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WATER QUALITY AND PROTECTION: ENVIRONMENTAL ASPECTS

# Water Quality Estimation Based on Histological Investigations of Fish: Case Study of Kenozero Lake

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**Abstract**—Specific hydrological and hydrochemical conditions in Kenozero Lake have led to changes in the morphology of fishes at a cellular level. The abnormalities in the function of the respiratory, digestive, and excretory systems were recorded. The degree of pathology depended on the ecological features of the fish. The pathological changes were most pronounced in the gills of the fish species (orfe, perch, and roach) characterized by active mode of life and sensible to oxygen deficiency. The changes in the epithelium of gill filaments had a compensatory significance and were aimed at increasing the area of the functional surface of the gills under the conditions of hypoxia.

## INTRODUCTION

The northwestern region of Russia is subject to anthropogenic pressure over many decades as a result of the mining and processing of mineral resources. Several areas in the Murmansk oblast, Arkhangelsk oblast, and republics of Karelia and Komi undergo an environmental crisis: wastewaters from large plants of the copper–nickel and mining complex and oil and coal production are discharged into the lakes and rivers of these regions. Their waters contain heavy metals (Ni, Cu, Zn, Sr, Al, Pb, and Cd), sulphates, suspended substances, flotation reagents, oil, nitrates, and other components. Therefore, freshwater ecosystems with natural characteristics are rare. One of such areas in the Arkhangelsk oblast is the Kenozero National Park, which has been included by UNESCO in the list of biosphere reserves with a worldwide significance. However, the anthropogenic activity in this area has been observed over several decades, and these ecosystems cannot be confidently regarded as undisturbed. Once this territory has the status of a National Park, it is important to evaluate the quality of environment in the lakes and rivers essential for the life of hydrobionts. According to the Directive of the European Parliament [13], the water quality should be characterized based on the hydrochemical and biological parameters. The goal of this study was to assess the efficiency of the use of several characteristics of fishes for the evaluation of water quality.

## AREA, OBJECTS, AND METHODS OF INVESTIGATION

Kenozero Lake is one of the largest lakes in the Arkhangelsk oblast, and it is the largest lake in the Kenozero Lake System (Fig. 1). The area of the lake is 74.1 km$^2$, and its specific feature is a complex relief of the bed with large depths alternated with banks and bars. Maximum depth of the lake is 63.8 m [2, 9]. The littoral zone is poorly developed, and shallow areas with the depth of up to 5 m are observed within a very narrow coastal line. Therefore, the production capacity of higher water plants is low [3, 4]. The water body is characterized by a weak water exchange (the conventional water replacement factor is 1.1.)

The values of pH, alkalinity, conductivity, as well as the concentrations of main ions and microelements (Zn, Ni, Cu, Sr, Al, Mn, Al, Cd, and Cr) were determined in this study. The analyses were conducted in the laboratory of the Institute of Problems of Industrial Ecology, Kola Scientific Center, Russian Academy of Sciences. The laboratory was licensed, and its equipment had been verified and intercalibrated with 22 laboratories from Western Europe.

The fish were collected with gill nets, mesh size from 10 to 50 mm. The organs of fishes with the most pronounced response to the change in the water quality were chosen for the histological analysis. These included the gills, kidneys, and liver. In total, 43 specimens were investigated. The number of samples for the histological analysis was 129. At the first step of the study, special attention was devoted to the correct sampling of the material. During the sampling, the fish were alive. The analyzed tissues were fixed instantly to prevent the changes occurring in the tissues isolated from the organism and preserve the initial structure of the tissues. The tissues were fixed in Bouin solution. The width of the fixed material did not exceed 1 cm. The samples were treated according to conventional histological methods [10].

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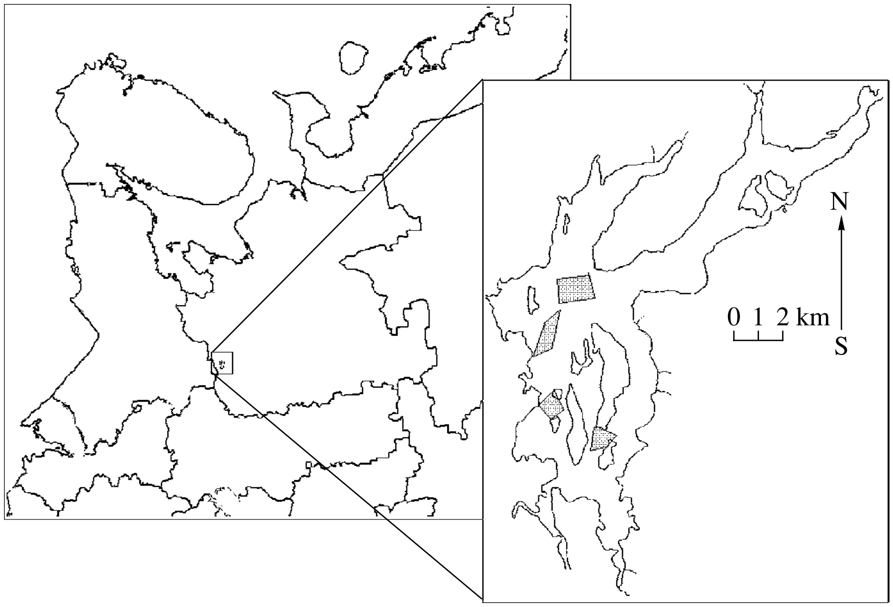



**Fig. 1.** Sampling sites in Kenozero Lake.

## RESULTS OF THE STUDY

*Main hydrochemical characteristics.* The waters of Kenozero Lake feature low salinity and belong to the calcium–hydrocarbonate class. The waters contain a large amount of organic substances of allochtonous origin, mainly of a humic nature. This can be supported by the coloration of water in the water body. Depending on the season, the coloration in the upper layer ranges from 77 to 109 degrees, which corresponds to a meso-polyhumous type of the water body. pH values (6.9–7.5) are close to neutral (table).

*Histological analysis of the fish organs.* The ichthyofauna of Kenozero Lake is represented by 21 fish species. They belong to seven families: whitefishes (European cisco, European whitefish, peled, and inconnu), smelts (smelt), pikes (pike), cyprinides (blue bream, bream, bleak, white bream, verkhovka, orfe, dace, minnow, roach, and goldfish), burbots (burbot), sticklebacks (nine-spined stickleback), and perchs (ruffe, perch, and sander). The objects for the histological analysis were the species prevailing in the community: bream, roach, perch, pike, and burbot.

### *Perch*

*Kidneys.* The pathological changes in the tissues of kidneys were connected with large hemorrhages in the parenchyma, which occurred, most likely, as a result of increasing blood supply, excessive pressure in the blood vessels, and breaking of their walls (Fig. 2b). These events led to the development of interstitial nephritis and caused subsequent maturation and transformation of infiltration cells, part of which transformed into fibroblasts. The latter cells formed a protein, protocollagen, representing the base for the formation of collagen fibres of the connective tissue. As a result, this tissue grew in the areas of necrosis of functional parenchyma (Fig. 2c), which could lead to the diffusive sclerosis of the organ.

The lipid dystrophy developing most often according to the infiltration type was recorded. This mechanism prevailed in the conditions of hyperlipidemy (increased lipid content of the blood). In this situation, the cells of the epithelium of nephric ducts and sometimes the cells of the kidney stroma infiltrated with a large amount of lipoids. However, in this case, most likely, the decomposition (destruction of membranes of internal cellular components with the discharge of lipoids) occurred, which had been recorded during the diseases caused by hypoxia. Due to the lipid dystrophy, the function of kidneys decreased.

*Liver.* In the liver of perch, no serious pathologies were recorded with the exception of small hemorrhages in the parenchyma.

*Gills.* Disturbance of the blood circulation was recorded in the gills of perch. It was connected with the widening of capillaries and preventing of blood supply (venous stasis) (Fig. 3b). As a result of these processes, the perfusion of tissues by blood was interrupted, and oxygen deficiency (hypoxia of tissues) formed. Numerous hemorrhages between gill filaments were observed (Fig. 3c). The change of the status of the blood system led to the deformation of gill filaments and gill lamellae. Angiomae caused by the disturbance of the vascular endothelium and disruption of walls of blood vessels were observed (Fig. 3d). The angioma was represented by a vascular tumor, which was localized, but was able to a rapid growth with the distribution onto

Hydrochemical parameters of Kenozero Lake (summer 2001)

| Parameter | Station 1 | | Station 2 | Station 3 | |
|---|---|---|---|---|---|
| | surface | bottom | surface | surface | water mass |
| pH | 7.40 | 6.98 | 7.10 | 7.18 | 7.52 |
| Conductivity, μS/cm | 44 | 46 | 43 | 46 | 91 |
| Ca, mg/l | 5 | 5.3 | 5 | 5.2 | 10.2 |
| Mg, mg/l | 2.00 | 2.21 | 1.93 | 2.07 | 3.96 |
| Na, mg/l | 1.57 | 1.56 | 1.57 | 1.57 | 2.57 |
| K, mg/l | 0.47 | 0.50 | 0.47 | 0.53 | 0.58 |
| $SO_4$, mg/l | 1.98 | 2.24 | 1.59 | 1.95 | 3.02 |
| Cl, mg/l | 1.35 | 1.72 | 1.44 | 1.81 | 2.44 |
| C, μg/l | 14.3 | 15.0 | 14.0 | 14.2 | 12.3 |
| Coloration, degrees | 93 | 109 | 92 | 92 | 77 |
| $NH_4$, μg N/l | 3 | 6 | 6 | 9 | 2 |
| $NO_3$, μg N/l | 5 | 83 | 5 | 9 | 1 |
| $N_{total}$, μg/l | 288 | 463 | 308 | 319 | 259 |
| $PO_4$, μg P/l | <1 | <1 | <1 | <1 | <1 |
| $P_{total\ f}$, μg/l | 6 | 7 | 6 | 6 | 6 |
| $P_{total\ nf}$, μg/l | 11 | 14 | 13 | 12 | 11 |
| Si, mg/l | 1.21 | 1.96 | 1.15 | 1.3 | 1.09 |
| Permarganate oxidation, mg O/l | 16.7 | 17.6 | 16.3 | 16.5 | 14.1 |
| Fe, μg/l | 236 | 380 | 450 | 270 | 230 |
| Al, μg/l | 88 | 220 | 105 | 97 | 130 |
| Sr, μg/l | 25.7 | 25.8 | 24.6 | 23.7 | 45 |
| Mn, μg/l | 10.1 | 19.5 | 15.7 | 15.9 | 9.8 |
| Zn, μg/l | 0.8 | 71 | 1.3 | 0.7 | 1.2 |
| Ni, μg/l | 0.6 | 1.5 | 0.6 | 0.5 | 0.7 |
| Cu, μg/l | 0.7 | 60 | 1.2 | 0.7 | 0.5 |
| Cr, μg/l | 0.5 | 1 | 0.5 | 0.5 | 0.5 |
| Co, μg/l | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |
| Cd, μg/l | <0.05 | 0.15 | <0.05 | 0.1 | 0.12 |

adjacent tissues. The presence of the tumor led to the appearance of thrombi and fibrosis of tissues, as well as to the adhesion between neighboring lamellae and their subsequent resorption. Finally, this situation could lead to the atrophy of the organ. In the majority of specimens, the hypertrophy of gill epithelium, hyperplasia of the epithelium of gill lamellae (Figs. 3e, 3f), desquamation of this epithelium, and adhesion between gill lamellae were recorded.

### *Roach*

*Kidneys.* Numerous parasitic invasion and related productive nephritis were the most pronounced anomalies in roach. The parasites were distributed in parenchyma causing serious necrotic and exudative changes of the tissue with subsequent proliferous reaction. As a result, capsules composed of the connective tissue were formed around the alien bodies. These capsules separated the places of nephritis from normal parenchyma (Fig. 2d). The zones of increased proliferation of the connective tissue, small hemorrhaged areas, and the signs of hemosiderosis (increased accumulation of hemosiderin) were recorded (Fig. 2e). Such a condition developed during extravascular hemolysis in the areas of hemorrhages, and it was connected with the disturbance in the transformation of hemoglobin pigments. The progressive hemosiderosis could lead to irreversible consequences due to the increased accumulation of ferritin with paralytic and hypotensive properties. As a result, the necrosis of tissues and atrophy of organs could occur. Changes were also recorded in the epithelial cells. The nephric ducts were occluded by the parasitic protozoa from the class Sporozoa. The reproduc-

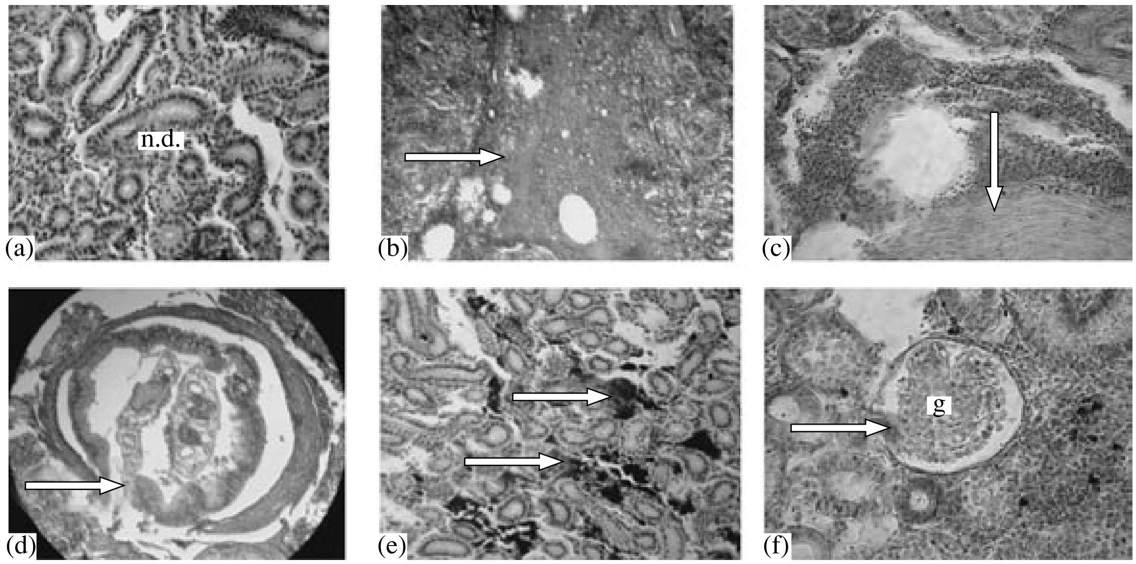



**Fig. 2.** Pathology of kidneys (see arrows). (a) Normal structure (n.d., nephric duct), ×200; (b) hemorrhage (perch), ×200; (c) proliferation of connective tissue (perch), ×320; (d) presence of the parasite (roach), ×200; (e) hemosiderin (roach), ×200; (f) parasitic protozoa in the kidney of roach (g, glomerulus), ×320.

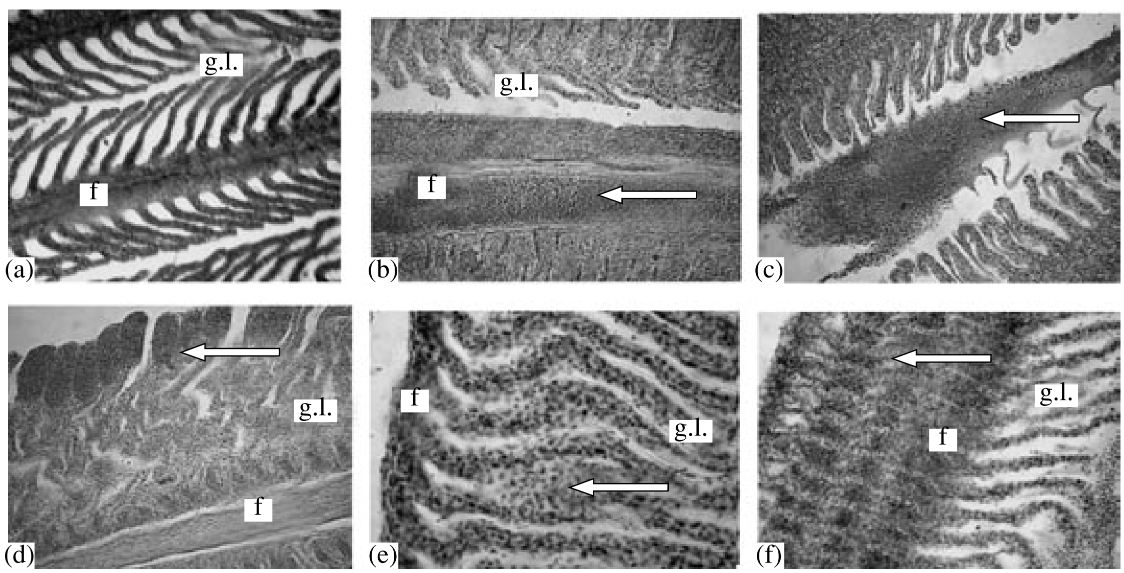



**Fig. 3.** Pathology of gills in perch (see arrows), ×160. (a) Normal structure (f, filament, g.l., gill lamella); (b) venous stasis; (c) hemorrhage between gill filaments; (d) angiomae and curved gill lamella; (e) hypertrophy of epithelium of the gill lamella (f) hyperplasia of the epithelium of the filament and fusion of gill lamellae.

tion of these organisms led to the nephritis and dysfunction of the nephric ducts. The same protozoa were observed in the space between the Bowman's capsule and glomerulus (Fig. 2f).

*Liver.* The microscopic examination of the liver showed that the architectonics of the organ was not disturbed, and the trabecular structure could be distinguished.

*Gills.* The changes in the structure of gill filaments in roach included the hypertrophy of all types of gill epithelium leading to the disappearance of free surface of gill lamellae contacted with external environment. The hypertrophy of the epithelium of gill lamellae and its desquamation were revealed.

*Bream*

*Kidneys.* Numerous hemorrhages were observed. They could lead to the interstitial nephritis and proliferation of the connective tissue, which replaced the tissue of parenchyma. Spots of necrosis of lymphoid tissue and occlusion of nephric ducts by parasitic protozoa were recorded.

*Liver.* The microscopic picture of the liver was normal. The architectonics was not disturbed, and the trabecular structure of the tissue could be seen.

*Gills.* The gills were disturbed to a lesser extent than in other fish species. A small adhesion between gill lamellae not leading to the dysfunction of the organ was observed.

### *Orfe*

*Kidneys.* Hemorrhages in the parenchyma and proliferation of the connective tissue in the areas of degenerated nephric ducts were recorded.

*Liver.* The necrotic processes together with the development of the capsules (from the connective tissue) separating the necrotic area from the normal tissue were revealed.

*Gills.* The heaviest pathological changes were recorded. They included the reduction and adhesion of gill lamellae, swelling of apical tips of gill filaments, desquamation of epithelium of gill lamellae, and hemorrhages as a result of the breaking of the capillary walls. In addition, the bifurcation of the gill filaments at the base of the branchial arch and their fusion in the apical parts were observed. This abnormality could be referred to the compensatory and adaptive reaction developing under the influence of hypoxia.

### *Pike*

*Kidney.* Hemorrhages, large zones of necrosis, disappearance of nephric ducts, as well as their occlusion were recorded.

*Liver.* The toxic dystrophy was diagnosed. It was associated with the pycnosis of nuclei and characterized by progressive necrosis of hepatocytes. In the areas of necrotic parenchyma, the proliferation of connective tissue (as rough seams) was recorded. This progressive sclerosis and structural reconstruction of the tissue could lead to the postnecrotic cirrhosis of the liver and subsequent development of dysfunction of the organ.

*Gills.* Insignificant structural changes connected with the adhesion of several gill lamellae were observed.

### *Burbot*

The microscopy of the organs and tissues of burbot showed that they were disturbed to a lesser degree in comparison to the changes revealed in other fish species from Kenozero Lake. Small sites of hemorrhages were recorded in the kidneys, the function of the liver was normal, and the adhesion of gill lamellae and necrotic sites in the epithelium of gill lamellae were observed.

## DISCUSSION

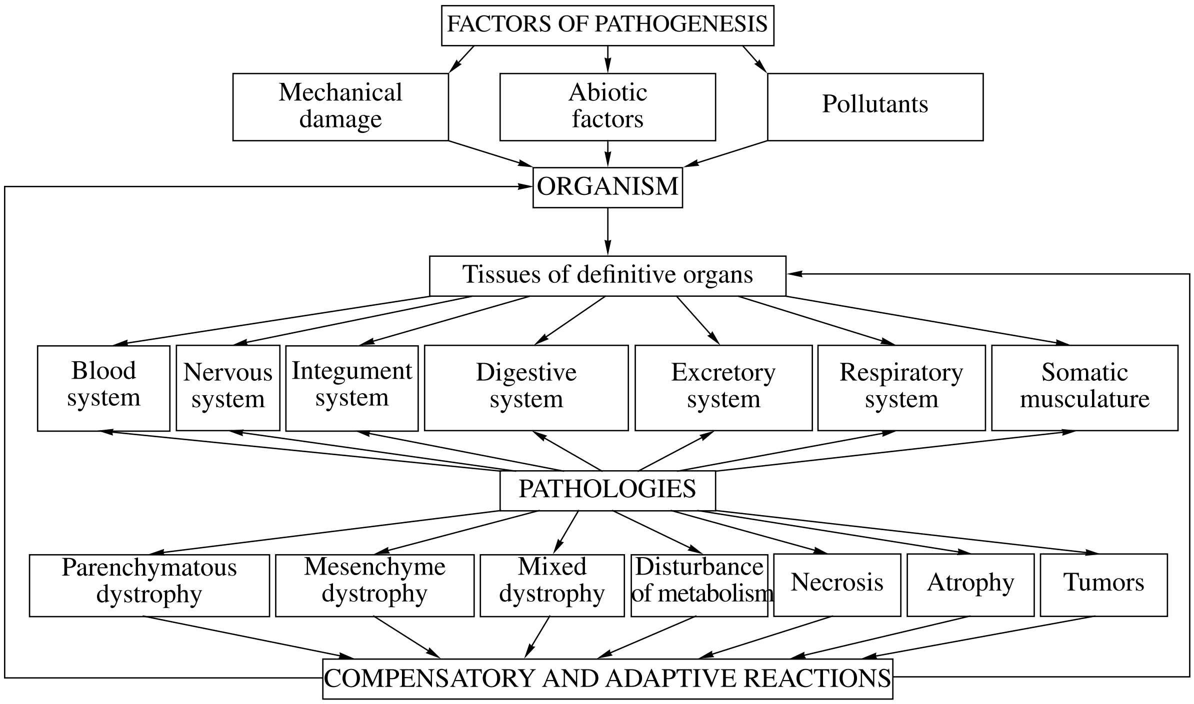
The hydrochemical investigations have established the occurrence of increased concentrations of Cu, Fe, Mn, and Al among analyzed chemical elements. It can be connected with the swampiness of the drainage basin area and the high concentration of these four elements in the geochemical province.

The only reliably established source of the negative anthropogenic load on the lake is timber rafting, which has been stopped. However, the results of hydrochemical analysis show the concentration of phenols in the water of Kenozero Lake to be comparatively high (from 3.4 to 9.7 μg/l, depending of the horizon). These values are five to ten times higher than the maximum permissible concentration (1 μg/l). In combination with a high level of water coloration, the increased concentration of phenols can be explained by the presence of humic substances represented by natural polyphenols. It can be suggested, that the consequences of the former timber rafting have an insignificant influence on the concentration of phenols, and the main source of phenols is humus.

According to the published data [2, 9], the quality of waters of Kenozero Lake has been relatively stable over several decades. The industrial and municipal wastewaters do not significantly affect the main hydrochemical parameters of the lake due to the low density of the population occupying several villages. The main source of organic matter, biogenic elements, and microelements is the territory of the drainage area. Nevertheless, the hydrochemical conditions and hydrochemistry of Kenozero Lake lead to the chronic stress in the fish inhabiting it.

The environmental factors that can induce pathologies are numerous. However, they lead to a pathogenous effect if the strength of their influence surpasses the defensive and adaptive properties of the organism. Acute intoxications lead to the development of compensatory and adaptive reactions aimed at the compensation of functions of damaged organs or systems. If the action of a pathogenous factor is not blocked and becomes chronic, the reactions of the organism are transformed into the irreversible degenerative processes. As a result, the organ or tissue is not able to implement its special function (scheme).

The histological analysis shows an occurrence of abnormalities in the structure and functions of some structures of the organism in all studied fish species from Kenozero Lake. The degree of the pathological changes depends on the ecological features of fish species. The most pronounced pathologies are recorded in the gills of orfe and perch (typical oxyphils with mobile mode of life and a high sensitivity to oxygen deficiency), as well as in the gills of roach (moderate riverine species). Burbot, bream, and pike are characterized by a low mobility, and the pathological changes of their gills are insignificant. The changes in the epithelium of gill filaments with an osmoregulatory function and in




Scheme of pathological development in fishes under the influence of negative environmental changes.

the epithelium of gill lamellae with a respiratory function have a compensatory significance, and they are directed toward an increase in the area of the functional surface under the conditions of hypoxia.

The pathological changes in the gills were aggravated by the prevention of normal blood circulation leading to hyperemia and blockage of the blood supply in the distal parts of branchial blood system. The observed accumulation of blood in the gills and formation of slime can have a compensatory significance. As is known, a large accumulation of blood can partly compensate for the negative effect of the hypertrophy of the gill epithelium on the gas exchange [15, 16, 19], and the presence of slime can reduce the negative cytological effect of metals [17, 18]. However, thick layers of slime reduce the total diffusive surface of the gills and, in addition, retard gas exchange, thus bringing about the development of respiratory and osmoregulatory stress [7, 21, 22]. Moreover, the increased secretion of slime by the hypertrophic cells leads to the appearance of a viscous layer, which is not usual for a normal gill. This layer represents a good substratum for a rapid development of pathogenous bacteria [14].

The most common pathologies of the kidneys and liver are necrosis and fibrosis. One of the main functions of nephric ducts is the secretion of bivalent ions. Therefore, the observed pathology of the epithelium of nephric ducts is most likely due to the contamination of waters by heavy metals, e.g., copper and cadmium [23]. In addition, the necrosis of tissues can be caused by parasitic protozoa and infectious diseases [11, 12].

Is it possible to conclude, based on the above data, that the anthropogenic load on the water body is heavy? The response of fishes to pollutants is characterized by general and specific features, depending on the environmental conditions and the properties of the organism itself. Many kinds of pollution can be diagnosed based on the “functions–targets”, and this method for revealing specific effects of pollutants is widely used in the international practice for the identification of the types of pollutants [1, 5, 6, 8, 20].

In the case of environmental pollution by several agents, the diagnostic of the changes in the organism is possible, but the separation of the effect of each agent based on the diagnosis seems exceptionally problematic, and additional analytical methods should be applied.

The investigation of fishes from Kenozero Lake suggests that the observed morphological and anatomical changes in the structure of the organism leading to the abnormalities in the function of respiratory, digestive, and excretory systems are the result of an unsatisfactory hydrochemical regime of the water body. Many abnormalities (necrotic processes and neoplasia), which have been recorded in the fish, are irreversible. However, such changes as hypertrophy, hyperplasia, reconstruction, and encapsulation represent the structural and functional bases for compensatory–adaptive reactions. Due to these reactions, the organism can pass to a new level of function that is helpful for the survival in the modified environmental conditions.

Thus, the analysis of the condition of fish organisms based on the histological methods can be successfully applied for the assessment of water quality. However, such analysis should be used together with the data of hydrological, hydrochemical, and hydrobiological observations.

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