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INFLUENCE OF THE HRAZDAN RIVER POLLUTION ON MORPHO-FUNCTIONAL FEATURES OF *PELOPHYLAX RIDIBUNDUS* INTESTINE

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Our study illustrates the histomorphological features of the intestine of frogs living in different surrounding areas of the Hrazdan River characterized by different degrees of pollution. The intestinal epithelium of frogs living near the most polluted parts of the river (villages Darbnik and Geghanist) illustrates phenomena of pyknotic changes and necrosis, the presence of a great number of lymphocytes and lymphoid tissue accumulations in the epithelium. An increasing amount of fibroblasts is observed in the mucous membrane. Our study indicates the increased activation process of the mast cells and the presence of their accumulations in submucosal layers of the intestines of frogs living in the most polluted parts of the river.

Keywords: *Pelophylax ridibundus*, small intestine, mitotic activity, mast cells.

Introduction. The Hrazdan River has different pollution in its parts, according to the data of the monitoring centre of MNP of RA [1]. In five points established by the centre (near Qaghsi, Argel, Darbnik, Geghanist villages and down Arzni Hydroelectric power station (HPS)) the water in 2009 enlisted the indicators of pollution illustrated in Tab. 1.

According to the data, the water near the Qaghsi and Argel villages can be considered relatively clean, because the maximum permissible concentration dominates only with vanadium and manganese ions. In the samples taken from different points of the river in 2014 the general quality of water varied between 3rd mediocre to 5th bad. The 3rd water pollution status was observed down the Qaghsi, Argel and Arzni HPS (Fig. 1) observatories connected with chemical oxygen demand, phosphate and nitrate ions. With bad 5th pollution status were characterised waters near the Geghanist and Darbnik villages, as well as waters near the Hrazdan River estuary connected with ammonium, phosphate and nitrate ions, the total inorganic nitrogen, total phosphorus [1].

Water pollution is also maintained at the Hrazdan Rriver nowadays [1].

Bioindication methods are successfully used nowadays in order to assess the degree and intensity of exposure of harmful components of the environment on the organisms including humans [2, 3]. One of these is the identification of morpho-functional changes of organs.

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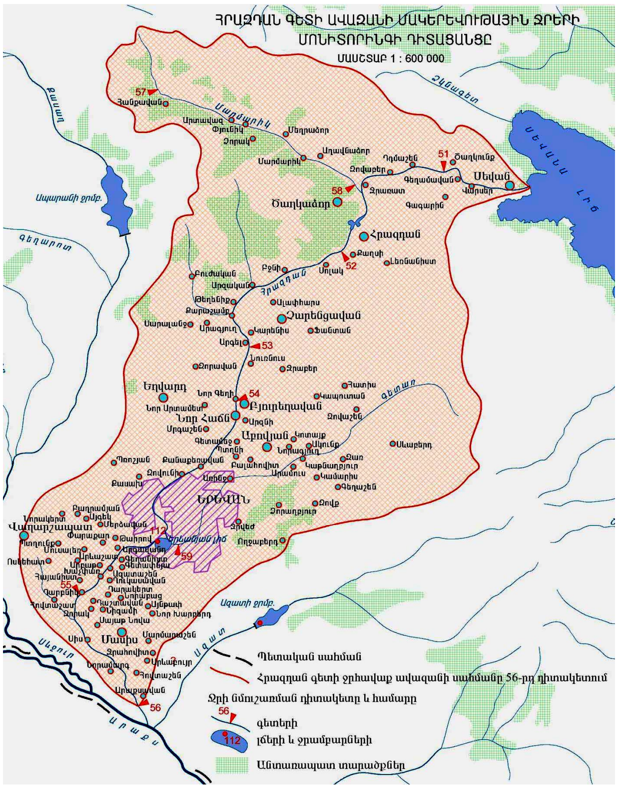

Fig. 1. Map of the Hrazdan River [1].

Table 1

The state of pollution in different points of the Hrazdan River in 2009

Points location (№ on the Map)	Q-ty of samples	MPC exceeded indicator's name	Number of cases exceeding MPC	Overflow of the average concentrations from MPC
0.5 *km* down the v. Qaghsi (52)	5	vanadium	5	5.0
		manganese	4	3.0
0.5 *km* down the v. Argel (53)	5	vanadium	5	9.0
		manganese	5	3.0
0.5 *km* down Arzni HPS (54)	5	manganese	5	9.0
		chrome	3	2.0
		manganese	4	9.0
		copper	3	4.0
Near the v. Darbnik (55)	12	nitrite ion	11	7.0
		ammonium ion	12	29.3
		5-day biochemical O demand	11	3.4
	5	vanadium	5	13.0
		manganese	5	7.0
		chrome	5	7.0
		copper	5	5.0
Near the v. Geghanist		nitrite ion	11	3.8
		ammonium ion	12	6.3
		5-day biochemical O demand	11	2.3
		vanadium	11	10.0
		manganese	12	5.0
		copper	12	4.0

P. ridibundus is considered to be a perfect object for evaluation of xenobiotics impact on organisms in an aquatic environment as it has water penetrable skin and unprotected ovules [4–6]. It absorbs various environmental pollutants with its skin during the respiration and with water, as well as with food, as it subsists both terrestrial and aquatic food [7–9]. Taking into account the above mentioned, our purpose is to identify the impact of the Hrazdan River pollution on *P. ridibundus* intestine.

Materials and Methods. 15 frogs of the *Pelophylax ridibundus* type served as material for the study (15–20 *g*, weight of each). The frogs were taken from 5 points of the Hrazdan River (Fig. 1).

For histological process the intenstine patterns were fixed in Bouin's and Carnoy's fluid. The fixed pattern underwent histological process and was enclosed in paraffin. 6 *μm* thick paraffin cuts were painted with hematoxylin and eosin, toluidine blue and were processed by silver nitrate according to Foote.

The mitotic activity of enterocytes was determined on the specimen, which were processed by hematoxylin and eosin. 2000 cells were counted in each animal's intenstine. The data was processed by a computer program "Statistica".

Results and Discussion. *P. ridibundus* intestinal wall has character typical to vertebrates. It is comprised of three membranes: mucous, muscular and serous membrane. The serous membrane is rather slender coated by mesothelium, blood vessels pass through it [10, 11].

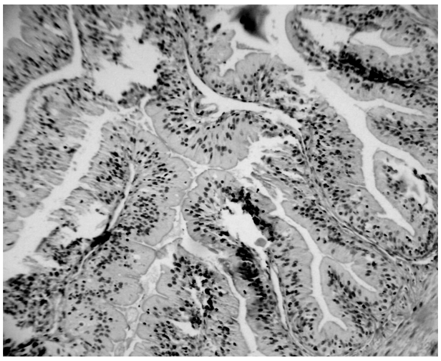

Fig. 2. A cut of the intestine of a frog. Hematoxylin-eosin staining, magnification ×100.

The internal surface of the intestinal wall is corrugated; it creates villi intestinals. The villi are relatively short. Unlike mammals the basic villi intestinals' foundational parts of *P. ridibundus* do not create crypts (Fig. 2). Two mucous layers can be distinguished into epithelial and submucosal. Epithelium is the thickest simple pseudostratified layer. Besides enterocytes in epithelium there are lots of goblet cells which are secrete mucus. The submucosal layer is comprised of loose connective tissue dominated by fibroblasts (a cell in connective tissue, which produces collagen and other fibres). There are also connective tissue cells and intracellular elements. In the connective tissue of submucosal layer especially under the basal plate there are "bunches" of argyrophilic thread. The mucous own plate is presented by a very thin layer, which comprises a small number of connective tissue cells and capillaries. The mucous muscular cell and the submucosal basis are intertwined and consist of 1–2 series of smooth muscle cells and collagen fibers.

P. ridibundus intestinal wall muscle membrane is composed of 2 layers: external longitudinal and internal ring-shaped. The ring-shaped layer exceeds the longitudinal layer 3–4 times by its thickness. The smooth muscle cells of ring-shaped layer are arranged oblique to the longitudinal plane. The data acquired from our observations witness the fact that the *P. ridibundus* intestine responds to the river's water pollution by a range of histomorphological changes, which are expressed in varying degrees in the intestines of frogs taken from different observatories.

In the intenstine of frogs living near the most polluted parts of the Darbnik and Geghanist Rivers, negative changes in epithelium are clearly observed.

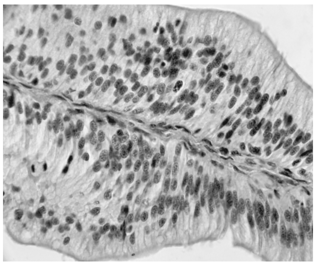

Fig. 3. Cut of the intestine of a frog. Hematoxylin-eosin staining, magnification ×400.

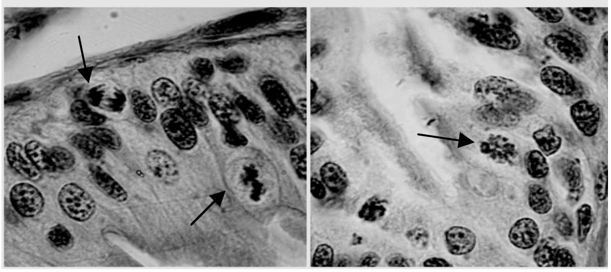

Fig. 4. Mitosys in *Pelophylax ridibundus*' gut epithelium. Hematoxylin-eosin staining, magnification $\times 10^3$.

There are often observed cells in pyknotic state with nucleus and necrosis in the epithelium. The reactive state of epithelium is also expressed by relatively a great number of cell height and epithelium expressed in oxyphilic reaction (Fig. 3). The recovery process of epithelium is also smaller. Tab. 2 illustrates the *P. ridibundus* gut epithelium cell mitotic index definition data in the basin of the Hrazdan River near the vv. Geghanist, Qaghsi, Argel, Darbnik and 0.5 *km* down Arzni HPS (Fig. 4).

Table 2

Mitotic activity of P. ridibundus' gut epithelium from different parts of the Hrazdan River (‰)

Location	v. Darbnik	v. Geghanist	v. Argel	v. Qaghsi	Arzni HPS
Mitotic activity	14.00 ± 1.155 $p<0.05$	19.00 ± 1.378 $p<0.05$	23.67 ± 1.453 –	20.33 ± 3.756 $p>0.05$	23.33 ± 0.882 $p>0.05$

As it is seen from the Table, the frogs' intestinal epithelium cells mitotic activity is smaller in the most polluted parts of the river, which are near the Geghanist and Darbnik villages compared with those living near the surrounding areas of the river, which are characterized with less water pollution. It directly points out the fact of epithelium regeneration reduction activity in the intestine of frogs living in the parts of the river having higher degree of pollution.

The comparative study of the intestine of frogs living in different areas of the Hrazdan River characterized by different degrees of pollution also gave an opportunity to discover adaptive-defensive changes, which are again stressed in the intestine of frogs caught from the most polluted parts of the river. First of all, it is expressed by the presence of lymphocytes and thrombocytes in gut epithelium.

An attention is also paid to the relatively frequent presence of lymphoid tissue islets in submucosal layer.

Our study has shown the fact that the mast cells activity in submucosal layer is higher in the intestine of frogs, living near the most polluted surrounding areas of the Hrazdan River near the Darbnik and Geghanist villages, which is manifested in the considerable increase of its number. They create accumulations in the intestine's submucosal layer. These cells are characterized by considerable polymorphism, which is expressed in a variety of sizes and shapes (Fig. 5). They are oblong, sometimes protruded the cytoplasmic grains can be of different density. Degranulated cells are also observed in cytoplasmic grains with different density. Taking into account the role of mast cells in the processes of inflammation, immunogenesis, blood clotting, circulatory regulation and in the local maintenance of homeostasis, it can be concluded that the animal's immune defense process is intensified.

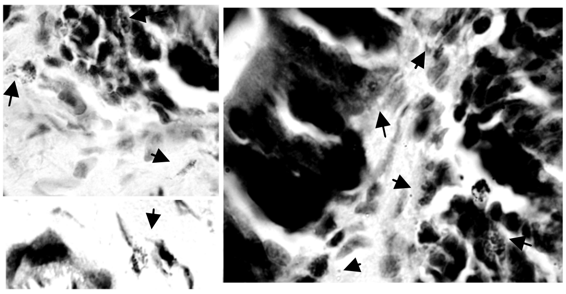

Fig. 5. Mast cells in *Pelophylax ridibundus*' gut epithelium. Pappenhaym staining, magnification ×10^3.

Conclusion. The data acquired from our study illustrates the histomorphological features of the intestine of frogs living in different surrounding areas of the Hrazdan River characterized by different degrees of pollution [1, Tab. 1]. The intestinal epithelium of frogs living near the most polluted parts of the river in the Darbnik and Geghanist villages illustrates regressive changes, which is indicated in the phenomena of piknotic changes and necrosis. Defensive phenomena are simultaneously displayed, such as the presence of a great number of lymphocytes and lymphoid tissue accumulations in the epithelium. An increasing amount of fibroblasts is observed in the mucous membrane. The comparative study of small intestines of frogs caught from different parts of the Hrazdan River indicates the increased activation process of the mast cells in the most polluted parts of the river. Our research has shown the presence of accumulations in submucosal layers of the intestines of frogs living in the most polluted parts of the Hrazdan River near the Darbnik and Geghanist villages. All the described defensive-adaptive phenomena witness the intensification of the body's/organism's immune defense processes in the most polluted environments.

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