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ESTIMATION OF DNA DAMAGE IN POPULATIONS OF MARSH FROG (*PELOPHYLAX RIDIBUNDUS*) BASED ON DNA COMET ASSAY

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Abstract.

Using the DNA Comet Assay, the level of DNA destruction in two populations of marsh frog (Pelophylax ridibundus) inhabiting biotopes with different anthropogenic pollution was assessed. Animal liver cells were used for the analysis. Five adult animals were selected from each biotope, in each of which minimum 100 cells were analyzed. The results showed a significant ($p<0.05$) increase in the DNA comet index in the contaminated zone (I_{dc} = 0.141±0.021). In addition, the presence of apoptotic cells was noted here. In the clean zone, the majority of the cells contained intact DNA (I_{dc} = 0.057±0.011). The obtained data demonstrate the possibility of using a swamp frog as an indicator of anthropogenic impact on ecosystems.

Keywords: *marsh frog, DNA damage, DNA comet assay, anthropogenic impact*

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INTRODUCTION:

One of the pernicious effects of a human on the environment is the deliberate or unintentional introduction of genotoxic xenobiotics into natural communities, which in turn can cause a change or destruction of the genetic material of living organisms [1, 2]. Various anthropogenic factors and chemical agents can disrupt the mechanism of transfer of hereditary information. The consequences of such interference can be very serious. Disorganization of the hereditary apparatus can cause chain reactions of self-destruction of the biota, and the person himself is given the role of the sufferer. Thus, to date, methods that allow diagnosing the degree of saturation of the environment with mutagenic components are in demand [3]. Among the available modern toxicology test systems for assessing the level of DNA degradation under the influence of various xenobiotics, alkaline gel electrophoresis of isolated cells, which was shortly called the Comet Assay [4- 6], is quite sensitive. For the overwhelming majority of cases, this approach is used in laboratory experiments *in vitro* and *in vivo*. But, gradually information is found on the application of this method to determine the degree of mutagenic load in various landscapes [2, 3, 7, 8]. Therefore, the question arises of acceptable organisms of bioindicators that can become objects of ecotoxicological monitoring. As such, we propose to use frogs *Pelophylax ridibundus* (Amphibia: Ranidae). The choice of these animals is due to the fact that they are an important part of ecosystems, they inhabit the boundaries of two environments. Meltwater and rainwater carry a huge number of pollutants absorbed during ontogeny by these animals and causing negative consequences in their organisms. There are a number of studies where this species is used as a bioindicator of anthropogenic pollution [9, 10].

Methodology:

Five adult animals *Pelophylax ridibundus* were selected from two sites around the city of Belgorod (Russia) for the analysis, which differed in the level of anthropogenic load. The first site (Sevrykovo) was located in the riverhead of the Razumnaya River (50°36'55.3"N 36°46'21.9"E). There was a rapid current and clear water. The second site (“Razumnaya") was located in the mouth of the Razumnaya River (50°31'48.0"N 36°38'59.5"E). There was slow flow, the water had an unpleasant smell, contaminated with solid household waste.

The animals were caught with a hydrobiological net and studied on the day of sampling. Liver tissue was used for the analysis. It is known that the liver is the main filter of the body, which neutralizes harmful chemical pollutants coming from the environment. Therefore, the toxic load first of all affects the violation of the cytogenetic stability of the cells of this organ.

Cell maceration was carried out in phosphate buffered saline (pH 7.5) containing 20 mM EDTA-Na_2 and 10% DMSO at temperature of +40 C. Cell suspensions in the fusible agarose composition were applied to agarose-coated slides at temperature of +420 C. The protein was lysed two hours at a temperature of +40 C (lysis buffer: 10 mM Tris-HCl (pH 10), 2.5 M NaCl, 100 mM EDTA-Na_2, 1% Triton X-100 and 10% DMSO). Electrophoresis was performed in a dark room, applying a neutral version of the method using Tris_EDTA_borate buffer (pH 8.9, 20 min, 1 V/cm). The fixed with ethyl alcohol (70%) and dried preparations were stained with SYBR Creen I. The images were analyzed on an epifluorescence microscope. The data was processed using CometScoreTM v 1.5 (http://www.autocomet.com/products_cometscore). The nuclei were ranked by the four stages of DNA destruction. At least 100 nuclei were counted on each specimen (Fig. 1).

The degree of DNA destruction was assessed using the Kruskel-Wallis criterion, sometimes expressed as DNA comet index (I_{dc}), using the formula:
$I_{dc} = (0n0 + 1n1 + 2n2 + 3n3 + 4n4) / \Sigma$,
where n0-n4 is the number of "DNA comets" of each type, Σ is the sum of the counted "DNA comets" [11].

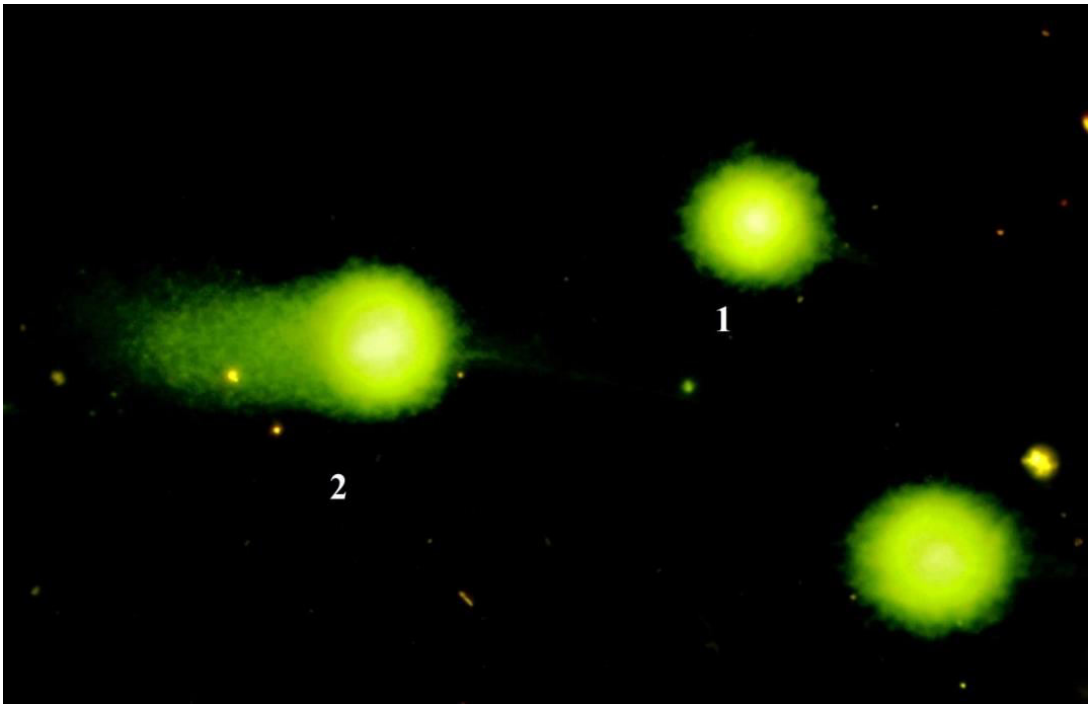

Fig. 1. The nucleus liver cells *Pelophylax ridibundus* in various stages of destruction (1 - unresolved nucleus, 2 - the second stage of destruction of the nucleus)

RESULTS AND DISCUSSION:

The results were summarized in Table 1. Cells sampled from "Sevrykovo" (1) site, in most cases, had intact nuclei. The nuclei with the first and second stages of DNA destruction are rarely found. The average value of the DNA comet index for this item was I_{dc} = 0.057±0.011. The animals from "Razumnaya" (2) site had cells with the nuclei at the third and fourth stage of DNA destruction. In addition, at this site, apoptotic cells were detected, which also indicates contamination of the water body by genotoxic pollutants. The average value of the DNA comet index for the item "Razumnaya" was I_{dc} = 0.141±0.021.

Table 1. DNA damage indicators in the studied groups of *Pelophylax ridibundus*

Point	Indicators	Number of animal				
		1	2	3	4	5
"Sevrykovo" (clean zone)	N	112	127	115	122	109
	Na	0	0	0	0	0
	0 stage	105	123	108	118	105
	1 stage	4	4	5	3	3
	2 stage	3	0	2	1	1
	3 stage	0	0	0	0	0
	4 stage	0	0	0	0	0
	I_{dc}	0.089	0.031	0.078	0.041	0.045
"Razumnaia" (dirty zone)	N	118	109	113	123	107
	Na	0	0	1	0	1
	0 stage	106	100	102	114	100
	1 stage	7	7	6	5	6
	2 stage	2	2	1	2	3
	3 stage	2	0	3	1	0
	4 stage	0	0	0	1	0
	I_{dc}	0.144	0.100	0.221	0.130	0.112

Note: N – Number of cells analyzed, Na – Number of apoptotic cells.

CONCLUSION:

The results of the studies showed significant differences in the degree of DNA destruction between the sites by the first probability threshold of error-free forecasts (Student's t-test = 3.5, $p<0.05$). This indicates an increase in the concentration of genotoxic elements in the liver of marsh frogs in “Razumnaya" (site 2). However, despite the strong urbanization of the study area, the level of DNA destruction in the studied groups of frogs can be considered insignificant, since the obtained average I_{dc} values do not reach even the first stage of destruction. This, on the one hand, may indicate the absence of strong destruction factors in the points of sampling that disturb the cytogenetic stability, and, on the other hand, suggest the existence of repair, homeostatic processes in animals that neutralize the adverse effects of environmental components. Nevertheless, certain trends towards an increase in the amount of destroyed DNA according to our data are observed.

SUMMARY:

Thus, the conducted analysis showed the possibility of using the DNA Comet Assay to assess the degree of genotoxicity of the environment in the ecosystems of the urbanized landscape. In addition, the use of marsh frogs as bioindication objects proved to be quite acceptable.

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