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The impact of environmental pollutants on viper venom *Macrovipera lebetina obtusa*

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Abstract
By the method of atomic absorption spectrometry, the trace element composition of venom *Macrovipera lebetina obtusa*, from different regions of Azerbaijan has been studied. Ions - Cr, Pb, Cd, Zn was revealed in venom of snake. The metal concentrations in the venom of snakes fluctuate within: Cr - (87.0± 0.049 - 103.1±2.793) mg / kg; Pb - (7.01±1.321 - 134,8±1.46) mg / kg; Cd - (1,6±0.177 - 24.2±0.985) mg/kg; Zn -(600.89±4.266 - 860.9 ± 0.129) mg/kg, respectively. γ – Radiospectrometric studies have shown that the venom samples contain radionuclides Ra ^{228} (0.08-0.174 mBq/g), Ra^{226} (0.35-2.48, mBq / g) K^{40} (1.35-23.4 Bq/kg) and Cs^{137} (MDA = 0.315. It was found that, in heterojunction "snake venom - p-InSe» viper venom be haves as a semiconductor p-type conductivity.

Keywords: *Macrovipera lebetina obtusa*, venom, heavy metals, γ-irradiation.

1. Introduction
The snake venom is a complex set of biologically active compounds. Integral part of venom, responsible for its toxic properties, consists of biologically active components of protein origin, including various enzymes [1, 2, 6-8].
Toxicity and specificity of action of snake venoms are mainly determined by the singularity of their constituent bioactive substances.
Considerable number of research works was devoted to the comprehensive study of the venom of venomous snakes [3-5, 9, 10]. However, there are some gaps in addressing of influence of ecological pollutants on the viper venom which require further theoretical and experimental studies [11].
Insignificant degree of a level of scrutiny of venom transcaucasian viper *Macrovipera lebetina obtusa* as representative of fauna of Azerbaijan being the large supplier of this invaluable medicinal raw material for a pharmaceutical industry has served as the precondition of its research.
They are the most dangerous toxic elements for fauna and animals and the people by entering in the basic biological circulation of substances.
Atmospheric pollution by industrial waste, including heavy metals, oils and other contributes to environmental deterioration. Transcaucasian viper *Vipera lebetina obtusa*, living on the contaminated territories of Azerbaijan, is adversely affected by environmental factors, and this is reflected in the quality and composition of the venom.
Studying of the given problem is the actual problem which has important value for medicine, biology and ecology.
The purpose of research influence the impact of environmental contaminants (heavy metals, radiation), electromagnetic radiation on the viper venom and study of the spectral characteristics of the venom under the influence of environmental factors.

2. Materials and Methods: By the method of atomic absorption spectrometry, the trace element composition of venom of *Macrovipera lebetina obtusa* was studied. Research of venom viper venom has been conducted on samples collected in the spring period 2010-2015 years. Metal ions (Cd, Ni, Zn and Pb) of venom samples at an excitation wavelength of 337.1 nm at a temperature range equal to 300K, and 77K by laser spectroscopy was studied. By radiothermoluminescence method, molecular mobility of venom irradiated at doses between 2 × 103 Gy and 104 Gy in air revealed changes was studied.

3. Results and Discussions: In this paper we studied the trace element composition of venom of *Macrovipera lebetina obtusa*, captured from different regions of Azerbaijan, differing in degree of contamination of man-made industrial emissions. By the method of atomic absorption spectrometry, metal ions Cr, Pb, Cd and Zn were revealed in samples of vipera venom (table 1).

It is shown that the metal concentrations in the venom of snakes fluctuate within: Cr - (87.0 ± 0.049 - 103.1 ± 2.793) mg / kg; Pb - (5.01 ± 0.285– 18.52 ± 1.758) mg / kg; Cd - (1.6 ± 0.177 – 5.42 ± 0.985) mg / kg; Zn - (250.0 ± 3.063 - 287.6 ± 8.402) mg / kg, and the content of metal of *Vipera* venom of different regions differ significantly. In this case, all the samples of the venom of *Viperas* caught from different regions of the Republic, have a certain amount of lead and zinc ions; Cr is present in the venom of samples of snakes of Sabirabad and Agsu and, and Cd were detected in samples of the venom of *Vipera* of Gobustan and Shamakhi regions. It was revealed that the concentrations of metals in the venom correlate with their content in soils.

Table 1: Data on the content of heavy metals in the samples, from the territory of Azerbaijan

Samples	The concentration of heavy metals in mg/kg(M±m)				
	Cr	Pb	Cd	Ni	Zn
Gobustan region (village Childag)					
plant	131.0 ± 1.300	23.0 ±4.480	2.05 ±0.058	37.99 ± 0.160	70.04 ± 0.091
soil	89.9 ±0.438	5.5 ± 0.079	0.70 ±0.001	35.15 ± 0.542	52.7 ± 0.049
venom		13.39 ± 0.033	1.9 ± 0.200		266.9 ± 0.034
Shamakhi district (village Maraza)					
plant	130.0 ± 1.20	20.9 ±3.480	1.99 ±0.038	39.36 ± 0.500	70.03 ± 0.090
soil	80.2±0.356	4.9 ± 0.030	0.53 ±0.025	34.15 ± 0.678	52.20 ± 0.071
venom		13.37 ± 0.029	1.6 ± 0.177		263.7 ± 0.027
Sabirabad region (village Karatugay)					
plant	66.5 ±1.290	4.9 ± 0.090	1.0 ± 3.480	40.4 ±0.670	28.01 ±0.040
soil	100.4 ±0.556	7.3 ± 0.027	0.6 ± 0.030	35.6 ±0.798	98.0 ±0.088
venom	87.0 ±0.049	8.70 ±0.030			269.0 ±0.076
Sabirabad region (village Shyhsalahly)					
plant	87.0 ±0,990	4.9 ± 0.487	0.5 ± 0.589	10.7 ±0.133	26.05 ± 0.440
soil	90.6 ±0.670	10.0 ±0.567	0.5 ± 0.131	33.9 ±0.228	67.09 ± 0.344
venom		5.01 ±0.285			260.9 ± 0.129
Agsu (village.Garagoyunlu)					
plant	153.0 ±1.316	8.5 ± 4.695	5.8 ± 0.063	33.7 ± 0.1685	69.02±0.050
soil	56.6 ± 0.459	9.5 ± 0.073	1.8 ± 0.004	28.0 ± 0.658	71.08±0.020
venom	103.1 ± 2.793	11.3 ± 6.560	5.42 ± 0.985		250.0±3.063
Neighborhood of the city of Baku (settlement Buzovna)					
plant	90.84 ± 3.080	8.17 ± 0.154	0.42 ± 0.010	25.80 ± 0.362	10.07 ± 2.14
soil	58.25 ± 0.60	52.08 ± 2.094	1.05 ± 0.042	20.12 ± 0.765	42.2 ± 6.418
venom		18.52 ± 1.758			260.89 ± 4.266
Neighborhood of the city of Baku (settlement Bina)					
plant	71.64 ± 3.01	7.80 ± 0.199	0.70 ± 0.028	40.42 ± 0.352	142.14 ± 0.81
soil	93.25 ± 3.58	11.05 ± 0.18	0.45 ± 0.048	37.78 ± 0.449	99.7 ± 0.269
venom		13.86 ± 2.36			274.7 ± 8.604
Neighborhood of the city of Baku(around the airport)					
plant	133.4 ± 2.528	22.6 ± 1.379	2.07 ± 0.064	40.36 ± 0.307	101.4 ± 0.303
soil	83.2 ± 4.842	5.6 ± 0.154	0.6 ± 0.007	36.15 ± 0.275	53.22 ± 0.098
venom		18.48 ± 1.46	1.8 ± 0.949		264.89 ± 3.99
Neighborhood of Sumgait					
plant	29.0 ± 7.714	16.9 ± 0.228	1.2 ± 0.039	39.0 ± 0.336	114.0 ± 0.034
soil	29.0 ± 1.888	16.8 ± 0.055	1.1 ± 0.056	38.0 ± 0.150	65.0 ± 0.410
venom		27.0 ± 1.321			287.6 ± 8.402

γ – radiospectrometric studies have shown that the venom samples also contain radionuclides as Ra^{228}, Ra226, K40 and 137 Cs, which are the specific activities of 228 Ra (0.08-0.174 mBq / g), Ra^{226} (0.35-2.48, mBq / g) K^{40} (1.35-23.4 Bq/kg), Cs^{137} (MDA = 0.315, respectively. Analysis of the data shows that the venom of *Viperas* caught from different regions of the Republic, are almost indistinguishable for the content of radionuclide's. Thus K^{40} is present in larger quantities in all samples of venom. All samples of the venom have a lower content of Ra^{228}.

In this paper by the method of EPR, electron paramagnetic spectra of venom at different temperatures was studied. EPR spectra of typical venom of vipera was revealed at amount of power at 1.58 mVt microwave field and the magnetic field strength in the range of 330 - 340 mTl (fig.1).

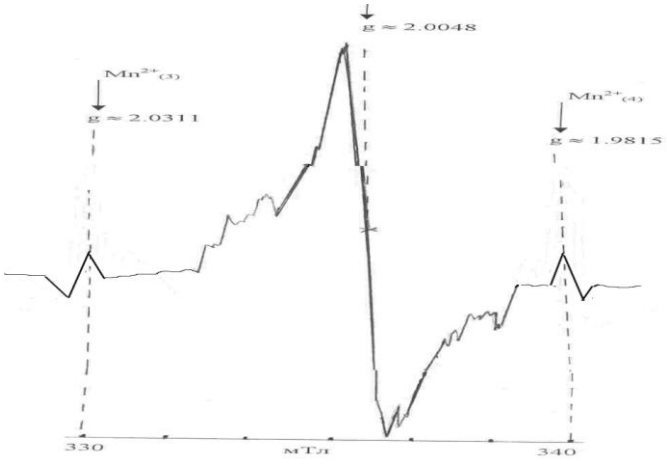

Fig 1: Spektr EPR the venom at T = 293 K

The revealed hyperfine structure, which is under the given temperature conditions symmetrical singlet with a g-factor of 2.0048, can be applied to identify the venom of vipera and its toxins. Thus, the EPR method opens up broad prospects for qualitative and quantitative determination of the whole venom and its components, as well as the possibility of applying this method in toxicology analysis for identification of snake venom toxins was identified.

By laser spectroscopy, spectral-luminescent characteristics of venom of *Vipera* inhabiting different degree of contamination areas of Azerbaijan were investigated. The photoluminescence peaks were identified in a snake venom samples at 520 nm and 400-500 nm at a temperature of 300K, and the maxima 440 and 470 nm at a temperature of 77K, characteristic ions of cadmium, zinc and lead (fig.2, 3).

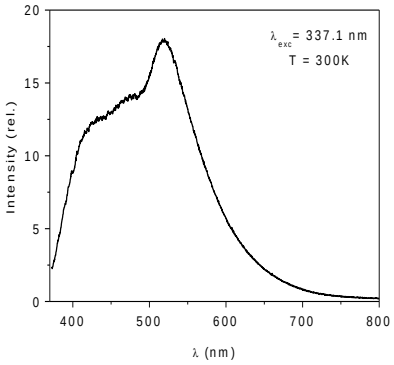

Fig 2: Spektr FL venom of viper at T = 300 K

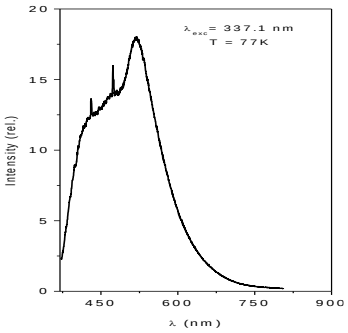

Fig 3: Spektr FL venom of viper at T = 77 K

We can assume that these data can be used in the identification of zootoxins and their metabolites, and these criteria can serve as a theoretical basis for the development of effective methods for diagnosis of poisoning zootoxins. For the first time, heterojunction monoselenide indium p-type conductivity and crystal viper venom (venom-p-InSe) established and it has been found that heterojunction in zootoxins behaves like a p-type semiconductor conductivity. It was found that studied heterojunction has significant photosensitivity in IR spectral range. The results of these studies can extend the range of materials used for the manufacture of photodetectors for the near infrared spectral range, and consider the use of snake venom in the instrument as a photodetector.

Summarizing the results obtained from the IR studies, it was concluded that the exposure to EMR low intensity within the 1000-7000 Vt/m^2 structural changes were not observed in samples of snake venom. However, when exposed to venom of EMR (high intensity 14000 - 20000 Vt/m^2) in the IR spectra shift of the absorption bands, which is undoubtedly due to structural changes in the protein molecule zootoxins are observed. Thus significant changes occur in the total amount of protein compared to venom specimens subjected to a low intensity electromagnetic radiation.

We believe that the data obtained may be used for authentication and quality characteristics of venom and preparations based thereon.

It has been established that the radiation dose (up to 1.35 kGy dose) for 3 minutes did not cause structural changes in the samples venom of *vipera*, but rather contribute to the stabilization of both toxicity and pharmacological activity while increasing the shelf life of aqueous solutions of *vipera* venom. At high doses (2.7, 4.05 and 5.4 kGy) γ-irradiation for 3 minutes there is a gradual decrease in toxicity (pharmacological activity of enzymes) of snake venom. We recommend that these facts should have been taken into account in the radiation sterilization of snake venom and drugs based on it.

By radiothermoluminescence method in the molecular mobility of γ - venom irradiated at doses between 2×10^3 Gy and 10^4 Gy in air revealed changes (fig.4).

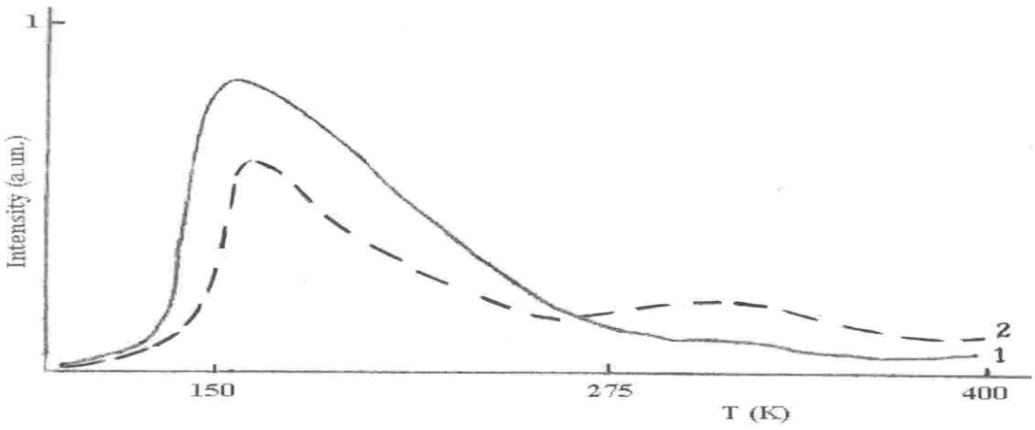

Fig 4: Spectrum radiothermoluminescence venom of *Macrovipera lebetina obtusa* (1 - 10^3 Gy, 2 – 10^4 Gy).

Study of the influence viper venom irradiated radioactive, electromagnetic radiation and treated at different temperatures, life expectancy of experimental animals have shown that with increasing doses of radiation, the intensity of electromagnetic radiation and the heating temperature of the samples, a decrease toxicity of the venom are observed, which is manifested in the increase in life expectancy experienced groups of mice. Furthermore, γ- irradiation followed by heat treatment leads to a significant reduction of toxicity up to complete neutralization of the venom. These facts should be considered in storage and preparation of drugs based on snake venom.

Thus atmospheric pollution industrial waste, in turn, adversely affects the physical and chemical properties of snake venom. Transcaucasian viper Vipera lebetina obtusa, living on the contaminated territories of Azerbaijan, is adversely affected by environmental factors, and this is reflected in the quality and composition of the venom. All these data should be considered in the production of drugs based on snake venom. The results can be used for identification and authentication of snake venom toxins.

4. Findings

1. It is shown that the basic metal ions present in the venom *Macrovipera lebetina obtusa* are Cr, Pb, Cd, and Zn. The concentration of these metals in the venom of snakes correlated with their content in soils: Cr - 87.0 ± 0.049 - 103.1 ± 2.793; Pb - 5.01 ± 0.285 – 18.52 ± 1.758; Cd – 1.6 ± 0.177 – 5.42 ± 0.985; Zn - 250.0 ± 3.063 -287.6 ± 8.402 mg / kg.
2. It is shown that Pb and Zn in standard samples present in snake venom concentrations 1.92 ± 0.01 and 180.0 ± 0.05 mg / kg, respectively, and Ni has only background concentrations.
3. The conclusion is made that electromagnetic radiation with a high intensity is a factor affecting the toxicity of snake venom.
4. The method by irradiation of laser spectroscopy venom samples at an excitation wavelength of 337.1 nm and the laser pulse duration 10 ns appearance of maxima of the photoluminescence at 520 nm and 400-500 nm at a temperature range equal to 300K., As well as maxima 440 and 470 nm at 77K due metal ions (Cd, Ni, Zn and Pb), which may be used to identify venom toxins and can be recommended for forensic analysis cadaveric material.
5. It was found that? in heterojunction "snake venom - p-InSe» viper venom behaves as a semiconductor p-type conductivity, thus expanding the range of materials used for the manufacture of photodetectors for near-infrared spectral range.
6. Method radiothermoluminescence detected changing the molecular mobility of viper venom after being exposed to γ-radiation doses 104Gr air. It was established that the irradiated samples viper venom have a characteristic asymmetric curve radiothermoluminescence with a maximum emission at 155K (-3.15mV) and c slowly decaying shoulder to a temperature of 300K. Revealed that with increasing dose intensity of the peak at 155 - 160K RTL reduced. We assume that these results can be used for study of radiation resistance viper venom, and may be used in the identification zootoxins and their metabolic products.

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