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Study of pathologies of the red blood cells of silver crucian (*Carassius gibelio* (Bloch, 1782)), by Samara river, Dnipropetrovsk region, Ukraine

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ABSTRACT

The study of hematological parameters is an important method of studying the health of fish in ecosystems that are exposed to the negative impact of the environment. In this work, we determined the presence of pathological changes in the red blood cells of the crucian carp of the Samara River under conditions of anthropogenic stress. The object of the study were six-year-old individuals of both sexes of silver crucian carp (Carassius gibelio (Bloch, 1782)). Morphological studies of erythrocytes were performed on blood smears, which were stained according to the method of Romanovsky-Gimza. During the analysis of the obtained data, the presence of cells with pathologies and the nature of these pathologies were determined. As a result of the conducted researches pathologies of erythrocytes which arise at toxicological poisoning of fish by heavy metals and organic compounds have been revealed. Were also recorded at all experimental points the pathologies indicating active adaptation of fish to environmental stressors.

***Keywords*:** silver crucian, blood, vacuolization, schistocytosis, micronuclei, nuclear shadow, poikilocytosis, erythrocyte pathology, *Carassius gibelio*

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1. INTRODUCTION

Fishery products are an important component of the human diet, so it is not surprising that the demand of the population in Ukraine for it is growing. This, in turn, contributes to the gradual revival of the industry.

In Dnipropetrovsk region, silver crucian was of secondary importance in the structure of industrial catches for until recently. However, in the last few years, this species of fish has become one of the priorities [1].

Silver crucian is unpretentious, able to withstand significant fluctuations in environmental conditions, including low temperatures and hypoxia. Food sources are diverse: benthos, phyto- and zooplankton, macrophytes, etc. This makes it not only a valuable species for fish farming, but also an ideal object for studying the factors of environment influencing on fish health, which, given the significant level of anthropogenic pressure on the region's reservoirs, is extremely relevant [2, 20].

Dnipropetrovsk region is known for its developed chemical and metallurgical industries. In addition, the region's reservoirs regularly receive a significant amount of pollutants from crop and livestock complexes, utilities, medical facilities and recreation facilities. The inflow of industrial and domestic effluents into rivers contributes to changes in the temperature and hydrochemical regime of aquatic ecosystems, which affects the productivity of industrial fish species [3].

Therefore, it is important to use methods of studying the health of fish, which allow to detect in the early stages of pathological changes in their physiological state, associated with the influence of environmental conditions, including food and the presence of infections or invasions. One of such methods is the study of hematological parameters, which, in combination with other methods, will be an effective tool for diagnosing the level of morbidity and stress resistance of fish [4].

Analysis of literature sources showed that information about pathological changes at different levels of the body caused by the impact of negative environmental factors on the ecosystem of the Samara River is limited.

For the most part, it belongs to the Samara Bay of the Zaporozhye Reservoir.

The purpose of this work was to determine the presence of pathological changes in the red blood cells of the silver crucian of the Samara River in the terms of anthropogenic loading.

2. EXPERIMENTAL / RESULT

2. 1. Characteristics of the study area

The Samara River is a left tributary of the Dnieper River. It is the main source of water supply for many industrial and commercial enterprises of the region, which leads to a high level of anthropogenic pressure on the river ecosystem. The environmental situation is exacerbated by the significant development of heavy, metallurgical, chemical industries, overpopulation in the region and outdated environmental infrastructure. All this contributed to the fact that today the Samara river basin is characterized by a high level of pollution and depletion of the ecosystem [3].

On the territory of Ukraine, the Samara River originates on the western slopes of the Donetsk ridge. Within the city of Dnipro, the river flows into the Zaporizhia Reservoir. The

length of the river is 311 km, the area of the basin is 22660 km². The river has a slope of 0,33 m/km. The width of the river in the narrowest place is 2,5 km, in the widest 12 km. The estuary of the Samara River is flooded by the waters of the Samara Bay.

There are many settlements and recreation facilities along the Samara riverbed, so it's actively used for urban and industrial water supply, as well as for agricultural purposes. All sections of the Samara River are characterized by a high level of water pollution by heavy metals, petroleum products and suspended solids. It should be noted that according to the State Food and Consumer Service, the concentration of nickel, cobalt, manganese and cadmium in most parts of the river exceeds the maximum allowable concentration. In some parts of the Samara River there is an increased content of nitrites and ammonium. The waters of the Samara River belong to the sulfate class of the calcium-sodium group, they are dominated by ions SO_4^{2-}, Ca^{2+} and Na^+, K^+. During the summer-autumn period, water salinity is 2,2 – 3,8 g/dm³ [5]. The bigest polluters of surface waters of the Samara River, which discharge untreated return waters into the river basin, are the enterprises of mining, chemical and household industries in Pavlograd, Novomoskovsk and Dnipro. Pollution waters of mine horizons should also be included as polluting factors [6].

Three sections of the Samara River within the Dnipropetrovsk region with different levels of anthropogenic load were selected for the study - the village of Khashchove, town Novomoskovsk and the village of Novoselivka (Fig. 1).

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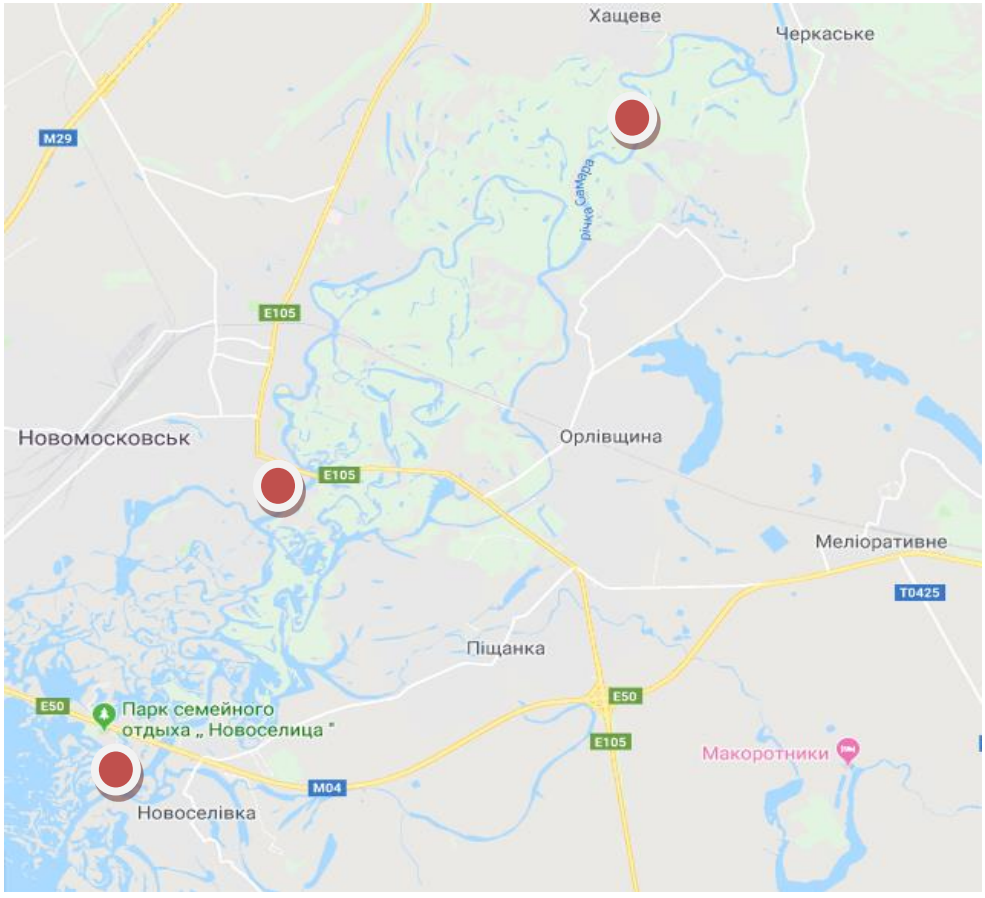

Figure 1. Control Sampling points on the Samara River

Within the first research point village Khashcheve (48° 42'11" N 35° 20'23" E), on the right bank of the river, there is a livestock farm and three recreation centers. The second research point is Novomoskovsk town (48° 38′16 35 N 35° 13′43″ E), the administrative center of Novomoskovsk district. The territory of the city occupies the right bank of the Samara River. Along the river within the city there are two children's camps and six recreation centers. Urban sewage also enters into the Samara River. Along the riverbed near the third research point village Novoselivka (48° 34′16" N 35° 14′31 ″ E), on the left bank, there is a children's camp, two recreation centers and a tourist complex. The river in this place forms numerous old rivers, estuaries, swampy reservoirs. The village is located at the confluence of the Samara River with Lake Samara Bay (lower part of the Zaporozhye Reservoir).

2. 2. Materials and methods

The object of the study were six-year-old individuals of both sexes of silver crucian (*Carassius gibelio (Bloch, 1782)*). Material for the study was collected using gill nets by research fishing in the summer-autumn period during 2019-2020. Blood was taken from the tail vein. Morphological studies of erythrocytes were performed on blood smears, which were stained according to the method of Romanovsky-Gimza. Blood smears were examined with a 40X magnification lens using a «Sciencelab T500 5.17 M» digital camera. 150 fields of view were viewed during the research.

During the analysis of the obtained data, the number of erythrocytes in the visual fields, the presence of cells with pathologies and the nature of these pathologies were determined.

Statistical processing of the obtained data was carried out according to the generally accepted methods using Excel 2010. The significance of the difference between the values between the experimental points was determined using the Student's test.

2. 3. Research results

Today there are many articles and materials about the effects on the body of fish various pollutants: ammonia, pesticides, heavy metals, crude oil, nitrogen and others. However, in natural reservoirs it is rare to find any one type of pollutants, mostly they form complexes. At the same time, unpredictable connections begin to operate between the components of such complex, which enhance or neutralize the action of pollutants. Therefore it is expedient to analyze the influence on the physiological state of aquatic organisms not only individual pollution factors, but of the whole complex [7].

Blood is one of the main indicators of the physiological state of the body. Pathological changes that occur with cells perfectly characterize the changes that occur in the body under the influence of the environment and stressors [8, 14]. Therefore, using of red blood test methods to determine the anthropogenic impact on aquatic organisms is relevant [22].

The concentration of erythrocytes in the blood of fish is on average in 5-10 times lower than in the blood of mammals. Their size, number and morphological features directly depend of nutrition and living conditions [15]. The main function of erythrocytes is oxygen transfer. It is known that smaller cells have a higher gas exchange rate [16]. In the ecological conditions of the studied points of the Samara River in the Dnipropetrovsk region, pathologies were detected in the erythrocytes of the silver crucian, which will be discussed in more detail below.

With the arbitrary deformation of the erythrocyte, the shape of the cell changes without changing its volume and area. The cell has an irregular shape, but its size is normal [9].

The study revealed the following forms of poikilocytosis: teardrop-shaped, rod-shaped, sickle-shaped, spindle-shaped (Fig. 2).

At drop-shaped deformation the cell of eritrocite is pointed on the one hand. The main part of the erythrocyte has a normal shape and width. With a rod-shaped deformation, the shape of the cell is close to cylindrical, it is slightly elongated and flattened on the sides. The area of the erythrocyte is almost unchanged. With crescent-shaped deformation, the erythrocyte cell has an elongated and concave shape resembling a sickle, the nucleus is displaced to one side [9, 12].

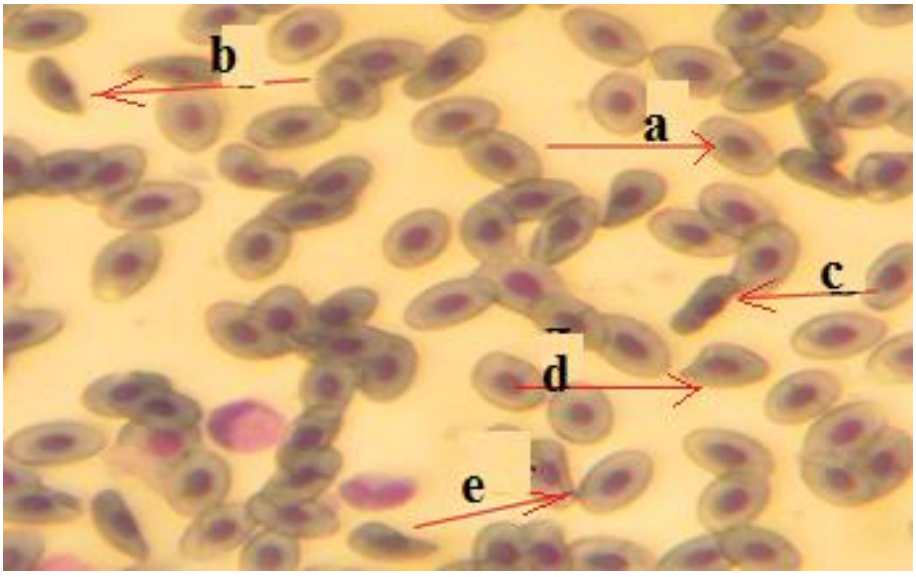

Figure 2. Forms of erythrocyte deformation: a - erythrocyte of normal shape, b - crescent-shaped deformation, c - rod-shaped deformation, d - teardrop-shaped deformation, e - arbitrary deformation.

The change in the shape of the erythrocyte may be the result of a decrease in the elasticity of the cell membrane. In turn, this may be an indirect consequence of changes in osmotic pressure [14, 20].

Nuclear pathologies of peripheral blood reflect well how favorable the aquatic environment is for fish at the time of its capture. It is known that when eliminating the factor influencing the body, its physiological responses to stress will return to normal [8, 10].

With an accentrically located, or parietal, nucleus, it is not in the center, but shifted to one of the poles of the cell. This pathology occurs not only among young but also among mature erythrocytes. It should be noted that it usually does not affect the size and shape of the cell. The parietal nucleus is observed in violation of cell turgor [9, 17].

Among the nuclear pathologies in the blood samples of the studied fish was observed deformation of the nucleus - a violation of its shape while maintaining normal size. This pathology is observed in fish that are exposed to high toxic effects of the environment [13, 21].

The following nuclear pathologies were also found in silver crucian: karyolysis - observed when dissolving part of the nucleus, pyknosis - occurs during cell aging at the apoptosis, fragmentation of the nucleus - it is separation from the nucleus its fragments, chromatinolysis

- decay of nucleus chromatin and loss of its structure, karyorexis - provided that the nuclear envelope is preserved, the nucleus disintegrates into individual fragments. In some individuals was observed the phenomenon of doubling of a nucleus or two nuclei . Such pathology is a kind of amitosis disorder [9, 17, 21].

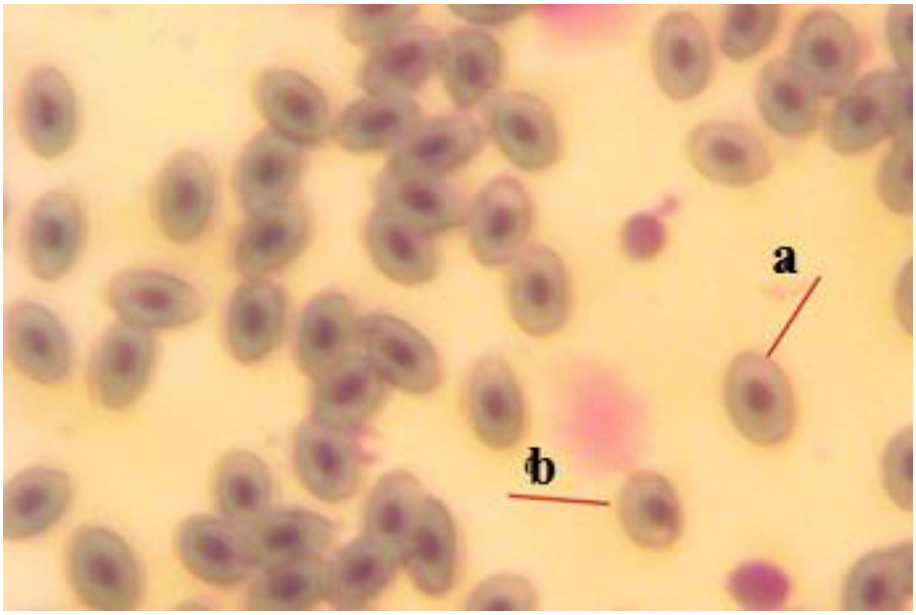

Figure 3. The main forms of erythrocyte pathology: a - erythrocyte with a normally located nucleus, b - erythrocyte with an acentrically located nucleus.

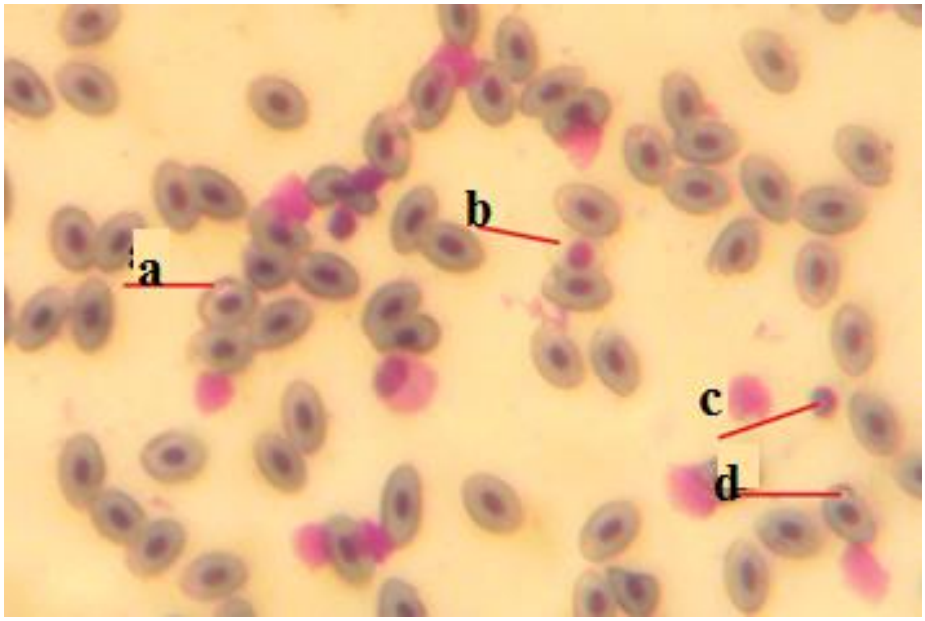

Figure 4. The main forms of erythrocyte pathology: a - erythrocyte edema, b - schistocytosis, c - nuclear shadow, d - vacuolation of erythrocytes.

It was also recorded the presence of micronuclei, which are separate fragments of the cell nucleus. Detection of these pathologies characterizes the unfavorable living conditions for silver crucian and is evidence of acute toxicological effects on fish [11, 18, 19].

The study revealed erythrocytes with vacuolation of the cytoplasmic membrane of the cell. The cytoplasm may contain from one to several vacuoles [9, 10].

Numerous cases of schistocytosis, a process that destroys a cell, have been identified in a blood test from all three points. At the same time the nucleus loses its structure, often there is no cytoplasm, wrinkling of a cover, often with breaks is observed. There are also erythrocytes with a scalloped edge. In this pathology, the cell membrane has one or more outgrowths filled with cytoplasm [9].

Almost all of the studied individuals of silver crucian in blood smears were found the nuclear shadows, which indicates the active processes of destruction of erythrocytes. The phenomenon of agglutination of red blood cells and the formation of "coin columns" was also detected in the samples [24].

There was no significant difference between the number of erythrocytes with pathologies in the blood of silver crucian from different experimental points (Table 1), but the highest proportion was in fish caught near the village Khashcheve. Most often erythrocytes with an accentric nucleus were observed in blood samples of fish from the village Khashcheve. Their number was 6.07 ± 0.50%. Rarely, this pathology occurred near the city of Novomoskovsk and was 3.97 ± 0.32%. There was a significant difference between the indicators of the number of erythrocytes with an accentric nucleus in silver crucian from Novomoskovsk and the village Khashcheve. Between the indicators of fish from the village Khashcheve and village Novoselivka, as well as from the village Novoselivka and city of Novomoskovsk significant difference was not found.

Table. 1. Pathology of erythrocytes of silver crucian in Samara River, %.

Pathology	Khashcheve	Novomoskovsk	Novoselivka
	M ± m	M ± m	M ± m
Acentric nucleus	6,07 ± 0,50	3,97 ± 0,32	4,59 ± 0,38
Vacuolization	6,59 ± 0,54*	4,73 ± 0,39	4,30 ± 0,35*
Swelling of the erythrocyte	3,99 ± 0,33*	2,57 ± 0,21*	2,28 ± 0,19*
Schistocytosis	9,18 ± 0,75*	6,72 ± 0,55	3,60 ± 0,29*
Poikilocytosis	10,63 ± 0,87*	9,45 ± 0,77	6,71 ± 0,55*
Nuclear deformation	2,82 ± 0,23*	2,19 ± 0,18*	1,31 ± 0,11*
Karyolysis	2,61 ± 0,21*	2,08 ± 0,17*	1,37 ± 0,11*
Pyknosis	2,19 ± 0,18*	1,84 ± 0,15*	0,56 ± 0,05*

Bifurcation of the nucleus	0,71 ± 0,06	0,34 ± 0,03	0,29 ± 0,02
Two nucleus	0,40 ± 0,03	0,38 ± 0,03	0,38 ± 0,03
No nucleus	0,00 ± 0,00	0,98 ± 0,08	0,00 ± 0,00
Karyorexis	3,58 ± 0,29*	2,11 ± 0,17*	0,58 ± 0,05*
Chromatinolysis	1,47 ± 0,12*	1,07 ± 0,09*	0,52 ± 0,04*
Nucleus fragmentation	1,94 ± 0,16*	2,04 ± 0,17*	0,96 ± 0,08*
Micronuclei	2,08 ± 0,17*	0,85 ± 0,07*	1,48 ± 0,12
Scalloped edge	10,37 ± 0,85*	6,21 ± 0,51*	4,45 ± 0,36*
Shell rupture	6,97 ± 0,57*	4,67 ± 0,38*	2,78 ± 0,23*
Doubling of an erythrocyte	1,10 ± 0,09*	1,03 ± 0,08	0,67 ± 0,06*
Erythrocyte wrinkling	1,34 ± 0,11	1,05 ± 0,09	2,04 ± 0,17
Nuclear shadow, pcs	2,55 ± 0,21	2,34 ± 0,19	3,59 ± 0,29

The difference is significant at $P < 0,05$*

We found a significant difference between fish with vacuolated erythrocytes obtained from catches in the village Khashcheve and village Novoselivka. Significant difference between other points in this indicator was not found. Most often this pathology occurs in silver crucian from the village Khashcheve and was 6,59 ± 0,54%, the least - in fish from the village Novoselivka (4,30 ± 0,35%). Often this pathology is accompanied by swelling of the erythrocyte and is associated with swelling of mitochondria and destruction of cell organelles and is the result of adverse living conditions.

The difference in the number of erythrocytes with swelling was significant in the blood of fish from city of Novomoskovsk and the village Khashcheve, and silver crucian from the village Khashcheve and village Novoselivka. The largest proportion of fish with this pathology was in the village Khashcheve (3,99 ± 0,33%), and the lowest - from the village Novoselivka (2,28 ± 0,19%). Swelling of erythrocytes may be the result of toxic effects, so it can be concluded that the most favorable conditions for fish are ecosistem of Samara River near the village Novoselivka.

Most numbers of erythrocytes, which showed signs of schistocytosis, were recorded in blood samples of silver crucian from the village Khashcheve (9,18 ± 0,75%). Much less often this pathology is fixed in blood samples of fish from village Novoselivka (3,60 ± 0,29%). The difference between the indicators of the number of cells with schistocytosis was not significant in the blood of fish from city of Novomoskovsk and the village Khashcheve. Since the phenomenon of schistocytosis shows the number of erythrocytes to be destroyed, it can be concluded that the conditions of the Samara River are not sufficiently favorable for this species of fish.

Deformation of erythrocytes was more common in silver crucian from the village Khashcheve (10,63 ± 0,87%), less often - from the village of Novoselivka (6,71 ± 0,55%). Significant difference between this indicator in fish from the experimental points was only for individuals of village Khashcheve and village Novoselivka. The phenomenon of poikilocytosis is a consequence of increased anthropogenic impact on the body of fish and the general deterioration of its physiological state. Therefore, we can assume that the best living conditions for the silver crucian were neare the village Novoselivka.

Among nuclear pathologies, we found a significant difference in the following indicators. Nuclear deformation, karyolysis, pyknosis, chromatinolysis and a fragment of the nucleus - between individuals from the village Khashcheve and village Novoselivka and silver crucian from city of Novomoskovsk and the village of Novoselivka. The phenomenon of nuclear doubling showed a significant difference between fish from city of Novomoskovsk and the village Khashcheve, and between village Khashcheve and village Novoselivka. According to the absence of the nucleus in erythrocytes, the significant difference was between the indicators of the blood of the silver crucian from city of Novomoskovsk and the village of Novoselivka. In terms of the number of erythrocytes in which the phenomenon of karyorexis was observed, the difference was significant in fish from all experimental points. According to the presence of micronuclei in erythrocytes, the study showed a significant difference between fish blood samples from city of Novomoskovsk and the village Khashcheve.

It is known that amitosis of blood cells is characterized as a pathological condition and refers to proliferative disorders of erythrocytes. The increase in the number of cells with amitosis can be seen as a manifestation of adaptation to heavy metals and radiation. In addition, the formation of cells with micronuclei may be a sign of pathological changes in the structure of erythrocytes due to the influence of stress anthropogenic factors [23].

Erythrocytes with a scalloped edge were most often recorded in blood samples of silver crucian from the village Khashcheve (10,37 ± 0,85%). Significant for this pathology was the difference between blood of fish samples from the village Khashcheve and village Novoselivka and between silver crucian from city of Novomoskovsk and the village of Novoselivka. Rupture of the erythrocyte membrane was most common in fish from the village Khashcheve (6,97 ± 0,57%), and least often in individuals caught near the village Novoselivka. The difference between this indicator in blood samples from all experimental points was significant. The presence of cells with these pathologies can characterize the state of toxicological poisoning of fish.

A significant difference between the number of blood cells with the pathology of red blood cell doubling, in contrast to other points, was not found in fish from the village Khashcheve and city of Novomoskovsk. Most often, this patology was recorded in silver crucian from the village Khashcheve (1,10 ± 0,09%), less often - from the village of Novoselivka (0,67 ± 0,06%).

Individuals with such pathology of red blood as wrinkling of erythrocytes were most often recorded in the village Novoselivka (2,04 ± 0,17%), and least often in the city of Novomoskovsk (1,05 ± 0,09%). The number of blood samples with nuclear shadow was the largest in the village Novoselivka, and the lowest in city of Novomoskovsk and was, respectively, 3,59 ± 0,29% and 2,34 ± 0,19%. Since the difference between blood samples from different research points for these pathologies was not significant, its presence in individuals can not be indicative. However, they indicate the presence of erythrocyte destruction processes, which may be a consequence about the death of old cells.

Due to the significant anthropogenic impact on the hydrochemical regime of the Samara River and its unsatisfactory ecological condition, the absolute number of individuals of silver crucian had various types of pathological changes in the structure of erythrocytes.

Table. 2. The number of individuals silver crucian with different types of pathologies of erythrocytes in Samara River

Research point	Number of individuals	Part of the fish with pathologies, %	
		1-2 pathology	more than two pathologies
v. Khashcheve	30	16,66	83,34
c. Novomoskovsk	30	13,33	86,67
v. Novoselivka	30	36,66	63,34

Most often fish with 1 - 2 pathologies in the structure of red blood cells are found in the village Novoselivka. The largest number of silver crucian with 3 or more pathologies was found near city of Novomoskovsk. Given that changes in the structure of erythrocytes of crucian carp were observed in all research points, as well as the presence of numerous enterprises in the mining, chemical, agricultural and housing and communal industries, we can assume a significant level of anthropogenic pressure on the reservoir [25-30].

3. CONCLUSIONS

The research results showed that the anthropogenic impact on the Samara River at all research points is quite high. Erythrocyte pathologies were detected in blood samples of crucian carp, which indicate the possibility of toxicological poisoning of fish with heavy metals and organic compounds. We also recorded pathologies in all research points that indicate the active adaptation of fish to environmental stressors.

Analyzing the obtained data, we can conclude that the most comfortable for the existence of crucian carp are the conditions of the Samara River near the village Novoselivka, which can be explained by a much lower level of anthropogenic impact than in the city of Novomoskovsk and the greater distance of this research point from the mine waters of Pavlograd. In contrast, the worst conditions were in the Samara River near the village Khashcheve. Despite the low anthropogenic load, this settlement is more affected by discharges from the mining industry.

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