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## THE IDENTIFICATION OF CLASTOGENIC IMPACTS OF WATER IN HRAZDAN RIVER BY STUDYING THE PERIPHERAL BLOOD ERYTHROCYTES OF LAKE FROG (*PELOPHYLAX RIDIBUNDUS*)

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To identify the possible clastogenic effects of the water environment in Hrazdan River, a micronucleus test was used on erythrocytes of peripheral blood of the frogs living in its various sections. Data on the effect of pollution of the aquatic environment were also obtained by studying the morphological features of the nuclei of erythrocytes.

According to the data obtained, a large number of erythrocytes with micronuclei, relatively high percentage of nuclei with protrusions, as well as vacuolated nuclei are found in frogs inhabiting in the sections of Hrazdan River that are characterized with a high degree of pollution (villages Darbnik and Geganist). A positive correlation was revealed between the data of the micronucleus test and the data obtained as a result of study of the morphology of the nuclei of erythrocytes.

Thus, the data obtained makes it possible to draw a conclusion on possible clastogenic effect of the waters of Hrazdan River in the areas of the villages Darbnik and Geghanist.

### *Pelophylax ridibundus – Hrazdan river – clastogenic impact – erythrocytes*

Ուսումնասիրվել է օրգանիզմների վրա Հրազդան գետի ջրային միջավայրի կլաստոգեն ազդեցության հնարավորությունը՝ դրա տարբեր հատվածներում բնակվող լճագորտի ծայրամասային արյան էրիթրոցիտներում միկրոկորիզային թեստի կիրառման միջոցով: Օրգանիզմների վրա ջրային միջավայրի աղտոտվածության ազդեցության մասին տեղեկատվություն ենք ստացել նաև էրիթրոցիտների կորիզների մորֆոլոգիական առանձնահատկությունների ուսումնասիրությամբ:

Ստացված տվյալները վկայում են Գեղանիստ և Դարբնիկ գյուղերի մոտ Հրազդան գետի շրջակայքում բնակվող լճագորտի էրիթրոցիտներում միկրոկորիզների բավական մեծ քանակության առկայության մասին, բավական մեծ տոկոս են կազմում արտափեղմներով կորիզներ ունեցող, նաև վակուոլացված կորիզներով էրիթրոցիտները: Հետաքրքիր է նշել, որ միկրոկորիզային թեստի և էրիթրոցիտների մորֆոլոգիական նշված փոփոխությունների ուսումնասիրության մեթոդով ստացված տվյալների միջև առկա է դրական կորելյացիա:

Ստացված տվյալները հնարավորություն են տալիս եզրակացություն անել Գեղանիստ և Դարբնիկ գյուղերի մոտ Հրազդան գետի ջրերի հնարավոր կլաստոգեն ազդեցության վերաբերյալ:

### *Pelophylax ridibundus – Հրազդան գետ – կլաստոգեն ազդեցություն – էրիթրոցիտներ*

Для выявления возможного кластогенного воздействия водной среды р. Раздан на организмы использовали микроядерный тест на эритроцитах периферической крови лягушек, обитающих в ее различных участках. Данные о влиянии загрязнения водной среды были получены также в результате исследования морфологических особенностей ядер эритроцитов.

Полученные данные свидетельствуют о наличии довольно большого количества микроядер в эритроцитах, высокого процентного содержания ядер с протрузиями, а также вакуолизированных ядер в эритроцитах крови лягушек, обитающих в районах сел Дарбник и Геганист, характеризующихся высокой степенью загрязненности. Выявлена положительная корреляция между данными микроядерного теста и данными, полученными в результате вышеуказанного исследования морфологии ядер эритроцитов.

Полученные нами данные дают возможность сделать заключение о возможном кластогенном эффекте вод р. Раздан в районах сел Дарбник и Геганист.

*Pelophylax ridibundus – р.Раздан – кластогенное воздействие – эритроциты*

Amphibians are successfully used nowadays for the biological monitoring of ecosystems [2, 3, 5-7]. In order to assess the degree of reservoirs' pollution for the identification of the mutagenic effect of the aquatic environment, the study of morphological changes of different elements is used as a reliable indicator criterion. In particular, the erythrocytes of the blood of amphibians are used, since they are very sensitive to the influence of adverse environmental conditions, including pollution [3].

Among the studies conducted for this purpose, a valuable information is obtained by using a micronucleus test on erythrocytes of the aquatic animals [5-9].

According to the data published by the Environmental Monitoring and Information Center (EMIC) of the Ministry of Nature Protection of Armenia, different sections of the Hrazdan River are characterized by different degrees of pollution. Samples of waters taken from Qaghsi and Argel are characterized by the second class ("good status").

The waters of Darbnik are characterized by the fifth class of pollution ("poor status"), which is caused by the existence of ions of an ammonium, phosphates and nitrites, of the common inorganic nitrogen [10].

The relevance of studying the impact of water pollution on organisms is due to the importance of Hrazdan River for the economy and the population of Armenia.

To identify the possible clastogenic effects of the water environment of the Hrazdan River, a micronucleus test was used on erythrocytes of peripheral blood of the frogs living in its various parts.

Data on the effect of pollution of the aquatic environment were also obtained by studying the morphological features of the nuclei of erythrocytes.

**Materials and methods.** 35 frogs of *Pelophylax Ridibundus* weighting 15-20 g were used in this research. The catching was carried out in 5 sections of Hrazdan River indicated by the Ministry of Nature Protection of the Republic of Armenia, in 2009. As control used frogs, catching from the **Ttu jur (Sour water)** near village Hanqavan. Smears were prepared from the samples of frogs' blood, which were fixed in ethyl alcohol for 30 min. The preparations were stained with Schiff's solution according to Feulgen. Hydrolysis was carried out in 5 N H-HCl at 22°C for 60 min. The counting of erythrocytes with micronuclei and percentage of erythrocytes with morphological anomalies of nuclei was carried out under the increase of microscope by 1000 times. 2,000 cells were counted on each animal's blood smears. The acquired/ obtained data were subjected to statistical processing using the computer program "Statistica 8" by the help of which the mean value, the quadratic deviation, the reliability of the values, and the correlation were determined.

**Results and Discussion.** Table 1 illustrates the obtained data on the number of micronuclei in the erythrocytes of peripheral blood of the lake frog living in different surrounding areas of Hrazdan River. The obtained data confirm the existence of a fairly large number of micronuclei in the erythrocytes of frogs' blood living in Hrazdan River near the villages Darbnik and Geghanist.

**Table 1.** The number of micronuclei in erythrocytes of peripheral blood of frogs living in different parts of the Hrazdan River

Name of the plot/place	Micronuclei number (‰)	P
village Hanqavan (Sour water)	0,50 ± 0,50	-
village Argel	1.333 ± 0,441	p < 0.05
village Qaghsi	1,833 ± 0,333	p < 0.05
Arzni hydroelectric power station	0,667 ± 0,167	p > 0.05
village Geghanist	3.333 ± 0,167	p < 0.05
village Darbnik	4,00 ± 0,764	p < 0.05

In comparison to erythrocytes of *Pelophylax Ridibndus* living near Hanqavan village there are statistically reliable difference.

As it is known, the micronuclei in cells are formed from nuclear chromatin, when under the influence of various factors the processes of the formation of spindle formation and chromosome segregation are disrupted.

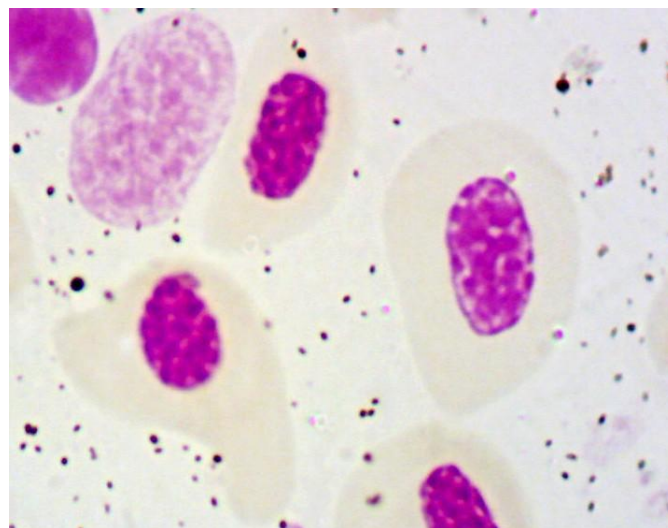
Micronuclei are found in the cytoplasm of cells in the interphase and represent rounded chromatin formations that are formed from chromosome parts.

Both the whole chromosomes and their certain parts can be included in micronuclei.

According to the data, obtained in our work, a large number of erythrocytes with micronuclei are found in frogs inhabiting in the parts of Hrazdan river which are characterized with a high degree of pollution, caused by the exceeding maximum permissible concentration of nitrite ions, ammonium ions, vanadium, manganese, chromium, copper, the amount of oxygen and chemical oxygen demand.

Technogenic environmental pollution disrupts the stability of ontogenesis of organisms and the ability of formation of genetically determined phenotype [4].

Thus the erythrocytes of frogs have an oval form. Under the influence of various harmful factors, their form can be violated (fig.1).



**Fig.1.** Erythrocytes of *Pelophylax Ridibndus*. 1. Erythrocyte with micronuclei, 2. Erythrocyte with protrusion. Schiff's solution according to Feulgen, 1000-fold magnification

The morphological study of the nuclei of erythrocytes of the lake frog, inhabiting in different parts of the Hrazdan River, allowed us to identify the following changes: nuclei with protrusions, vacuolated nuclei, picnotically altered nuclei. The data illustrated in tab. 2 indicate the presence of a relatively high percentage of nuclei with protrusions, as well as vacuolated nuclei in the erythrocytes (red blood cells) of frogs living in the areas of the villages Darbnik and Geganist.

**Table 2.** The number of violated nuclei in erythrocytes of peripheral blood of frogs living in different parts of the Hrazdan River

Observatory name	Nuclei with protrusions (‰)	Vacuolized nuclei (‰)	Picnotic nuclei (‰)
village Hanqavan (sour water)	3,00 ± 1,00	1.834 ± 0.334	1.334 ± 0.167
village Argel	4,67 ± 1,167	1,00 ± 0.289	2.50 ± 0.764
	p < 0.05	p < 0.05	p < 0.05
village Qaghsi	6,167 ± 0.167	0.667 ± 0.667	1.50 ± 0.289
	p < 0.05	p < 0.05	p > 0.05
Arzni hydroelectric power station	2,50 ± 0,289	0.834 ± 0.334	1.834 ± 0.334
	p > 0.05	p < 0.05	p > 0.05
village Geghanist	11,33 ± 1,856	3.834 ± 0.601	2.00 ± 0.50
	p < 0.05	p < 0.05	p < 0.05
village Darbnik	15,00 ± 2,517	3.334 ± 1.093	4.167 ± 1.424
	p < 0.05	p < 0.05	p < 0.05

It is interesting to note that a positive correlation was revealed between the data of the micronucleus test and the data obtained as a result of the above stated study of the morphology of the nuclei of erythrocytes. Based on this factor, it can be concluded that the morphological study can also be used for environmental monitoring purposes.

Thus, the data obtained makes it possible to draw a conclusion about the possible clastogenic effect of the waters of the Hrazdan River in the areas of the villages Darbnik and Geghanist.

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