

## MORPHOLOGICAL AND GENOTOXIC ANALYSIS OF FISH OF THE OKHCHUCHAY RIVER IN ZANGILAN DISTRICT

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**In July 2022, monitoring of the transboundary Okhchuchay River was carried out from the borders with Armenia deep into the Zangilan District of Azerbaijan. The Okhchuchay River is playing the role of an industrial waste collector, as it is constantly polluted with waste from the Kafan and Gajaran mining industries in Armenia. From the five monitoring points studied, only two specimens of *Oxynoemacheilus brandtii* were caught. For morphological and genotoxic studies, samples of peripheral blood, liver, and gills were taken. The analysis showed that in the gills of *Oxynoemacheilus brandtii*, hyperplasia of the interlamellar epithelium and respiratory epithelium of secondary lamellae, complete fusion of secondary lamellae, uneven distribution of hyperplasia of secondary lamellae, and detachment of the respiratory epithelium were detected. Expansion of sinusoids, melanomacrophage centers, and necrotic changes were revealed in the liver. When examining the blood, among the changes in the structure of the nucleus of erythrocytes, karyolysis, karyorrhexis, deformation of the nucleus, parietal nuclei, eccentric nuclei, and hemoglobin breakdown were observed. The obtained data confirm the catastrophic ecological state of the Okhchuchay basin.**

**Keywords:** Zangilan District, Okhchuchay River, *Oxynoemacheilus brandtii*, blood, gills, liver.

### INTRODUCTION

Okhchuchay is a river in the south of the Lesser Caucasus and a left tributary of the Araz River. It starts on the territory of Armenia. The river, most of which flows through the historical region of Azerbaijan, Zangezur, through the Zangilan District, liberated from occupation, and flows finally into the Araz River. The main water flows are formed from snow (46%), rain (10%), and groundwater (44%).

The transboundary Okhchuchay River has constantly been polluted on the territory of Armenia with wastes from the Kafan and

Gajaran mining industries, playing the role of a collector of industrial wastes. The Zangezur copper-molybdenum plant, whose main shareholder is the German company Kronimet Mining, which is engaged in mining at the Gajaran mine, creates a serious threat to the environment and human health [1].

In July 2022, employees of the Laboratory of Ecological Physiology of the Academician Abdulla Garayev Institute of Physiology participated in the monitoring studies of the Okhchuchay River for the purpose of determining the state of health of the ichthyofauna in this river. To determine the

degree of impact of various environmental factors on hydrobionts, gills, and livers, as well as pathologies of peripheral blood erythrocytes, are conventionally used as bioindicators [4, 8]. In this regard, the purpose of this work was to assess the histopathological changes in the gills and liver, as well as the genotoxic state of erythrocytes in fish from the Okhchuchay River.

## MATERIAL AND METHODS

During the monitoring studies, fishing was carried out at 5 points along the Okhchuchay River from the border with Armenia for a length of 35 km. The net for catching fish at each point blocked the entire river along its width. However, only in two of the five studied points it was possible to catch one specimen of *Oxynoemacheilus brandtii*, which had a body length of 11 and 11.5 cm, and a body weight of 10 and 8 g.

For histological examination, gill and liver samples taken from two specimens of *Oxynoemacheilus brandtii* were fixed in 10% neutral formalin. Processing of histological samples and staining of preparations with hematoxylin-eosin (according to Ehrlich) were carried out according to the generally accepted method [6]. The resulting preparations were studied under a NU-2 light microscope (Karl Zeiss, Jena) and photographed with a Motic digital microscope.

Genotoxic analysis was undertaken using a micronucleus test, which, due to its simplicity and the possibility of rapid analysis, has become one of the main methods for studying the influence of the environment on cytogenetic activity [16]. The advantage of the method is that in addition to micronuclei it allows the identification of such nuclear pathologies as condensation of the nucleus (karyopyknosis) with its subsequent dissolution (karyolysis) or disintegration into condensed clumps (karyorrhexis). The more cells possess such damage, the fewer cells have micronuclei.

From the tail vein, with the help of an insulin syringe, a drop of blood was taken and a smear was prepared on a glass slide. The smear

was dried at ambient temperature and fixed in methanol.

The resulting preparations were stained by the Giemsa-Romanowsky method with a standard kit obtained from Sigma.

Pathological changes in blood erythrocytes were determined according to the recommendations of Zhiteneva et al. [3].

The proportion of cells with micronuclei (MN %) was determined by the ratio of the number of cells with micronuclei to the total number of analyzed erythrocytes. Similarly, the proportion of cells with other types of nuclear pathology was assessed.

Pathologies were counted under a light microscope at a magnification of 12×100 times. For each individual, 2000 cells were counted. The frequency of occurrence of cells with nuclear pathologies (FCNCP) was calculated using the formula:

$$\text{FCNCP} = n / N \times 100\%$$

where **N** is the total number of counted cells; **n** is the number of cells with nuclear pathology.

For each individual, the average number of cellular pathologies was calculated.

## RESULTS AND DISCUSSION

**Histological examination of the gills.** As a result of a histopathological study of the gills of *Oxynoemacheilus brandtii* from the first point of the Okhchuchay, hyperplasia of inter-lamellar and respiratory epithelium, complete fusion of secondary lamellae, uneven distribution of hyperplasia of secondary lamellae, and detachment of respiratory epithelium were found (Fig. 1). In the gills of *Oxynoemacheilus brandtii* from the second point, uneven hyperplasia of the respiratory epithelium, fusion of secondary lamellae, and detachment of the respiratory epithelium were revealed (Fig. 2).

Histopathological changes are assessed by identifying structural alterations in four types of reactions: circulatory disorders, regressive and progressive changes, and inflammatory processes. In addition, there are three degrees of gravity of alterations that determine the depth of these pathologies: 1 - minimal pathological

value, the lesion is easily reversible; 2 - moderate pathological gravity, the lesion is reversible in most cases, if the stress factor is taken off; 3 - severe pathological gravity, the lesion is irreversible [14].



**Figure 1.** Gills of *Oxynoemacheilus brandtii* from the first point. Detachment of the respiratory epithelium, hyperplasia of the inter-lamellar epithelium, complete fusion of secondary lamellae x240



**Figure 2.** Gills of *Oxynoemacheilus brandtii* from the second point. Uneven hyperplasia of secondary lamellae, detachment of the respiratory epithelium, a fusion of secondary lamellae x240

Pathologies, identified in the gills of *Oxynoemacheilus brandtii* harvested from both points, have a similar character. These changes may be a manifestation of the organism's adaptation at the tissue level, directed to increasing the distance between the circulatory system and the polluted environment [12].

Literature data indicate changes in the gills similar to our data obtained in monitoring studies [10,13] as in the studies conducted under laboratory conditions [2,11].

The alterations revealed in this study are the changes of the progressive type referred to 1<sup>st</sup> degree of gravity, i.e., they are easily reversible changes. At the same time, in the gills of *Oxynoemacheilus brandtii* from the first point the hyperplasia of the inter-lamellar epithelium is so pronounced in loci that it becomes the cause of the complete fusion of secondary lamellae. Complete fusion of secondary lamellae is a moderate pathology and is considered as a 2<sup>nd</sup> degree of gravity. As noted above, changes in the 2<sup>nd</sup> degree of gravity in the case of taking off of the stressful factors can be reversible.

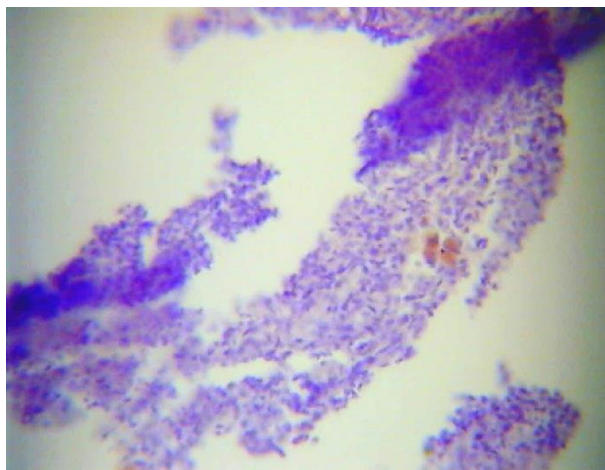
Thus, the histopathological changes revealed in the gills of *Oxynoemacheilus brandtii* from two points of the Okhchuchai River present the primary reaction of the respiratory system to the effect of a stressful factor and bear a protective-adaptive nature preventing the penetration of pollutants into the gills.

#### **Histological examination of the liver.**

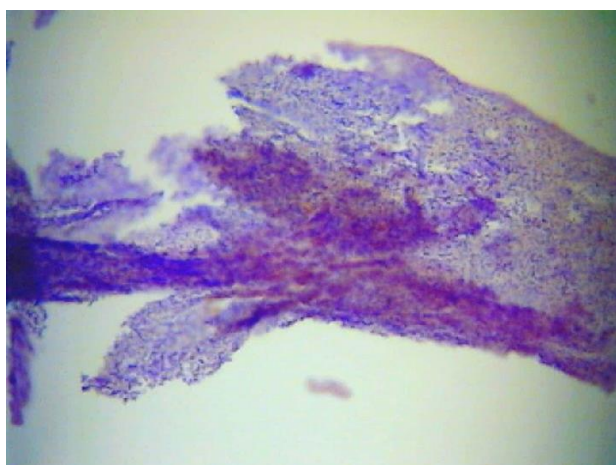
When opening the body cavity of the studied fish, we noticed that the liver was a dark brown, almost black color. During the histological study of the liver of *Oxynoemacheilus brandtii* from both points, the structural organization was somehow modified. The sizes of hepatocytes, as well as their nuclei, were increased. The expansion of sinusoids and melanomacrophage centers was revealed in the liver tissue. Necrotic changes, detected during the histological study of liver samples of *Oxynoemacheilus brandtii* from both points, are irreversible changes and indicate an unfavorable state of the environment for the survival of specimens [17].

Similar melanomacrophage centers and expansion of sinusoids have been described in the liver tissue of the carp and in the liver tissue of the cichlid fish *Pelvicachromic pulcher*, exposed for 15 days to a 40% concentration of water-soluble fractions of crude oil. An opinion is expressed that an increase in the number of melanomacrophage centers and their sizes

occurs in response to changes in the environment, and hence, macrophages can serve as reliable biomarkers of water quality in the case of chemical pollution [14, 17].



**Figure 3.** Liver of *Oxynoemacheilus brandtii* from the first point. Melanomacrophage centers, expansion of sinusoids, necrotic changes. x240



**Figure 4.** Liver of *Oxynoemacheilus brandtii* from the second point. Necrotic changes in hepatocytes. x240

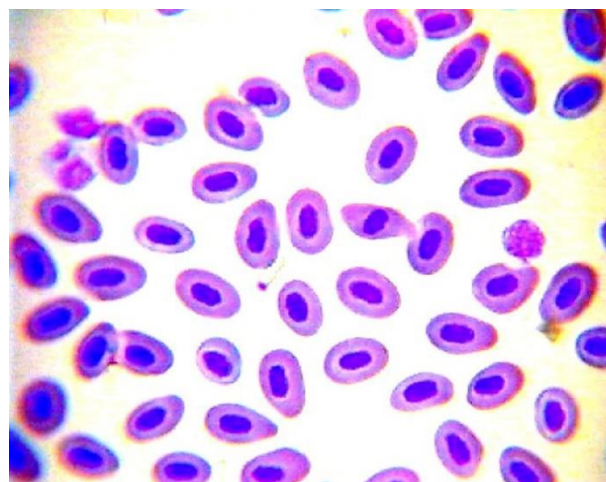
Pollutants enter the body of fish through the gills, skin, and orally through food and water. Adsorbed through the gills, skin, and intestinal mucosa, toxicants enter the bloodstream and either are broken down to the final products of water and carbon dioxide or turn into substances more toxic than the original ones. Here they are either excreted with bile through the excretory organs (gills and kidneys) or accumulated in the liver [17] Toxic substances accumulated in the liver for a long

time, in addition to destructive changes in the tissue itself, can have a negative effect on the body.

The micronucleus test revealed multiple pathologies of peripheral blood erythrocytes in captured fish. With a normal pathology not exceeding 5%, up to 70% pathology of peripheral blood erythrocytes was detected in the studied fish. Among the violations of the structure of the nuclei of erythrocytes, the following abnormalities were established: karyolysis, karyorrhexis, nucleus deformation, parietal nuclei, eccentric nuclei (the nucleus is displaced from the center), as well as the decomposition of hemoglobin. It should be especially noted that some of the erythrocytes were cigar-shaped, which may be indicative of an increased background of radioactivity in the river [9, 18].

**Table 1**

Point.1. Per 1000 erythrocytes	
Micronuclei amount	Nuclear pathologies
0	59.9
0	57.1

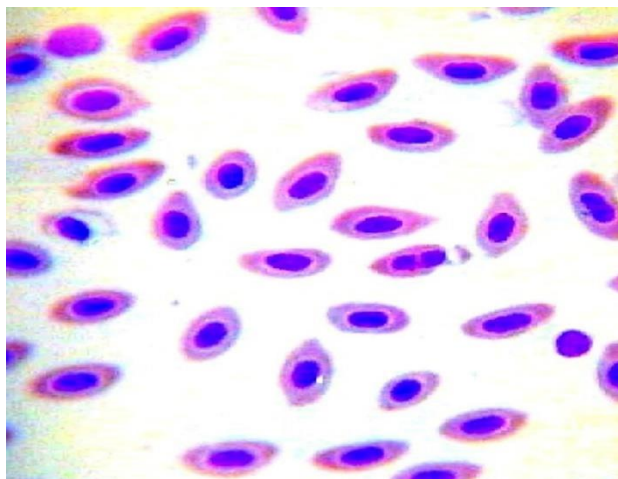


**Figure 5.** For *Oxynoemacheilus brandtii* from the first point the following pathologies were typical: hemoglobin breakdown, change in the shape of erythrocytes, displacement of the erythrocyte nucleus, cigar-shaped nuclei.

**Table 2**

Point.2. Per 1000 erythrocytes	
Micronuclei amount	Nuclear pathologies
0	58.1
1	67.8





**Figure 6.** For *Oxynoemacheilus brandtii* from the second point the following pathologies were typical: a change in the shape of erythrocytes; displacement of the nucleus; cigar-shaped (stick prominent) nuclei and one cell with a micronucleus.

## CONCLUSION

Due to transboundary water flows, approximately 70% of the land and water resources of Azerbaijan are formed in neighboring countries. At the same time, Armenia and Georgia have not yet acceded to the Helsinki Convention for the Protection of Transboundary Water Basins. This international document was adopted in Helsinki in 1992. It plays the role of a mechanism for the environmentally sound management of transboundary surface and groundwater and needs to strengthen international cooperation and national measures targeted at their protection. As issues from the data of earlier monitoring indicate, the transboundary Okhchuchay River is constantly polluted in the territory of Armenia with wastes from the Kafan and Gajaran mining industries, which have industrial importance. The Zangezur copper-molybdenum plant poses a serious threat to the environment and human health, polluting the Okhchuchay River on a catastrophic scale. These industrial wastewaters are discharged into the river untreated, which increases the level of pollution many times. Therefore, these water resources are considered unsuitable for use on the territory of our country. The Ministry of

Ecology and Natural Resources of Azerbaijan has conducted monitoring of the rivers flowing through the territory of the Zangilan region liberated from occupation. In water samples, an elevated level of heavy metals was found, in particular copper, molybdenum, iron, zinc, chromium, and manganese. According to monitoring reports, the content of the copper-molybdenum compound in the river water was twice as much as the norm, iron was 4 times higher, and nickel was 7 times higher than the maximally adopted concentrations. Due to pollution, the color of the water in the river periodically changes, and in the summer, when the water temperature rises, a strong, chemical smell comes from the river [1].

It should be noted that the Okhchuchay flows into the second major river of the South Caucasus, the Araz, which in turn is the largest tributary of the Kura, and it is also a transboundary river with Armenia and Iran. All these rivers play a decisive role in irrigating the sown areas of Azerbaijan. However, due to a serious change in the quality of water in these transboundary rivers, its use for domestic and agricultural needs can have an extremely negative impact.

The data obtained in the course of this monitoring confirm the catastrophic state of the Okhchuchay River basin.

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## ZƏNGİLƏN RAYONU OXÇUÇAY ÇAYINDA YAŞAYAN BALIQLARIN MORFOLOJİ VƏ GENOTOKSİK ANALİZİ

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2022-ci ilin iyul ayında Ermənistanla sərhəddən Azərbaycanın Zəngəlan rayonunun dərinliklərinə keçən Oxçuçay transsərhəd çayında monitorinq aparılıb. Oxçuçay Ermənistanda Qafan və Qacaran mədən sənayesinin tullantıları ilə daim çirklənən sənaye tullantılarının toplayıcısı rolunu oynayır. Beş monitorinq məntəqəsindən yalnız iki fərd *Oxyneomacheilus brandtii* tutulmuşdur. Morfoloji və genotoksik tədqiqatlar üçün periferik qan, qaraciyər və qəlsəmə nümunələri götürülmüşdür. Aparılan tədqiqatın nəticəsi göstərdi ki, *Oxyneomacheilus brandtii* qəlsəmə aparatında lamellərarası epitelinin və ikincili lamellərin tənəffüs epitelisinin hiperplaziyası, ikincili lamellərin tam birləşməsi, ikincili lamellərin hiperplaziyasının qeyri-bərabər paylanması, tənəffüs epitelisinin ayrılması aşkar edilmişdir. Qaraciyərdə sinusoidlərin genişlənməsi, melanomakrofaq mərkəzləri və nekrotik dəyişikliklər aşkar edilmişdir. Qan nümunələri tədqiq edildikdə eritrositlərin nüvələrinin strukturunun pozulması arasında karioliz, karioreksis, nüvənin deformasiyası, divarönü nüvələr, eksentrik nüvələr və hemoglobinin parçalanması müəyyən edilmişdir. Əldə edilən məlumatlar Oxçuçay hövzəsinin fəlakətli vəziyyətini təsdiqləyir.

**Açar sözlər:** Zəngilan rayonu, Oxçuçay, *Oxyneomacheilus brandtii*, qan, qəlsəmə, qaraciyər.

## МОРФОЛОГИЧЕСКИЙ И ГЕНОТОКСИЧЕСКИЙ АНАЛИЗ РЫБ РЕКИ ОХЧУЧАЙ В ЗАНГЕЛАНСКОМ РАЙОНЕ

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В июле 2022 года был проведен мониторинг трансграничной реки Охчучай от границ с Арменией вглубь Зангеланского района Азербайджана. Река Охчучай постоянно загрязняется на территории Армении отходами Кафанского и Гаджаранского горнодобывающих производств, играя роль коллектора промышленных отходов. Из пяти мониторинговых точек только в двух были выловлено по одной особи *Охуноемачейлус брандtii*. Для морфологического и генотоксического исследований были взяты образцы периферической крови, печени и жабр. Проведенный анализ показал, что в жаберном аппарате *Охуноемачейлус брандtii* были выявлены гиперплазия межламеллярного эпителия и респираторного эпителия вторичных ламелл, выраженное слияние вторичных ламелл, неравномерное распределение гиперплазии вторичных ламелл, отслоение респираторного эпителия. В печени были выявлены расширение синусоидов, меланомacroфаговые центры и некротические изменения. При исследовании крови среди нарушений структуры ядер эритроцитов установлены кариолизис, кариорексис, деформация ядра, пристеночные ядра, эксцентричные ядра, распад гемоглобина. Полученные данные подтверждают катастрофическое состояние бассейна реки Охчучай.

**Ключевые слова:** Зангеланский район, река Охчучай, *Охуноемачейлус брандtii*, кровь, жабры, печень

Çara təqdim etmişdir: Arif Əli-Övsəd oğlu Mehdiyev (redaktor), b.e.d., dosent.

Redaksiyaya daxil olma tarixi: 14.09.2022.

Təkrar işlənməyə göndərilmə tarixi: 26.09.2022.

Çara qəbul edilmə tarixi: 10.12.2022.

<https://ajp.az>



