10,7±0,133	66,05±0,440
Soil	90,6±0.670	10,0±0.567	0,5±0,131	43,9±0,228	67,09±0,344
Venom		50,1±0,285			860,9±0,129

Table 8. The data content of heavy metals in investigated samples

Territory	**District Sabirabad Village Ahtachi**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	65,0±0,560	4,2±0,074	1,0±0,898	39,2±0,879	282,0±0,235
Soil	96,0±0,076	7,1±0,333	0,61±3,670	43.3±2,30	99,5±0,004
Venom		87,0±4,35			362,0±0,800

Table 9. The data content of heavy metals in investigated samples

Territory	**District Sabirabad Village Uladjali**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	64,0±0,009	4,0±0,789	1,3±0,956	38,9±0,451	282,0±0,760
Soil	95,0±0,435	6,9±1,345	0,60±1,68	42.4±0,289	99,9±0,390
Venom		89,0±0,300			360,8±0,190

Table 10. The data content of heavy metals in investigated samples

Territory	**District Kurdamir Village Atakishili**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	67,6±0,010	4,8±0,700	1,1±0,8906	41,5±0,400	284,3±0,799
Soil	102,3±0,336	7,6±1,355	0,7±0,489	46,7±0,8,90	99,0±0,480
Venom		89,1±0,280			371,1±0,230

Table 11. The data content of heavy metals in investigated samples

Territory	**District Kurdamir Village Hirdapay**				
	Concentration, mg/kg (M±m)				
Samples	Cr	Pb	Cd	Ni	Zn
Plant	88,4±0,045	4,6±0,680	0,6±0,542	11,9±0,484	67,05±0,6500
Soil	91,4±0,501	9,3±1,500	0,6±0,980	44,9±0,389	68,05±0,200
Venom		49,13±0,478			863,6±0,315

We had spent experimental measurements of the maintenance of radio nuclides in samples of venom of viperas, caught with various degree of impurity districts of Azerbaijan. The spectrum defining activity of radionuclides in venom of snakes has been removed on spectrometer Camberra. The radiating background of radionuclides (uranium, caesium),

defined in venom of snakes is presented in the table 12-14. The radiating background of radionuclides in venom of snakes from various zones was identical.

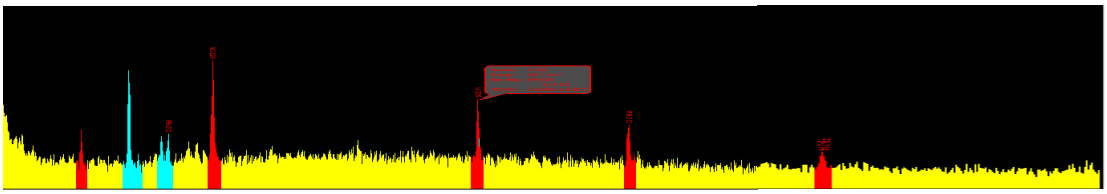

Fig. 1. A spectrum of activity of radionuclides in venom of snakes, caught in territory of Gobustan and Absheron peninsula of Azerbaijan

In the table the radioactive elements which are available as a part of snake venom are presented.

Table 12. Radiating activity of elements in snake venom

Elements	Radiating activity, Bk/g
Ra 228	0.174 ± 0.090
Ra-226	2.48 ± 0.05
Cs 137	MDA=0.315

Table 13. Radiating activity of elements in investigated samples

The crystals were Venom	Sample 1	Sample 2	Sample 3
The name radionuclide	Ra^{226}	K^{40}	Ra^{226} = Pb^{212}, U^{235}, U^{238}
Energy radionuclide	186.2 keV	1460.8 keV	185.97 keV
Radiating activity	0.539663 Bk/g	1.44382 Bk/g	
Radiatsionno-chemical exit	3.28%	10.67%	5.26%
The peak area with a margin error	356; 10.98%	483 4.897%	77, 770
Mass	56 mg	60mg	55 mg
Specific activity	-	-	0.427, 0.26, 12.64 Bk/kg
Width of semiheight	0.936 keV	1.501 keV	

Natural radionuclides of low activity again have been defined in samples of snake venom (fig.1-6). The revealed activity of an element of uranium in a spectrum of venom grows out of a radiating background which is formed by influence of environment. Therefore protection of environment from technogenic pollutants on value ranks now with such problems as protection of soils. It is necessary to note that an increase of a radiating background of environment which throughout last several millenia remained rather stable. The spectrum of activity of radionuclides in soil is presented in the drawing.

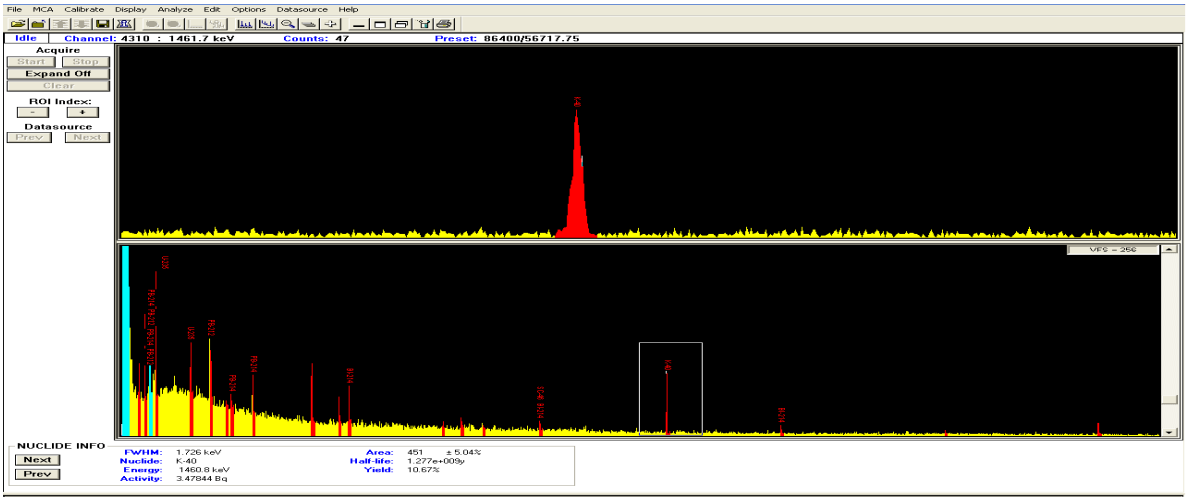

Fig. 2. A spectrum of activity of radionuclides in the snake venom, caught in territory of Shamakhi of Azerbaijan

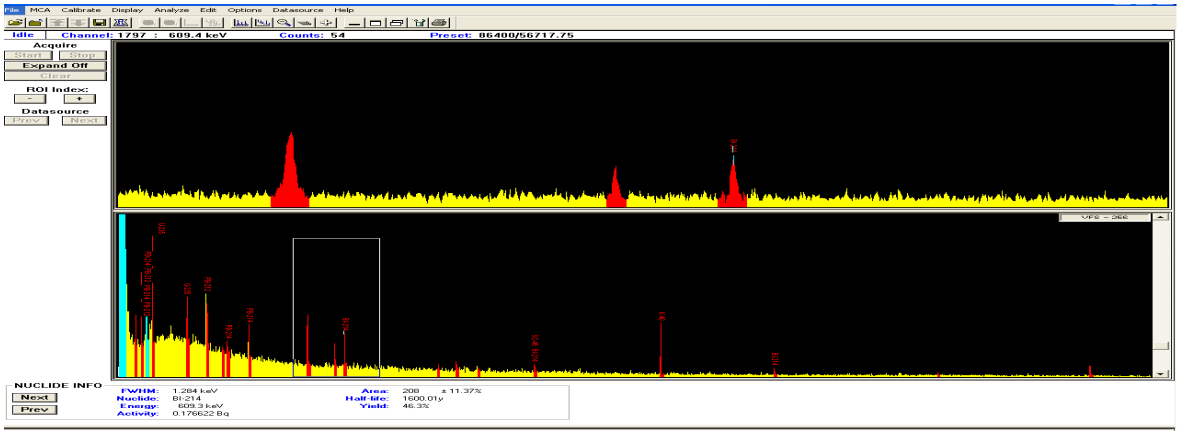

Fig. 3. A spectrum of activity of radionuclides in the snake venom, caught in territory of Sabirabad and Kurdamir

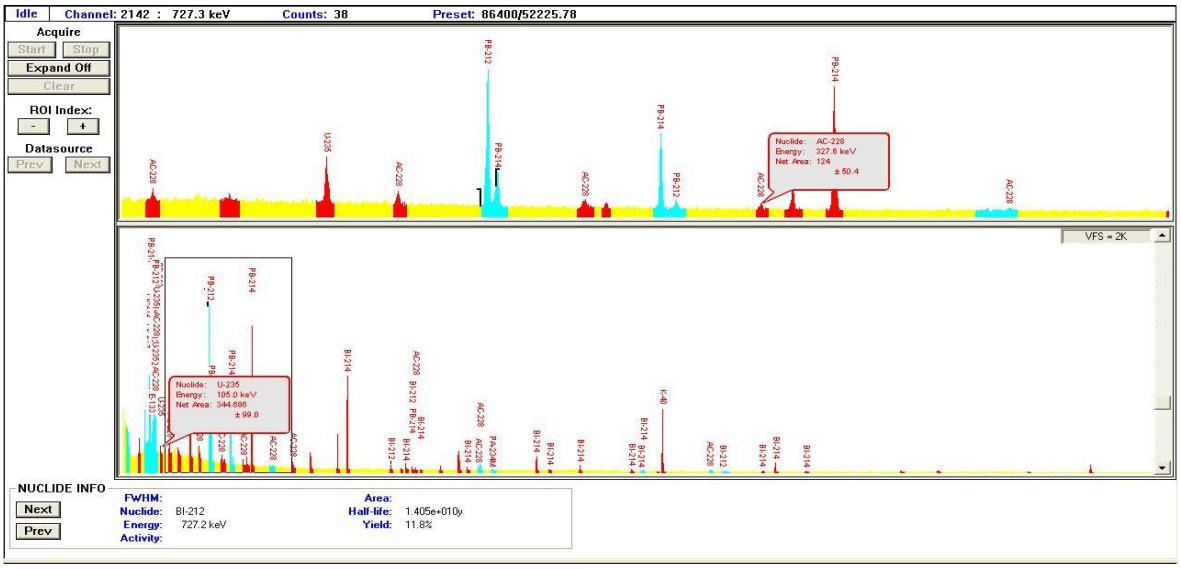

Fig. 4. A spectrum of activity of radionuclides in soils of Sabirabad and Kurdamir

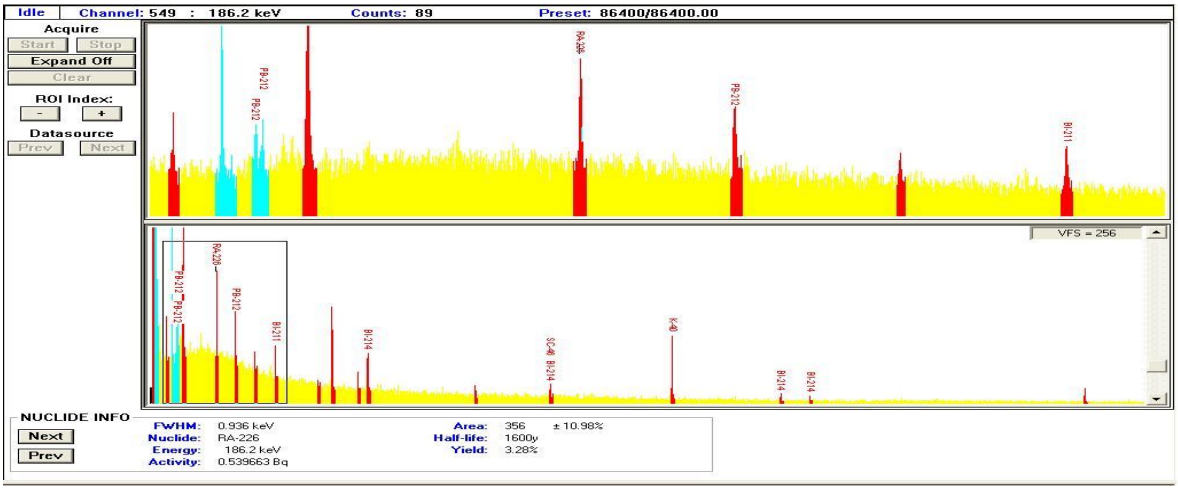

Fig. 5. A spectrum of activity of radionuclides in soils from vicinities of Bina

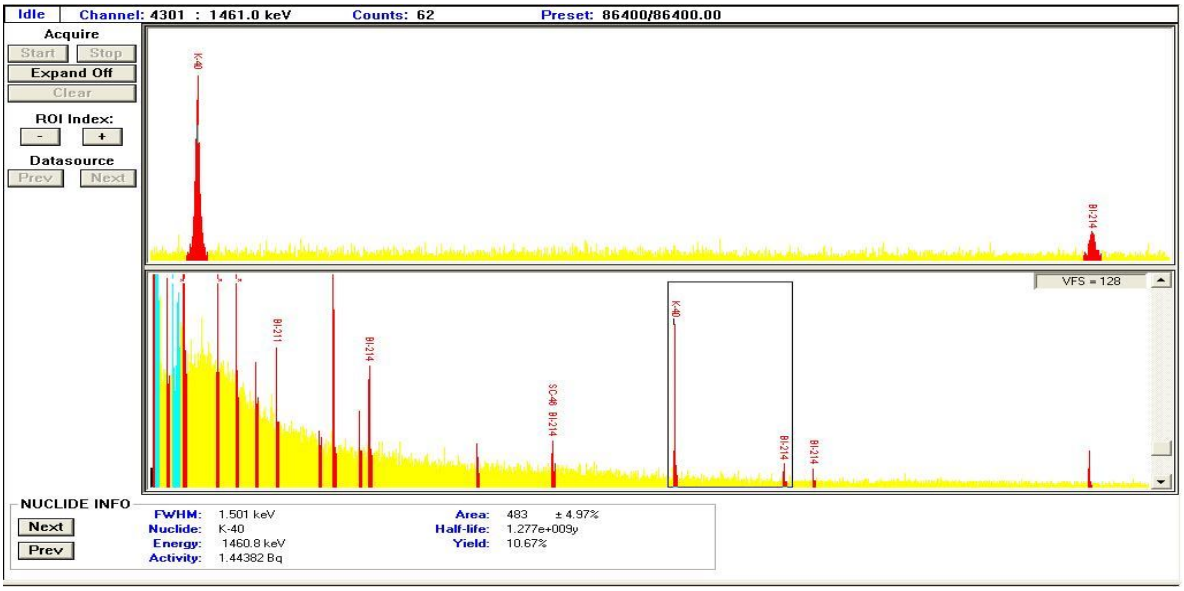

Fig. 6. A spectrum of activity of radio nuclides in soils from vicinities of Baku - the Airport

Table 14. Radiating activity of elements in investigated samples

Soil	Sample 1	Sample 2	Sample 3
The name radionuclide	B^{212}	K^{41}	B^{214}
Energy radionuclide	727.2 keV	1460.4 keV	628.3 keV
Radiating activity	-	1.47844 Bk/g	0.176622 Bk/g
Radiatsionno-chemical exit	11.8%	10.57%	46.7%
The peak area with a margin error	-	451 5.04%	208 11.37%
Mass	170 mg	110mg	155 mg
Specific activity	-	-	-
Width of semiheight	-	1.726 keV	1.205 keV

On the basis of the received data it is possible to ascertain that depending on degree of impurity of district where catching, milking venom, sampling of vegetation and soils fluctuations in the maintenance of heavy metals has been marked. However radiating activity of elements in snake venom and soils did not change almost. Natural radio nuclides of low activity again have been defined in samples of snake venom (table12-14). *The* revealed activity of an element of uranium in a spectrum of Venom grows out of a radiating background which is formed by environmental influence. Therefore protection of environment from technogenic pollutants on value ranks now with such problems as protection of soils. Thus, influence of ecological factors (biotic, abiotic), on biochemical indicators, pharmacological and toxicological properties of Venom Caucasian Viperas is studied and optimum doses of radiating sterilization of Venom are established. The method atom-absorbtcnion spectrophotometry (AAS-300, Perkin-Elmer) in vipera venom, caught from ecologically polluted sites of Absheron, defines the maintenance of heavy metals-pollutants. As a result Chemical compound changes, and also influence of heavy metals on protein structure, fermentative activity and other biochemical indicators of vipera venom are established. Influence of the metals-pollutants, ionising radiation and electromagnetic radiation on biochemical parametres of vipera venom , on fermentative activity and toxicity of venom have been experimentally revealed. Doses - radiations have been established and revealed for sterilisation of both snake venom, and its water solutions. Influence of ecological factors (biotic, abiotic), on biochemical indicators, pharmacological and toxicological properties of Venom Caucasian vipera has been studied and optimum doses of radiating sterilization of venom have been established. The method of atom-absorbtion spectrophotometry (AAS-300, Perkin-Elmer) in vipera venom, caught from ecologically polluted sites of Absheron, defines the maintenance of heavy metals-pollutants. On the basis of the received data it is possible to ascertain that depending on degree of impurity of district where catching, milking of snakes, vegetation sampling has been spent and soils fluctuations in the maintenance of heavy metals have been marked. However. Radiating activity of elements in snake Venom and soils didn't change almost. Thus, in comparative aspect of influence of ecological factors on venom of Transcaucasian viperas has been revealed. Concentration of heavy metals in venom of vipera, caught in various biotopes of Azerbaijan, and also in vegetative and soil tests have been defined. As a result chemical compound changes, and also influence of heavy metals on protein structure, fermentative activity and other biochemical indicators of venom of viperas have been established. The revealed values will be applied at preparation of preparations on the basis of venom of snakes. It will be in turn recommended for pharmaceutical industry by manufacture of injections on the basis of venom of snakes. Thus influence of metals-pollutants, ionizing radiation and electromagnetic radiation on biochemical parameters of venom of vipers, on fermentative activity and toxicity of venom have been experimentally revealed. Doses of -radiation for sterilization both of venom of snake, and its water solutions have been established and revealed. The revealed values can be applied at preparation of preparations on the basis of snake venom. It can be recommended for a pharmaceutical industry at manufacture of injections

on the basis of venom of snake. The effect of environmental pollution on Caucasian Macrovipera Lebetina Obtusa venom has been studied. It has been revealed that pollution by radioactive substances could be considered as a new abiotic factor on products of snake venom gland. İt is established by method of atom-absorbing spectrometry that the maintenance of heavy metals in venom of snake changes depending on degree of impurity of district of dwelling and corresponds: Pb (49.13-134.9), Zn (360.8-863.6), Cd (1.6-1.9) mg/kg. Natural radioactive elements in investigated samples - isotopes Ra^{226}, Ra 228 and K^{40} have been revealed. Insignificant activity of isotope Ra ^{228} and Ra^{226} in investigated samples is defined, minimum activity was 0.174 Bk/g and 2.48 Bk/g. Minimum activity of isotope Ra^{226} was in limits 0.539663Bk/g. The minimum activity of isotope K^{40} was 1.44382Bk/g and activity of other isotopes was insignificant.

4.CONCLUSIONS

- It has been established by method of atom-absorbing spectrometry that the maintenance of heavy metals in venom of snake changes depending on degree of impurity of district of dwelling and corresponds: Pb (49.13-134.9), Zn (360.8-863.6), Cd (1.6-1.9) mg/kg.
- Natural radioactive elements -isotopes Ra^{226}, Ra ^{228} and K^{40} have been revealed in investigated samples of venoms of snakes taken from polluted areas.
- Minimum concentration of radionuclides Ra ^{228} and Ra^{226} in investigated samples of snake venom has been defined as 0.174 Bk/g and 2.48 Bk/g.
- Minimum radiating activity of isotope Ra^{226} in snake venom was in limits 0.539663Bk/g. The minimum radiating activity of isotope K^{40} was 1.44382Bk/g

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