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**THE KARYOLOGIC STUDY OF FROGS (*PELOPHYLAX
RIDIBUNDUS*) HEPATOCYTES LIVING IN SURROUNDING AREAS
OF HRAZDAN RIVER CHARACTERIZED BY DIFFERENT
DEGREES OF POLLUTION**

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The main purpose of this research was studying the peculiar features of frogs (*Pelophylax ridibundus*) livers' glandular cell nucleus and nucleolar (nucleolus) DNA quantity, mitotic activity, and the formation peculiarities of micronuclei in them, which live in Hrazdan rivers' surrounding areas, characterized by different pollution degrees. Results of the study show that in the livers of frogs which live near Hrazdan River with medium or heavy pollution status have acquired adaptive, countervailing changes, which find their expression in proliferative activity of cells, in activation of transcription processes and increase in body mass. At the same time in areas described with the highest pollution – “bad” status/level a disruption of normal mitotic separation process of cells is observed, the result of which is the formation of micronuclei.

Pelophylax ridibundus – liver – DNA quantity – mitotic activity – micronuclei

Աշխատանքում նպատակ էր դրված ուսումնասիրել Զրազդան գետի աղտոտվածության տարբեր աստիճաններով բնութագրվող հատվածների շրջակայքում բնակվող լճագորտի յարդի գեղձային բջիջների կորիզային և կորիզակային ԴՆԹ-ի քանակության, միտոտիկ ակտիվության, դրանցում միկրոկորիզների առաջացման առանձնահատկությունները: Ստացված տվյալները վկայում են այն մասին, որ Զրազդան գետի աղտոտվածության «միջակ» և «վատ» կարգավիճակ ունեցող հատվածների շրջակայքում բնակվող գորտերի յարդում դիտվում են հարմարողական, փոխհատուցողական փոփոխություններ, որոնք իրենց արտահայտությունն են գտնում բջիջների պրոլիֆերատիվ ակտիվության, տրասկրիպցիայի պրոցեսների ակտիվացման, օրգանի զանգվածի մեծացման մեջ: Միաժամանակ, աղտոտվածության ամենաբարձր՝ «վատ» կարգավիճակով բնութագրվող հատվածներում դիտվում է բջիջների միտոտիկ բաժանման պրոցեսների բնականոն ընթացքի խաթարում, որի հետևանքը միկրոկորիզների առաջացումն է:

Pelophylax ridibundus – յարդ – ԴՆԹ քանակություն – միտոտիկ ակտիվություն – միկրոկորիզներ

Целью настоящей работы является изучение количества ядерной и ядрышковой ДНК, митотической активности гепатоцитов, а также особенностей образования микроядер в гепатоцитах озерной лягушки, обитающей в разных участках реки Раздан, характеризующихся различной степенью загрязненности. Полученные данные свидетельствуют о том, что в печени лягушек, обитающих в участках со “средним” и “высоким” уровнем загрязненности, наблюдаются компенсаторно – приспособительные реакции, выражающиеся в активации пролиферации клеток, процессов транскрипции и увеличении массы органа. Одновременно у животных из участков с высокой степенью загрязненности воды наблюдается образование микроядер в результате нарушения процессов митотического деления клеток.

Pelophylax ridibundus – печень – количество ДНК – митотическая активность – микроядра

According to the data released by the Environmental Impact Monitoring Center of the Ministry of Nature Protection of Armenia, water pollution is observed in different sections of Hrazdan River [12].

In five sampling points of the Center (near Qaghshi, Argel, Darbnik, Geghanist villages and down the Arzni Hydropower plant) in 2009 the following values of indicators of pollution were recorded (tab. 1).

Table 1. Values of pollution parameters at different sampling points on Hrazdan river for 2009

Water facility	Indicator's number on themap	Points (station) location	number of samples	MPC exceeded indicator's name	The number of cases exceeding MPC	Overflow of the average concentrations from MPC
Hrazdan	52	0,5 km down the village Qaghshi	5	vanadium	5	5,0
				manganese	4	3,0
	53	0,5 km down the village Argel	5	vanadium	5	9,0
				manganese	5	3,0
	54	0,5 km down the Arzni Hydroelectric - power station	5	manganese	5	9,0
				chrome	3	2,0
				manganese	4	9,0
				copper	3	4,0
	55	Near the village Darbnik	12	nitrite ion	11	7,0
				ammonium ion	12	29,3
				The five-day biochemical oxygen demand	11	3,4
				vanadium	5	13,0
			5	manganese	5	7,0
				chrome	5	7,0
				copper	5	5,0
		Near the village Geghanist		nitrite ion	11	3,8
				ammonium ion	12	6,3
				The five-day biochemical oxygen demand	11	2,3
vanadium				11	10,0	
manganese				12	5,0	
copper				12	4,0	

According to the data shown above, the water near the villages Qaghshi and Argel can be considered relatively clean because the maximum permissible concentration exceeds only for vanadium and manganese ions.

In the samples taken from different sampling points at the river in 2014 the general quality of water varied between 3rd (medium) to 5th (bad). The 3rd water pollution status was observed downstream the villages Qaghshi, Argel and Arzni HPP due to chemical oxygen demand, phosphate and nitrate ions. With Waters near to villages Geghanist and Darbnik, as well as waters near Hrazdan river source were characterized by "bad" pollution status due to ammonium, phosphate and nitrate ions, total inorganic nitrogen, and total phosphorus (12).

The method of morphophysiological indicators is nowadays successfully used to study the unfavorable exposure of harmful environment components on organisms, including humans.(1, 2, 3, 7).

Pylophylax ridibundus which runs the coastal as well as an aquatic lifestyle is a very suitable object for assessing the environmental pollution exposure as it absorbs various environmental pollutants with its skin during breathing and with water as well as with food because it eats terrestrial and aquatic food. (5). Therefore the disruptions of different morphophysiological features of these frogs can be used as a reliable testing criteria for measuring terrestrial and aquatic environments harmful impact degree (4).

The liver hepatocytes are extremely sensitive to the environmental impact of man-made pollution, which plays an important role in maintenance of organisms' homeostasis. (10,11):

The main purpose of the given work was to study the peculiar features of frogs (*Pylophylax ridibundus*) livers' glandular cell nucleus and nucleolar (nucleolus) DNA quantity, mitotic activity, and the formation peculiarities of micronuclei in them, which lives near Hrazdan rivers' surrounding areas, characterized by different pollution degrees. Micronuclei test is successfully used to test/assess the environmental harmful impact. The micronuclei appear in cells from nuclear chromatin because of different harmful impact, when the processes of spindle formation and chromosome segregation are violated. These are the round chromatin structures detected during the interphase in the cytoplasm of cells which arise from the parts of chromosomes. Micronuclei may include complete chromosomes as well as its separate parts.

Materials and methods. Bacteria, bacterial growth, membrane vesicles. As a material for given work were used 15 frogs with weight of 15-20 g belonging to *Pylophylax ridibundus*. The frogs were euthanized, smears were prepared from their livers, which were put in ethyl alcohol for 30 minutes and were painted by Schiff solution according to Feulgen.

The DNA quantity in nuclei and in nucleolar was decided in conventional units in 575 nm wavelength conditions, by the cells commensurate method, on image trace analyzer which was created on the basis of SMP 05 Microscope photometer (OPTION, Germany) and was connected to the computer.

The quantity of DNA in nuclei and nucleolar was determined in each animals liver's 100 cells.

The peripheral blood lymphocytes and diploid hepatocytes of frogs living near Argel village were used as a DNA diploid standard.

On the basis of comparison of DNA quantity with standards the hepatocytes distribution was established by the nuclei ploidy, the surface of nuclei and nucleolar, DNA mass and the quantity of nucleolar was also determined.

The photometric examination of samples/micro-preparations revealed that the quantity of DNA varies no more than of 10 percent.

Mitotic activity of hepatocytes was determined and number of hepatocytes with micronuclei was counted on the micro-samples of liver.

The numerical data were developed by a computer program Statistica, the degree of differences probability between the compared values was also determined.

Results and Discussion. Anthropogenic pollution of water creates extreme conditions for living organisms. This creates an additional load on organisms and the level of metabolism is changed. The liver cells play a vital role for preserving homeostasis and providing desintoxication.

The data obtained in our study prove the fact of increase in relative liver weight of frogs living in Hrazdan rivers' surrounding area-villages Geghanist and Darbnik described with the highest water pollution level [fig.1]. There are numerous data in literature which show that the presence of toxic substances in environment cause an increase in relative liver weight [8, 10]:

As it follows from the karyologic study a considerable role belongs to DNA synthesis and the increase of mitotic activity. The data from tab. 2 show the increase of nucleolar quantity in hepatocytes of frogs living in Hrazdan river's surrounding areas characterised by high level of pollution.

This indicates the activation of transcription processes in cells. Besides that in livers of frogs living in points down the Arzni power station, where the waters of Hrazdan river have medium pollution level, the percentage increase of hepatocytes having nucleus DNA 3c hepatocytes quantity is observed (fig 2). We may assume that these are the cells that are in S phase of cell cycle, in which a DNA synthesis occurs.

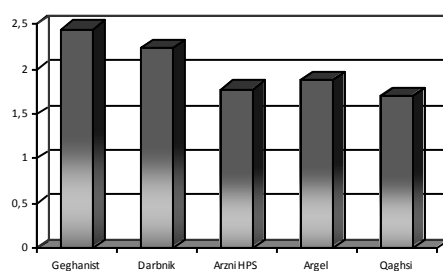


Fig. 1. The relative liver weight of frogs living in Hrazdan river.

Table 2. Nucleolar quantity in hepatocytes of frogs living in Hrazdan river's surrounding areas

Observatory name	0 nucleolus	1 nucleolus	2 nucleolus	Average number of nucleoli per nucleus
Argel village Qaghsi village	36	62	2	0.7±0.05
Arzni HPS	29	65	6	0.8±0.05
Geghanist village	30	64	6	0.8±0.06
Darbnik village	30	62	8	0.8±0.06

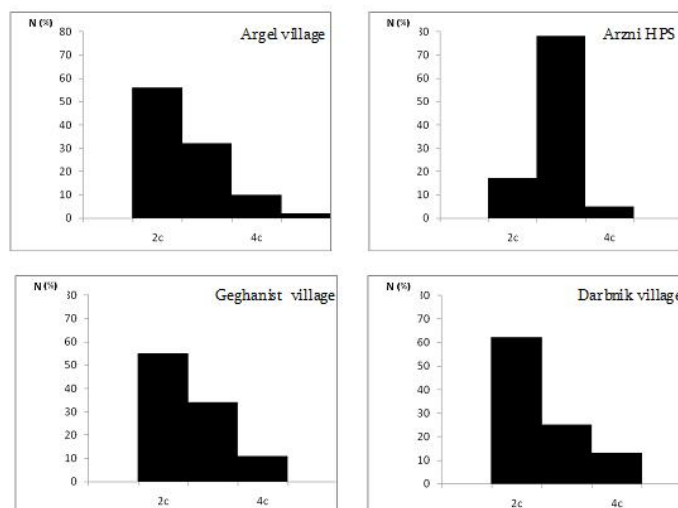


Fig. 2. Ploidy distribution of nuclei in frog liver cells from Hrazdan rivers' surrounding areas, characterized by different pollution degrees

This assumption is also confirmed by the existence of high level of mitotic activity of cells in the livers of frogs caught from these observatories (tab. 3).

In comparison to the livers of frogs living near Argel village this indicator has statistically significant difference.

In cases of extremely high doses of toxic substances disruptive factors prevail over the protective mechanisms.

In rivers' areas described with the highest pollution level the mitotic activity of hepatocytes is smaller in comparison to the livers of frogs living in areas with medium pollution status. Moreover in the livers of the frogs living in this observatories the amount of cells containing micronuclei is statistically significant.

Table 3. The mitotic activity of hepatocytes in livers of frogs living in different surrounding areas of Hrazdan rivers observatories

Observatory name	Mitotic activity (%)	p
Argel village	8.67 ± 3.56	
Qaghsi village	6.67 ± 1.47	p > 0.05
Arzni HPS	17.00 ± 0.71	p < 0.05
Geghanist village	14.67 ± 8.89	p > 0.05
Darbnik village	6.67 ± 2.86	p > 0.05

Table 4. The quantity of cells containing micronuclei in livers of frogs living in different observatory surrounding areas of Hrazdan river.

Observatory name	M ± m, %0	P
Argel village	8.00 ± 1.41	
Qaghsi village	8.67 ± 2.68	p > 0.05
Arzni HPS	7.00 ± 0.71	p > 0.05
Geghanist village	16.00 ± 2.45	p < 0.05
Darbnik village	15.67 ± 2.20	p < 0.05

This fact illustrates that the normal mitotic process of separation in that cells is destroyed.

Thus the data obtained come to witness the fact that in the livers of frogs which live near Hrazdan river waters with medium or bad pollution status have acquired adaptive, countervailing changes, which find their expression in proliferative activity of cells, in activation of transcription processes and increase in body mass.

At the same time in areas described with the highest pollution – “bad” status/level a disruption of normal mitotic separation process of cells is observed, the result of which is the formation of micronuclei.

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