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## EVALUATION OF SOIL GENOTOXICITY IN KARVACHAR AND KAPAN USING THE COMET ASSAY IN URAL FIELD MOUSE (*APODEMUS URALENSIS*)

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The aim of the present study was evaluation of soil genotoxicity from Karvachar (Nagorno-Karabakh Republic) and Kapan (Republic of Armenia) using the single cell gel electrophoresis (comet assay) on peripheral blood leukocytes of the local Ural field mouse (*Apodemus uralensis*). DNA damage in mice leukocytes from Karvachar was significantly higher compared to Kapan. It is worth to mention, that in the soil samples from Karvachar the content of Fe, Mg and Ca was significantly higher compared to Kapan indicating potential genotoxic influence. The obtained results confirm that the assessing of genotoxicity by the Comet assay in free-living mice is valuable test in monitoring of environmental pollution.

*Comet assay – Ural field mouse – leukocytes – biomonitoring*

Աշխատանքի նպատակն էր Քարվաճառի (Լեռնային Ղարաբաղի Հանրապետություն) և Կապանի (Հայաստանի Հանրապետություն) հողի զենաթունայնության գնահատումը փոքր անտառային մկան (*Apodemus uralensis*) ծայրամասային արյան լեյկոցիտներում եզակի բջիջների ժել-էլեկտրաֆորեզի (ԴՆԹ-կոմետ մեթոդ) կիրառմամբ: Քարվաճառի անտառային մկների լեյկոցիտներում ԴՆԹ-ի վնասվածքները հավաստիորեն ավելի բարձր են, համեմատած Կապանի մկների հետ: Հարկ է նշել, որ Քարվաճառի հողի նմուշներում Fe, Mg և Ca պարունակությունը զգալիորեն ավելի բարձր է, համեմատած Կապանի հետ, ինչը վկայում է հնարավոր զենաթունային ազդեցության մասին: ԴՆԹ-կոմետ մեթոդի կիրառումը փոքր անտառային մկան ծայրամասային արյան լեյկոցիտներում արդյունավետ մոտեցում է հողի զենաթունայնության գնահատման համար:

*ԴՆԹ-կոմետ մեթոդ – փոքր անտառային մուկ – լեյկոցիտներ – կենսամշտադիտարկում*

Целью настоящего исследования являлась оценка генотоксичности почв Карвачара (Нагорно-Карабахская Республика) и Капана (Республика Армения), с применением метода гель-электрофореза единичных клеток (метод ДНК-комет) в лейкоцитах периферической крови у малой лесной мыши (*Apodemus uralensis*). Уровни повреждений ДНК в лейкоцитах мышей из Карвачара были значительно выше по сравнению с мышами из Капана. Стоит отметить, что в пробах почвы из Карвачара содержание Fe, Mg и Ca было значительно выше по сравнению с образцами почв из Капана, что указывает на потенциальное генотоксическое влияние. Полученные результаты подтверждают, что оценка генотоксичности почв с применением метода ДНК-комет у малой лесной мыши является эффективным подходом для мониторинга окружающей среды.

*Метод ДНК-комет – малая лесная мышь – лейкоциты – биомониторинг*

Environmental pollution causes a variety of effects, including increasing level of DNA damage. For the evaluation of environmental genotoxicity chemical analysis of pollutants should be combined with the analysis of DNA damage in testing species.

Rodents are routinely used as bioindicators of environmental contamination due to their ubiquitous distribution, high relative abundance, ease of trapping and

handling, and close association with the soil [8]. Earlier mice from the genus *Apodemus* have been successfully applied as pollution bioindicators using micronuclei test [7] and chromosomal aberrations [12].

The Comet assay, also known as a “single cell gel electrophoresis”, has become an important tool for assessing DNA damage and repair in single cells [4]. This method is established as a valuable tool in genetic ecotoxicology in wild animal populations [13], including free living mice [6].

In the present study the Comet assay was applied on the peripheral blood leukocytes of Ural field mouse (*Apodemus uralensis*) sampled from two areas with different levels of contamination: Kapan, the center of the Syunik Province, Republic of Armenia and Karvachar or Kalbajar, Nagorno-Karabakh Republic (NKR) for detection of soil genotoxicity.

**Materials and methods.** Soil chemical analysis was realized in Institute of Chemical Physics of NAS RA, according to APHA [1]. Levels of Ca, Mg, Mn, Fe, Co, Cr, Cu, Zn, Mo, Pb, Cd, P and V were measured in soil samples from Kapan and Karvachar in 2014.

In total, 26 mice were caught in 2014 using live traps and transported to the laboratory: 11 (10 males: 1 female) from Kapan and 15 (10 males: 5 females) from Karvachar. To avoid interindividual differences related to age, only adult specimens were examined. Blood samples were collected from each mouse by cardiac puncture, placed in vials with heparin as an anticoagulant.

Alkaline Comet assay was applied with some modifications [11]. A mixture of 20 µl of whole blood sample with 80 µL low melting point agarose (0.5%) (Sigma) was pipetted onto a microscope slide precoated with 1% normal melting agarose (Sigma). After the solidification of gel layer the slides were immersed in lysis solution (2.5 M NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mM Tris and 1% Triton X-100, pH 10.0) for 24 hour at 4°C. Subsequently, the slides were incubated in freshly made alkaline buffer (300 mM NaOH and 1mM Na<sub>2</sub>EDTA, pH>13) for 20 min for DNA unwinding, and electrophoresed in the same buffer for 15 min at 300 mA and 25 V. Slides were washed with neutralization buffer (0.4 M Tris, pH 7.5) for 10 min and stained with 20 µg/mL ethidium bromide (Sigma). The images were evaluated by fluorescence microscope (ZEISS, Germany) in conjunction with a digital camera. DNA damage was assessed using a Comet Assay IV imaging system (Perceptive Instruments, UK). 150 cells scored from each mouse. To characterize DNA damage the parameters % DNA in tail, Tail moment and Tail length was used.

Statistical analysis of obtained data was performed using nonparametric Mann–Whitney U test and Chi-Square (x<sup>2</sup>) test by StatgraphicsCenturion 16.2 (StatPoint Technologies, Inc. USA; Warrenton, VA) program.

**Results and Discussion.** The chemical composition of soil from two sampling sites is shown in and data of data of maximum allowable concentration (MAC) [9] for soils of Armenia are shown in tab. 1.

Chemical analyses of soil samples demonstrated differences in concentrations of pollutants between sites. In soil samples from Karvachar the content of Fe, Mg and Ca was significantly higher compared to Kapan.

DNA damage values assessed by the Comet assay in leukocytes of mice sampled in Kapan and Karvachar are shown in tab. 2. Data are presented pooling together the sexes, since the preliminary analysis did not show significant differences between female and male animals within the same group. This result coincides with literature data [8].

Nonparametric Mann–Whitney U test showed that the comet parameters (% of DNA in the tail and tail moment) in mice leukocytes from Karvachar were significantly higher compared to Kapan (p<0.05). Significant difference between two sites by the tail length was not revealed (tab. 2). This can be explained by the fact that the tail length does not linearly related to DNA break frequency [4].

**Table 1.** Concentrations of chemical elements (mg/kg) in the soil samples from Kapan and Karvachar and MAC – “maximum allowable concentrations of chemical elements” for soils of Armenia.

Chemical elements	Kapan	Karvachar	MAC
Ca	0.35	96.26*	No data
Mg	0.05	92.82*	No data
Mn	0.30	1.10	<1500
Fe	19.62	58.72*	No data
Co	0.01	0.03	No data
Cr	0.02	0.49	<90
Cu	0.31	0.04	<132
Zn	0.19	0.10	<220
Mo	0.03	0.00	<132
Pb	0.04	0.01	<65
Cd	0.00	0.00	<2
P	0.60	1.59	No data
V	0.20	0.06	<150

\* – denotes significant difference compared to Kapan ( $p < 0.001$ )

Increased level of DNA damage in mice from Karvachar compare with Kapan could be related with higher content of Fe, Mg and Ca. Earlier the genotoxicity Fe, Mg and Ca was shown [2, 5, 10]. Chemical composition assays of environmental habitat give important information on the extent of its contamination. However, they do not show the real effect on biological systems, because the combination of different factors acts simultaneously and reveals a summary biological effect. For this reason, biological assays of genotoxicity evaluation have been elaborated [3].

Thus, the assessment of DNA damage by Comet assay in leukocytes of mice *Apodemus uralensis* can be used as a valuable tool for assessment of genotoxic hazards of environmental pollution.

**Table 2.** DNA damage in leukocytes of mice *Apodemus uralensis* collected from Kapan and Karvachar

Sampling site	Number of mice	% DNA in tail		Tail moment		Tail length	
		Mean $\pm$ SE	Median	Mean $\pm$ SE	Median	Mean $\pm$ SE	Median
Kapan	11	6.64 $\pm$ 0.22	2.84	0.99 $\pm$ 0.03	0.43	31.74 $\pm$ 0.21	30.49
Karvachar	15	7.59 $\pm$ 0.19	4.25*	1.19 $\pm$ 0.03	0.68*	31.04 $\pm$ 0.17	29.17

\* – denotes significant difference compared to Kapan ( $p < 0.001$ )

The present study showed that DNA damage in mice leukocytes from Karvachar was significantly higher compared to Kapan. It was also found that soil samples from Karvachar more polluted with Fe, Mg and Ca. Thus, soil chemical analysis data agreed with the data of the analysis of DNA damage.

The results obtained demonstrate the sensitivity of free living mice *Apodemus uralensis* as biomarkers of environmental genotoxic pollution.

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