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INFLUENCE OF ENVIRONMENTAL POLLUTANTS TO THE VENOM OF *MACROVIPERA LEBETINA OBTUSA*

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Abstract: It has been studied element content of venom of *Macrovipera lebetina obtusa*, taken from different regions of Azerbaijan by atomic absorption spectrometry method. It was revealed Cr, Pb, Cd and Zn ions in the samples of venom. The metal concentrations in the snakes venom are within: Cr – 87.0 ± 0.049 - 103.1 ± 2.793 mg/kg; Pb – 5.01 ±0.285– 19.0 ± 1.321 mg/kg; Cd –1.6 ± 0.177 – 2.42 ± 0.985 mg/kg; Zn – 250.0±3.063 - 377,6 ± 8.402 mg/kg. γ –radiation analysis have shown that the venom contains radionuclides ^{228}Ra (0.08-0.174 Bq / kg), ^{226}Ra (0.35-2.48, Bq/kg), ^{40}K (1.35-23.4 Bq/kg), ^{137}Cs (MDA = 0.315). It was revealed by laser spectroscopy method, that venom may be used to identify venom toxins and can be recommended for analysis of material.

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

1. Introduction

The snake poisons is a complex set of biologically active compounds. Integral part of venom responsible for its toxic properties, are biologically active components of protein origin, including various enzymes [1, 2, 6, 7, 8].

Toxicity and specificity of action of snake venoms are mainly determined by the singularity of their constituent bioactive substances.

The comprehensive study the venom of venomous snakes devoted considerable number of research works [3, 4, 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.

Purpose of research is to study the impact of environmental contaminants (heavy metals, radiation), electromagnetic radiation on the viper venom, study of the spectral characteristics of the poison under the influence of environmental factors.

2. Experimental

In this paper 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 was studied. By the method of atomic absorption spectrometry, metal ions Cr, Pb, Cd and Zn was revealed in samples of viper venom (Table 1)

Table 1. The content of heavy metals in the samples

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	2.42 ± 0.985		250.0±3.063
Settlement Buzovna, Baku city					
plant	90.84 ± 3.080	8.17 ± 0.154	0.42 ± 0.010	25.80 ± 0.362	100.7 ± 2.14
soil	58.25 ± 0.60	52.08 ± 2.094	1.05 ± 0.042	20.12 ± 0.765	142.2 ± 6.418
venom		18.52 ± 1.758			300.89 ± 4.266
Settlement Bina, Baku city					
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			354.7 ± 8.604
Around the airport, Baku city					
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		364.89 ± 3.99
Sumgait city					
plant	29.0 ± 7.714	26,9 ± 0.228	1,2 ± 0.039	19,0 ± 0.336	214,0 ± 0.034
soil	29.0 ± 1.888	26,8 ± 0.055	1,1 ± 0.056	23,0 ± 0.150	65,0 ± 0.410
venom		19,0 ± 1.321			377,6 ± 8.402

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 metals of viper venom of different regions differ significantly. In this case, all the samples of the venom of vipers 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 was detected in samples of the venom of viper of Gobustan and Shamakhi regions. It was revealed that the concentrations of metals in the venom correlate with their content in soils.

γ – radiospectrometric studies have shown that the venom samples also contain radionuclides as Ra^{228}, Ra^{226}, K^{40} and Cs^{137}, which are the specific activities of 228 Ra (0.08-0.174 Bq/kg), Ra^{226} (0.35-2.48, Bq/kg) K^{40} (1.35-23.4 Bq/kg), Cs^{137} (MDA = 0.315), respectively. Analysis of the data shows that the venom of vipers 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 were studied. EPR spectra of typical venom of viper 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).

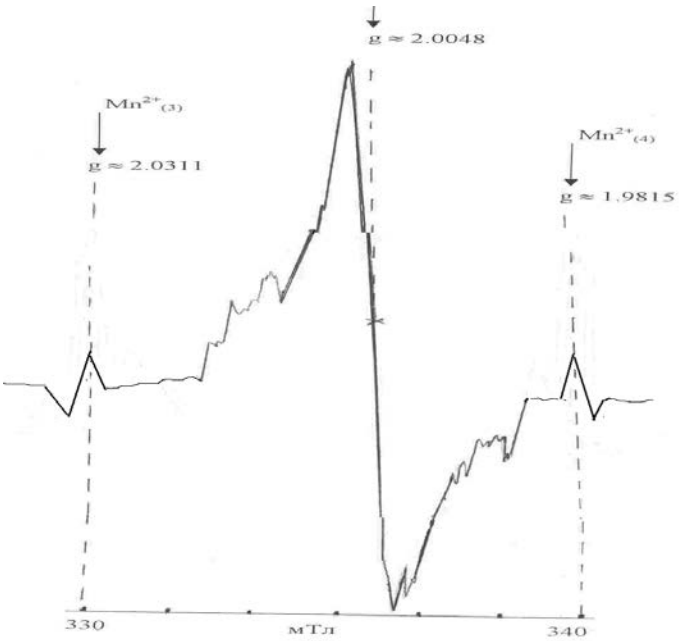

Fig.1. EPR spectrum of the venom at T = 293 K

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 was investigated. The photoluminescence peaks are 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, Fig.3).

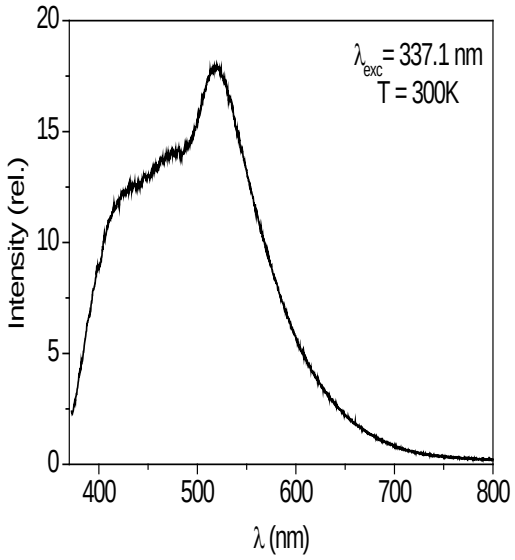

Fig.2. FL spectrum of the viper venom at T = 300 K

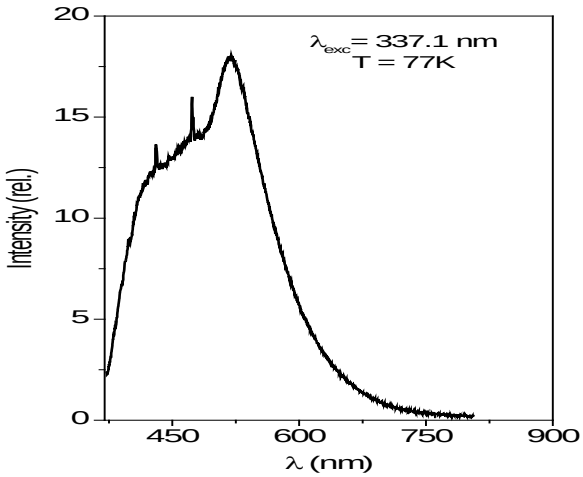

Fig.3. FL spectrum of the viper venom at T = 77 K

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 was 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 was 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).

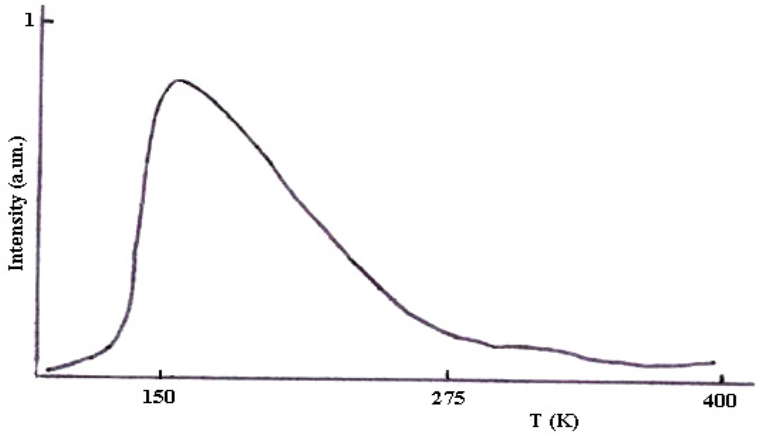

Fig.4. Radiothermoluminescence spectrum of venom of Macrovipera lebetina obtuse

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.

3. Results

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– 19.0 ± 1.321; Cd –1.6 ± 0.177 – 2.42 ± 0.985; Zn – 250.0±3.063 - 377,6 ± 8.402 mq/kq.

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.

1. The conclusion is made that electromagnetic radiation with a high intensity is a factor affecting the toxicity of snake venom.

2. 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.

3. 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.

4. Method radiothermoluminescence detected changing the molecular mobility of viper venom after being exposed to γ-radiation doses 104Gr. Is 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 to study radiation resistance viper venom, and may be used in the identification zootoxins and their metabolic products.

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ВЛИЯНИЕ ЭКОЛОГИЧЕСКИХ ФАКТОРОВ НА ЯД ГЮРЗЫ *MACROVIPERA LEBETINA OBTUSA*

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Резюме: Изучен элементный состав яда гюрзы взятых из различных регионов Азербайджана методом атомно-абсорбционной спектрометрии. Были выявлены ионы Cr, Pb, Cd и Zn в образцах яда. Концентрации металлов в яде змеи были в пределах: Cr - 87,0 ± 0,049 - 103,1 ± 2,793мг/кг; Pb - 5,01 ± 0.285- 1.321 ± 19,0 мг/кг; Cd -1.6 ± 0,177 - 2,42 ± 0,985 мг/кг; Zn - 250,0 ± 3.063 - 377,6 ± 8,402 мг/кг. Анализы γ-излучения показали, что яд содержит радионуклиды ^{228} Ra (0.08-0.174 Бк /кг), ^{226}Ra (0.35-2.48,Бк/кг), ^{40}K (1.35-23.4Бк/кг), ^{137}Cs (MDA=0,315). Методом лазерной спектроскопии, был определен, что яд может быть использован для идентификации токсинов яда и может быть рекомендован для анализа материала.

Ключевые слова: *Macrovipera lebetina obtusa*, яд, тяжелые металлы, γ-излучение.

EKOLOJİ FAKTORLARIN *MACROVİPERA LEBETİNA OBTUSA* İLAN ZƏHƏRİNƏ TƏSİRİ

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Xülasə: Azərbaycanın müxtəlif bölgələrindən götürülmüş *Macrovipera lebetina obtusa* ilan zəhərinin element tərkibi atom absorbsiya spektroskopiyası üsulu ilə öyrənilmişdir. Bu zəhər nümunələrində Cr, Pb, Cd və Zn ionları aşkar edilmişdir. İlan zəhərinin tərkibində metalların konsentrasiyası Kr - 87.0 ± 0.049 - 103.1 ± 2,793 mq/kq; Pb - 5.01 ± 0.285- 19.0 ± 1.321 mq/kq; Cd -1.6 ± 0,177 - 2,42 ± 0.985 mq/kq; Zn - 250.0 ± 3,063 - 377,6 ± 8,402 mq/kq olmuşdur. γ -radiasiyanın təhlili göstərmişdir ki, zəhərin tərkibində aşğıdakı radionuklidlər vardır: ^{228}Ra (0.08-0.174 Bq / kg), ^{226} Ra (0.35-2.48, Bq / kg), ^{40}K (1.35-23.4 Bq / kg), ^{137}Cs (MDA = 0,315). Lazer spektroskopiya metodu ilə müəyyən edilmişdir ki, zəhər toksinlərin identifikasiyası üçün istifadə edilə bilər və materialların təhlili üçün tövsiyə edilə bilər.

Açar sözlər: *Macrovipera lebetina obtusa*, zəhər, ağır metallar, γ-şüalanma.