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Investigation of influence of pollution of the biosphere of Azerbaijan on the physico-chemical properties venom of snake Macrovipera lebetina obtusa

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Abstract

As a result of investigation development of geological ecology of snakes we can predict venom properties practically from any region, to make a catch of snakes in certain populations and necessary level of ferment activity. Progressive pollution of biosphere of Azerbaijan by technogen waste products lead to its significant saturation by toxic chemical elements, including heavy metals (Ni 41-142, Pb 21-38, Cr 89-213, Zn 18-147, Cu 20-51, Cd 0.8-4.2 mg/kg). Comparative study of metal concentration in venom composition and soil cover allows to establish the correlation between pollution and toxic composition in venom in natural conditions and in captivity. Thus, to explain the relationship to soil ecology conditions of venom property change one can pay attention on elementary composition of zootoxins and toxin content in given specific conditions.

Key words: Macrovipera lebetina obtusa, venom, luminescence, ecology, heavy metals

1.Introduction.

The intensive studies on venom led to attraction of attention of some authors on variation of venom features obtained from the same species. There are poor and discrepant literature information on dependence of zootoxin's features on various habitat conditions of venomous snakes [1,2,7,8].

The problem of pollution of an atmosphere, including ground and vegetative cover, by toxic substances and heavy metals (HM) has arisen with receipt in biosphere technogenese discharges of the industrial enterprises, oil refining, oil-extracting, chemical, metallurgical, building and power enterprises of Azerbaijan becoming one of its constant and irreversible components, and their study - ecological factor of an environment [4,5].

In connection with increasing studies on venom, the more and more wide scope is accepted by researches of properties of zootoxins, including venom of Macrovipera lebetina obtusa depending on the various ecological factors [1,6,9].

At the present stage of development of a human society increase speed of urbanization and industrialization has a large influence on biosphere. Chemicalization of an agriculture is accompanied by a large amount of atmospheric emissions.

Half-willow of agricultural cultures by waste water, manufacture of petroleum, entering under agricultures of organic fertilizers rich of toxic elements (cadmium), phosphoric fertilizers rich of impurity of uranium and lead, application of pesticides containing certain amount of mercury cause gradual increase of pollution of soil. At waste water irrigation of soil the pollution by heavy metals (HM) and natural radioactive elements occurs. It is necessary to note that the gross forms of HM are difficulty soluble in water and do not represent direct threat to an environment. However they are potential sources of pollution, which as a result of long interaction with components of soil environment can partially pass in the mobile forms and increase the contents of HM in a vegetative part and transferred to an animal and man by the food circuits [3].

2. Materials and the methods of study

Materials research was the standard poison taken from the snakes in herpetology center and venom taken from snakes Macrovipera lebetina obtusa captured from different areas Azerbaijan.

The methods 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.

Study of fluorescent properties and photoluminescence spectra viper venom was performed by laser spectroscopy with a spectrometer SDL-1

3. Results and their discussion

For recent years progressive pollution of biosphere of Absheron peninsula of Azerbaijan by technogen waste products lead to its significant saturation by toxic chemical elements, including heavy metals as: Pb,Hg,As,Cd,Cr,Ni,Sn,Cu and etc.

Being in regular interaction with the atmosphere and hydrosphere, soil cover takes and gives up suspended or solute substances assimilated by plants during its growth, and subsequently by biological edible chains pass to animal and man.

Absheron peninsula is different from high variety of flora and fauna including reptile. As an object for determination of space propagation regularities, vertical migration, accumulation and behavior in system of soil-plant, character and intensity of trace element inclusion into biological edible chains Macrovipera lebetina obtusa have been selected. Such selection is due to venom composition of Vipera which contains the following metals: Cd, As, Cu, Ni, Hg, Pb, Zn, Cr. For the present there is no data on influence of pollution of the biosphere of Azerbaijan on the physico-chemical properties venom of snake Macrovipera lebetina obtusa and this problem is very high-priority one.

Investigation results have been given on geochemical ecology of Viperas on which basis one can practically forecast properties of zootoxins from any region, to trap Viperas in certain populations and to produce venom with the given toxicity and necessary level of fermentative activity.

Development of ecochemical maps offers scope for establishment of correlation between the content of heavy metals in soil and quality of produced venom by Viperas.

For the recent several ten years all progressing pollution of biosphere by technogen discharge of industrial, energy, chemical, oil-refining entertainment and by motor transport lead to its significant saturation by toxic chemical and radioactive elements including heavy metals such as: lead, mercury, cadmium, chromium, nickel, tin, zing and etc.

Pollution of biosphere by technogen discharge leads to formation of ecogeochemical anomalies, which sizes increase with the intensification of work of industrial and energy complexes and motor transport. As a result of it there have been found local anomalies containing uprated qualities of heavy metals (HM) in soil, water and plants exceeding multiply their maximum permissible concentration (MPC).

Top-soil being in constant interselation with atmosphere and hydrosphere takes and gives them neighted or soluted substances which are assimilated by plants during the processes of its development and growth and subsequently through biological edible chains pass to animals and human beings.

From above-mentioned study of regularities of space spread, vertical migration, accumulation and behavior in soil-plant system, character and intensity of content of HM in biological edible chains are very important for forecasting and production of most valuable Macrovipera lebetina obtusa venom with given toxinity and high level of fermentative activity.

Study of chemical composition of soil and plants of Absheron peninsula, making up ecogeochemical maps on this base, brought to local anomalies of certain population of venomous Viperas allows to trap them and produce venom with necessary pharmacological properties.

Table 1.

Content of total forms of heavy metals (mg/kg) in soil (0÷20 cm) of Absheron peninsula

Soil	Sample	PH	Ni	Pb	Cr	Zn	Cu	Cd
Grey-brown virgin soil and flat plant	Baku city, 5 km to the west from the industrial zone, 11 km away from city boundaries							
	1	7.4	141	28	213	132	31	2.5
	Baku city, 25 km to the west from the industrial zone, 31 km away from city boundaries							
	2	7.2	98	38	129	111	48	0.9
	3	7.7	82	36	106	147	31	1.1
	4	7.7	75	34	109	114	28	1.1
	Baku city, 30 km to the west from the industrial zone, 36 km away from city boundaries							
	8	7.8	44	26	110	101	25	1.0
	9	7.9	47	29	110	91	28	1.4
	10	7.9	42	30	150	86	21	1.2
	11	8.0	46	37	140	69	29	1.2
	12	7.6	41	30	89	72	20	0.8
	13	7.8	44	21	110	69	25	0.8
	Baku city, 10 km to the west from the industrial zone, 20 km away from city boundaries							
Grey-brown sowings and flat plant	14	7.5	141	48	212	18	50	4.2
	15	7.5	142	39	192	66	51	2.3
	16	7.5	103	47	168	54	48	2.2
	17	7.4	90	36	144	49	46	3.1
	18	7.3	97	76	140	45	45	3.7

For determination of real level of HM in soil and plants there have been carried out field and reconnaissance investigations on which key sections are marked. Soil specimen, plant samples are selected from these sections. Venomous Viperas have been traped simultaneously for establishment of correlation between the content of HM in soil and venom of Vipera trapping is carried out in neighborhood of villages: Nardaran, Bilgah, Mashtagi, Fatmai, Buzovna and etc.

By the method of atomic absorption in soil specimen content of HM: lead, mercury, nickel, arsenic, tin, zinc, copper, manganese, cobalt, molybdenum and vanadium have been determined (Table 1).

Obtained results of analysis show that in soils of this region being under the constant effect of technogen discharge, significant concentration of HM exceeds multiply MPC in upper layer of soil (0÷20 cm).

On the base of soil classification in content of weight forms of HM maps of: lead, cadmium, nickel, arsenic, tin, zinc, copper, manganese, cobalt, molybdenum and vanadium have been made.

There have been distinctly traced areas of HM scattering of contamination sources connected with direction of the wind on Absheron peninsula (rose of winds) and distance from sources of technogen discharge on maps.

4.Physical Investigation

For analysis of venom properties and its metabolism products, chemical composition, qualitative element correlations , spectral and luminescent characteristics of the whole venom and its proteins have been determined by laser spectroscopy method , which are separated by method of gel and ion-exchange chromatography.

Photoluminescence spectra and time dependence of each maximum of proteins and toxins of venom are taken (Fig.1;2).

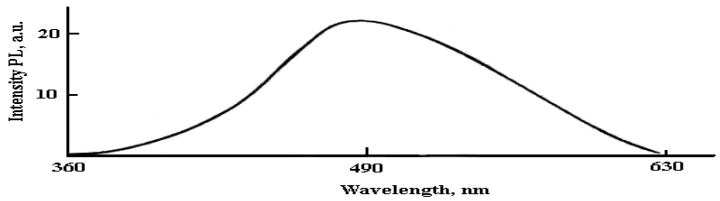

Fig.1. Photoluminescence spectrum of protein of Vipera venom for maximum λ_{max}=490 nm

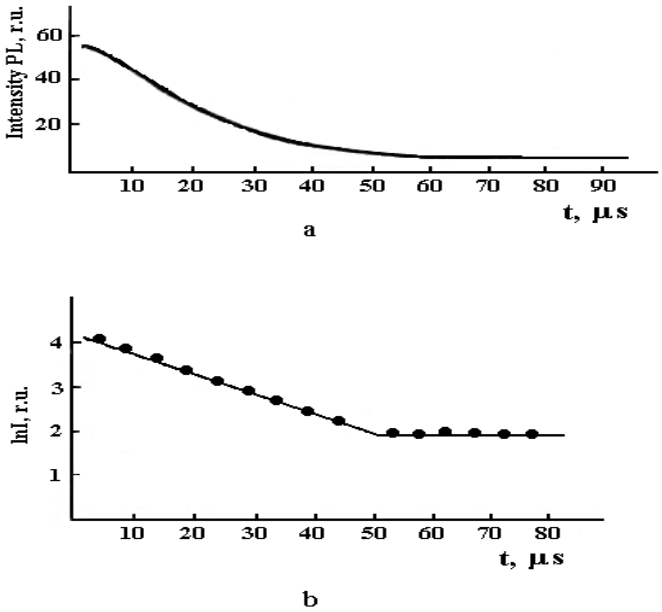

Fig.2. Time dependence of each maximum of proteins and toxins of venom

By method of spectral and luminescent analysis photoluminescence (PL) spectra characteristics have been determined as metabolites extracted from organism (Table 2; 3).

In dynamic conditions the samples are excited by pulse nitrogen laser of LGI-21 model (wavelength 337.1 nm, duration of pulse 10 ns). PL spectra range over 360÷650 nm. On PL spectra there have been found maximum at 527÷455 nm.

Time dependence of each maximum (t=3·10^{-5} sec) is taken. Pl intensity in wide range of wavelength decrease to exponential law with time after interruption of excitation.

Pl spectra of venom takes the range of 360÷630 nm with the wide maximum at 530 nm.

Table 2.

Spectral-luminescent parameters of venom and its proteins

Mol.mass.of venom proteins, kD	Half-width spectrum Photoluminescence, eV	λ_{max}, nm
149±2.14	0.70	490
146.5±0.98	0.70	490
132.5±3.45	0.74	525
101±2.30	0.68	510
99±0.86	0.72	510
92.5±0.09	0.70	505
79±1.04	0.62	510
66±0.76	0.75	525
56.5±0.93	0.65	520
51.5±0.03	0.66	530
45±0.01	0.72	510
35±0.25	0.78	508
32±0.09	0.79	513
20±0.01	0.69	510
Snake's venom	0.83	530

Table 3

Spectral-luminescence parameters of metabolism products of venom Vipera's, excreted from organs and tissues of the poisoned mice.

Mol.mass.of metabolits, kD	Half-width spectrum Photoluminescence, eV	λ_{max}, nm
153±3.56	0.75	510
150±4.04	0.68	495
146±2.01	0.74	495
142±0.99	0.71	500
139±2.02	0.67	480
136± 1.99	0.78	480
132±6.01	0.66	490
113±4.03	0.68	510
102± 2.04	0.77	485
92± 1.90	0.81	495
89±0.90	0.85	485
80±2.01	0.88	485
69±0.08	0.63	510
63±0.06	0.72	510
43±01.01	0.71	495
35±0.95	0.77	510
29±0.08	0.75	500
11±0.01	0.74	520
2.5±0.03	0.83	520

Time dependence of PL maximum of venom of Vipera is given in Fig.6. analysis of the obtained results show that PL intensity in wide range of wavelength decreases at times according to the exponential law after interruption of excitation:

$$I = I_0 \cdot e^{\frac{t}{\tau_D}}$$

I - intensity by time t; I_0 - intensity by $t=0$; τ_D - constant time characterized by the lifetime of excitation state of luminescence centers.

Date presented in polylogariphmic scale $\ln I = f(t)$ consist of two straight lines with different slopes. For the time of excitation state $t = 3 \cdot 10^{-5}$ sec. was obtained from these date for venom of Vipera.

Investigations show that intensity and maximum in PL spectra, also the time of excitation state of luminescence centers change depending on quality and quantity of microelements in venom of Vipera.

So venom of Vipera can be identified and analyzed by means of luminescent analysis. The influence of various ecological factors of microelementar composition under different ecogeochemical conditions can also be investigated according to luminescent analysis.

Comparative analysis of initial products offers scope for study of distribution, pharmakinetics of venom and identifying products of zootoxin metabolism in venom of vipera trapped in sections with high content of HM and in "background sections" being at certain distance from technogene discharge sources.

There have been noted direct correlative bond between chemical composition of venom and content of HM in soil and plants. In venom obtained from Viperas trapped in soil containing HM exceeding multiply MPC, intensity and maximum in PL spectrum, also time of excited states of luminescence centers are changed and differed significantly from zootoxin properties, produced from Viperas trapped in "background sections".

Thus conducted investigations on geochemical ecology of venom Viperas offers scope for forecasting venom properties practically from any place of inhabiting, to trap Viperas in certain population and produce venom with given toxicinity and necessary level of fermentative activity.

5.Conclusions

- Obtained results of analysis show that in soils of Absheron peninsula of Azerbaijan being under the constant effect of technogen discharge, significant concentration of HM exceeds multiply MPC in upper layer of soil (0-20 cm).
- Progressive pollution of biosphere of Absheron peninsula of Azerbaijan by technogen waste products lead to its significant saturation by toxic chemical elements, including heavy metals (Ni 41-142, Pb 21-38, Cr 89-213, Zn 18-147, Cu 20-51, Cd 0.8-4.2 mg/kg).
- PL spectra of snake venom range over 360÷650 nm. On PL spectra there have been found maximum at 527÷455 nm.
- Pl spectra of venom takes the range of 360÷630 nm with the wide maximum at 530 nm.
- There have been noted direct correlative bond between chemical composition of venom and content of HM in soil and plants.

Conflict of Interest

The authors declare no conflict of interest.

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