Pelophylax ridibundus complex as bioindicator of the level of anthropogenic pollution of the environment

Zhannat Bazarbayeva[1], Oralbek Ilderbayev[1,*], Ainash Suleimenova[1], Gaziza Nurmukhambetova[1], Rauza Olzhayeva[2], Asiya Dukenbayeva[1], Ayauzhan Issina[1]

[1] Department of General Biology and Genomics, L.N. Gumilyov Eurasian National University, Astana, Kazakhstan
[2] Department of Biochemistry and Chemical Disciplines, Semey Medical University, Semey, Kazakhstan

*Corresponding author e-mail: ilderbayev_oz@enu.kz

Received: 2 January 2024 / Accepted: 14 May 2024

Abstract. The work presents the relationship between the degree of pollution of water bodies and changes in the structure of internal organs of the marsh frogs, Pelophylax ridibundus complex, inhabiting these bodies of water. To determine this relationship, two natural lakes and one artificial water reservoir located in an industrialised area near the city of Astana in Kazakhstan were investigated and conclusions were drawn regarding the gradient of anthropogenic pollution. In addition, histological examinations of the gastrointestinal tract of frogs caught from these water bodies differing significantly in the gradient of heavy metal concentration were carried out. It was found that of the analyzed organs, the stomach and liver were the most sensitive to pollution. The concordance of the intensity of damage to these organs with the degree of pollution of the lakes and the reservoir, as determined by physicochemical analysis of the water, indicate that the studied species can be considered a bioindicator of early pollution of aquatic biotopes.

Keywords: aquatic biotopes, amphibians digestive system, environmental bioindicators, heavy metals pollution, histopathological changes.

1. Introduction

In connection with the growing problem of environmental pollution, the study of amphibians as early bioindicators of the state of the environment makes it possible to assess the dynamics of ongoing changes in aquatic biotopes (Bazarbayeva et al., 2011; Jozwiak & Jozwiak, 2014). The main pollutants in surface water bodies are salt composition ions, biogenic and organic compounds, heavy metals, phenols, and suspended solids. Excess values of permissible concentrations are due to anthropogenic and natural-climatic factors, wastewater discharge from enterprises and industries (Chulenbaeva et al., 2018, 2022; Iztleuov et al., 2020; RSE “Kazhydromet”, 2021).

Contaminants that subsequently settle on the soil can be carried along with municipal runoff to nearby water bodies, streams and rivers. Of particular interest are such contaminants as heavy metals and insecticides, which have acute toxicity and can accumulate in biological systems and cause histopathological changes in the internal organs of amphibians (Don-Pedro et al., 2004; Tapbergenov et al., 2013; Taiwo et al., 2014; Iztleuov et al., 2019; Azizishirazi et al., 2021, Păunescu et al., 2010; Jayawardena et al., 2017.

As negative factors impact on the environment, the biodiversity and bioproductivity of natural biogeocenoses decrease.

Biomonitoring is one of the stages of the consistent process of studying the state of the ecosystem. Therefore, the aim of biomonitoring is to establish the level of biota pollution in order to alert and prevent, diagnose and predict.

Amphibians that live on the outskirts of settlements might be affected by a number of anthropogenic factors. The semi-permeable and highly vascularized skin enables natural respiration in amphibians and thus gives them a high propensity to accumulate environmental contaminants in their tissues from water and moist environments (Purucker et al., 2023). The earliest reactions to unfavorable environmental factors can be identified during macro- and micromorphological studies at the cellular and tissue levels of the most important internal organs. Of great interest is the study of the organs of the digestive system, which first comes into contact with toxicants that enter the body with food and water. With the help of bioindicators, it is possible to determine the degree of danger of the corresponding state of the environment for all living organisms, including humans.

Bioindication provides for the detection of environmental pollution that has already taken place or is taking place according to the morphological and functional indicators of individuals. In general, biomonitoring methods, being relatively low-cost, allow one to reveal the degree of pollution of individual biotopes, and to choose environmental measures, to make adjustments to economic activities. In bioindicative methods, the main advantages over the methods of physical and chemical analysis can be listed. The first is that the responses of organisms reflect important data about the environment as a whole; detect the presence of a complex of pollutants in the environment; bioindicators respond to very weak anthropogenic impacts; they can be used to determine the rate of ongoing changes in the environment; serve as an indicator of contaminated places of various kinds in accumulations and the probable routes of entry of these substances into the human body.

Features of the biology of amphibians make them the most convenient objects for biomonitoring. They are able to withstand large anthropogenic loads, while the structure and composition of the community do not change. Ease of collection and observation, longevity, abundance, optimal body size, high fecundity and vitality, close relationship with the habitat, ecological plasticity, morphostructural mobility, and ease of laboratory maintenance also favor bioindication (Lutsenko, 2001; Lutsenko & Vasiliev, 2007; Polo-Cavia et al., 2016; Brady et al., 2022; Burgos-Aceves et al., 2022).

Currently, the ecological state of water, air and soil in various regions of the Akmola region and Astana is being actively studied. Nevertheless, we did not find works on biomonitoring of various biotopes by studying cellular and tissue changes in the amphibian digestive system.

The purpose of our work was to conduct a histological study of the digestive organs of the marsh frog of the *Pelophylax ridibundus* complex from Lake Maibalyk, Lake Zhaltyrkol and the Koyandy reservoir of the Akmola region, and to explore the potential of using frogs as environmental bioindicators in this industrialised area.

2. **Materials and Methods**

The material for this work was collected in the period June-August 2022 in the Akmola region (Koyandy reservoir (51.337398° 71.713656°), Lake Zhaltyrkol (50.992356° 71.818213°), Lake Maibalyk (51.02711° 71.495383°). The material for the study was the tongue, esophagus, stomach, duodenum and liver of the marsh frog. Pieces of organs were fixed in 10% neutral formalin and processed according to the generally accepted histological technique. Ten specimens of marsh frog (30 in total) were caught from each studied water body and 150 histological preparations were prepared. The protocol of the study was reviewed and approved by the Local Ethics Committee of the NAO "Semey Medical University", Kazakhstan (Protocol No. 6 dated 05/11/2022). Microphotography of histological preparations stained with hematoxylin-eosin was carried out on an inverted fluorescent microscope EVOS FLc, specialized for visualization of cellular structures. Heavy metals in water were determined by atomic spectrometry.

3. **Results**

Tongue. The tongue of the lake frog was a muscular organ covered with a mucous membrane and multi-row ciliated epithelium. Muscle tissue consisted of striated muscle fibers. In the epithelium, there were unicellular glands – goblet cells in small quantities. In addition to unicellular glands, there were also simple tubular multicellular glands in the mucous membrane of the tongue.

Histological examination of the tongue of the marsh frog from Lake Zhaltyrkol revealed enlarged glands, different heights of the epithelium, and inflammatory infiltrates were found at the end of the papillae (Fig. 1).

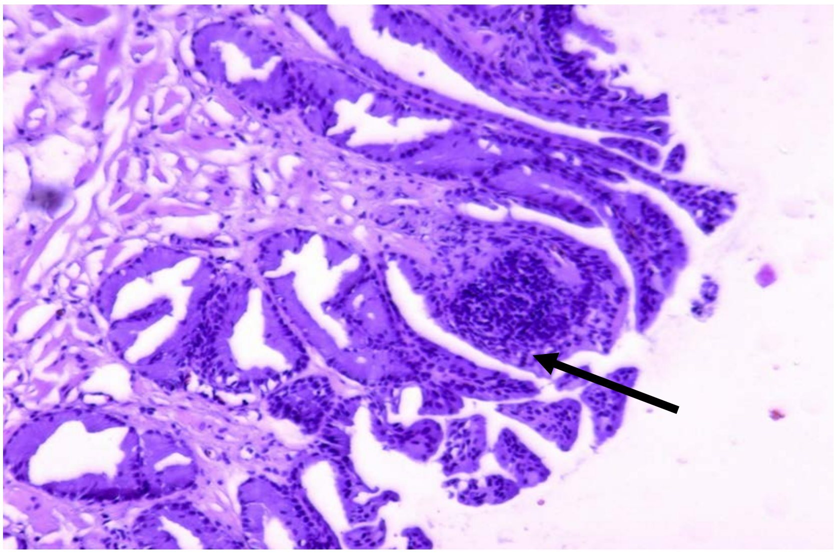

Figure 1. Tongue of the marsh frog from Lake Zhaltyrkol. Inflammatory infiltrate at the end of the papilla (shown by arrow). Hematoxylin-eosin, magnification ×40

In a frog sample from the Koyandy reservoir, edema was observed in the muscle tissue of the tongue (Fig. 2).

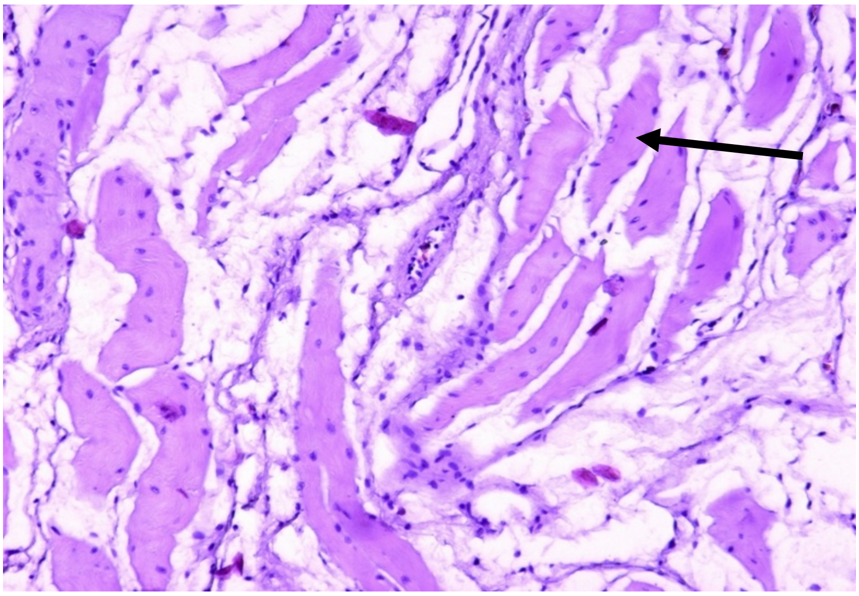

Figure 2. Tongue of the marsh frog from the Koyandy reservoir. Edema in the muscle tissue. Hematoxylin-eosin, magnification ×60

Esophagus. The mucous membrane of the esophagus was covered with multi-row ciliated epithelium (Fig. 3).

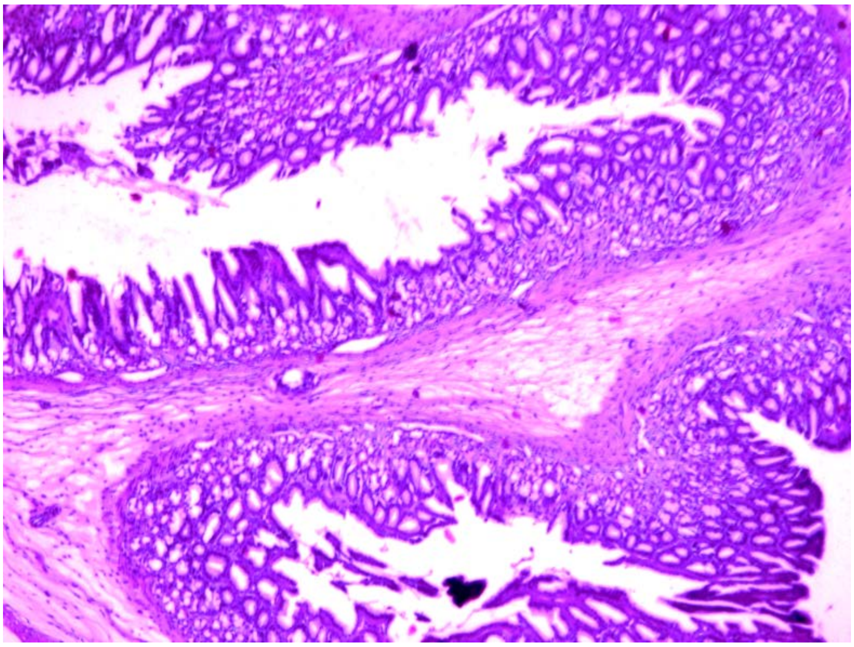

Figure 3. Esophagus of the marsh frog from Lake Zhaltyrkol. Hematoxylin-eosin, magnification ×40

This layer consisted of three groups of cells: goblet, ciliated, and intercalated cells. Two types of glands have been observed in the mucosa of the esophagus: unicellular goblet cells or mucocytes and multicellular simple tubular glands. The mucous membrane of the esophagus forms large longitudinal folds (Fig. 4).

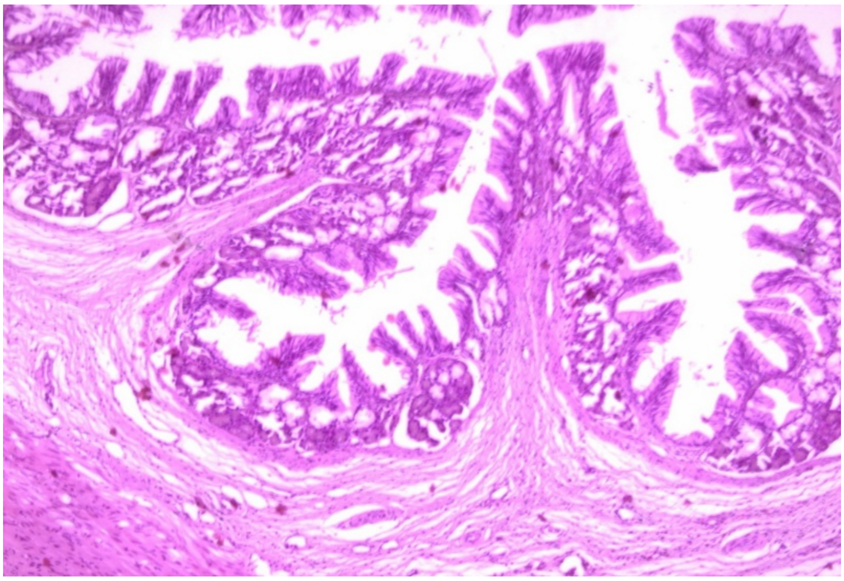

Figure 4. Esophagus of the marsh frog from Lake Maibalyk. Hematoxylin-eosin, magnification ×40

The muscular coat is well developed, which is associated with the function of moving the food bolus into the stomach.

When studying the esophagus of the marsh frog from the Koyandy reservoir, an increase in the number of goblet cells was found, indicating the presence of external stimuli (Fig. 5).

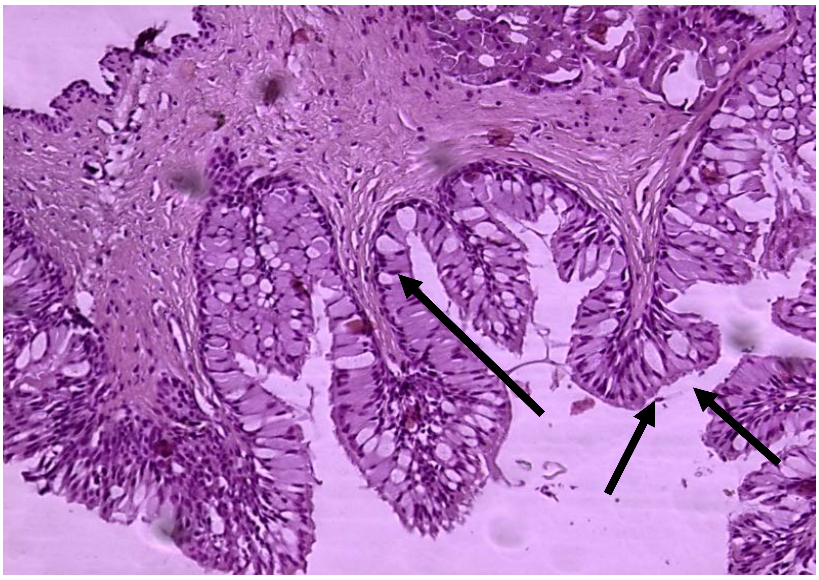

Figure 5. Esophagus of the marsh frog from the Koyandy reservoir. Increase in the number of goblet cells. Hematoxylin-eosin, magnification ×40

The frog's **stomach** was a thick-walled tube with a left or large curvature and a right or small curvature. The wall of the stomach consisted of a mucous membrane, submucosa, muscular and serous membranes. The inner surface of the stomach was covered with a mucous membrane, forming rather large longitudinal, high folds and fields. The surface of the mucous membrane was covered from the outside with a cylindrical single-layer epithelium. The epithelium lay on the basement membrane. Under it in the mucous membrane were tubular glands. Histological study of the stomach of the lake frog from Lake Maibalyk did not reveal any pathological changes. There were fatty inclusions in the muscle tissue. The glands of the stomach are numerous; their lumens are enlarged (Fig. 6).

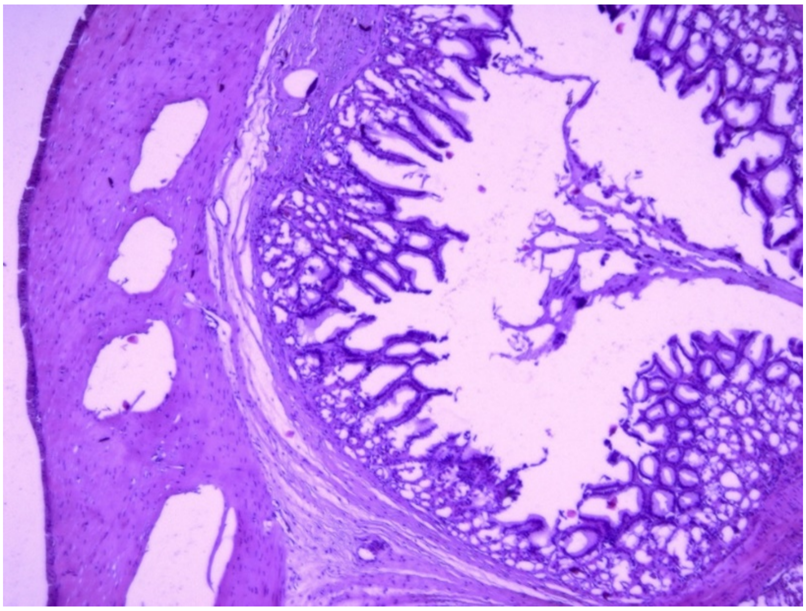

Figure 6. Stomach of the marsh frog from Lake Maibalyk. Hematoxylin-eosin, magnification ×20

The muscle layer was two-layered, the inner one was annular and the outer one was longitudinal. In some areas, severe damage was found in the digestive glands. The epithelium of the glands was devoid of nuclei; the cytoplasm was destroyed in the form of fragments. Desquamation of the integumentary epithelium with exposure of the basement membrane and inflammatory infiltration was observed in the stomach of the marsh frog from Lake Zhaltyrkol (Fig. 7).

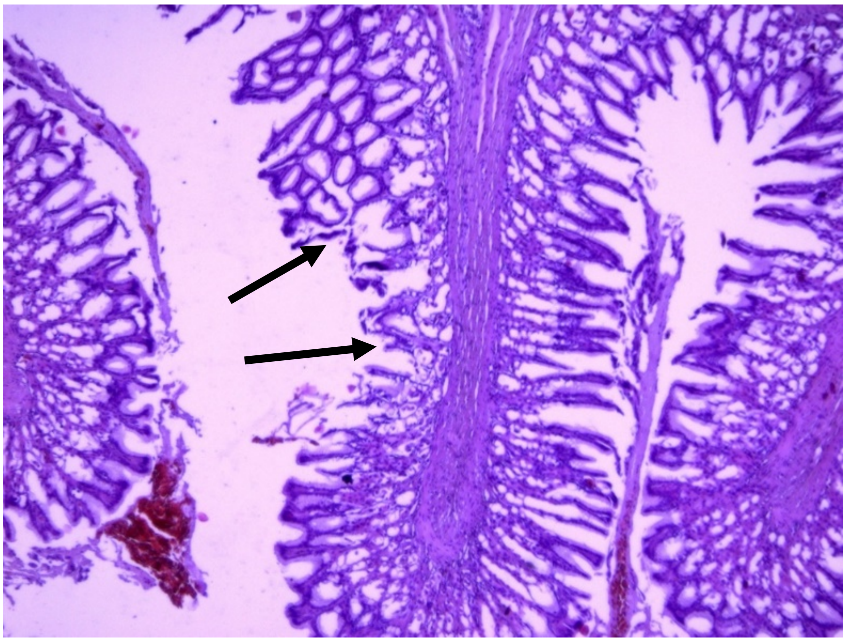

Figure 7. Stomach of the marsh frog from Lake Zhaltyrkol. Desquamation of the integumentary epithelium with exposure of the basement membrane (indicated by arrow). Hematoxylin-eosin, magnification ×20

A histological study of the duodenum of the marsh frog from Lake Zhaltyrkol showed a normal structure. The outer serous layer of the intestine was formed from the connective tissue and mesothelium that covered the outer part of the intestine. The muscle layer consisted of two layers: the inner – annular layer, and the outer – longitudinal one. The epithelium was single-layered and contained cylindrical enterocytes and goblet cells (Fig. 8).

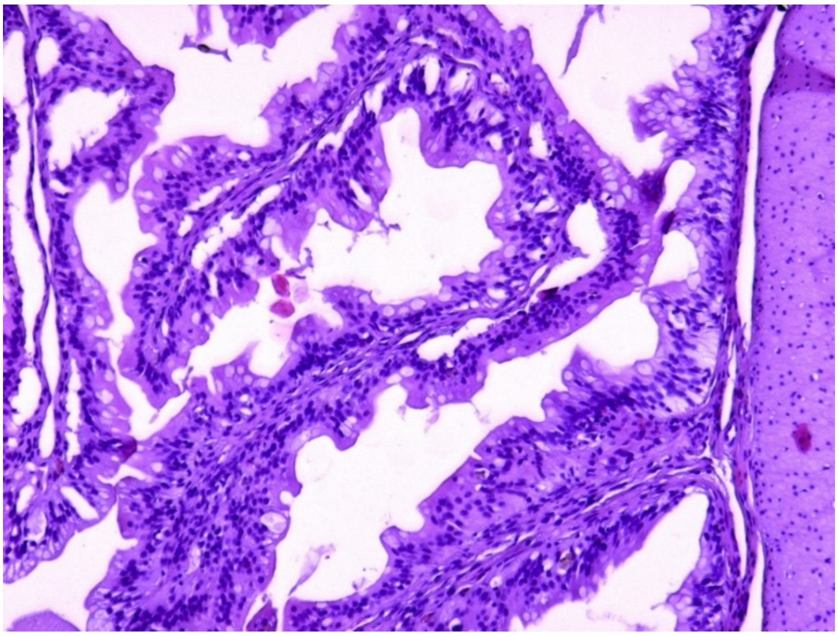

Figure 8. Duodenum of the marsh frog from Lake Zhaltyrkol. No pathological changes. Hematoxylin-eosin, magnification ×20

Histological examination of the duodenum of frogs from the Koyandy reservoir revealed the following changes. Desquamation of the epithelium occurred at the tips of the villi, edema and desquamation of the integumentary epithelium were observed (Fig. 9).

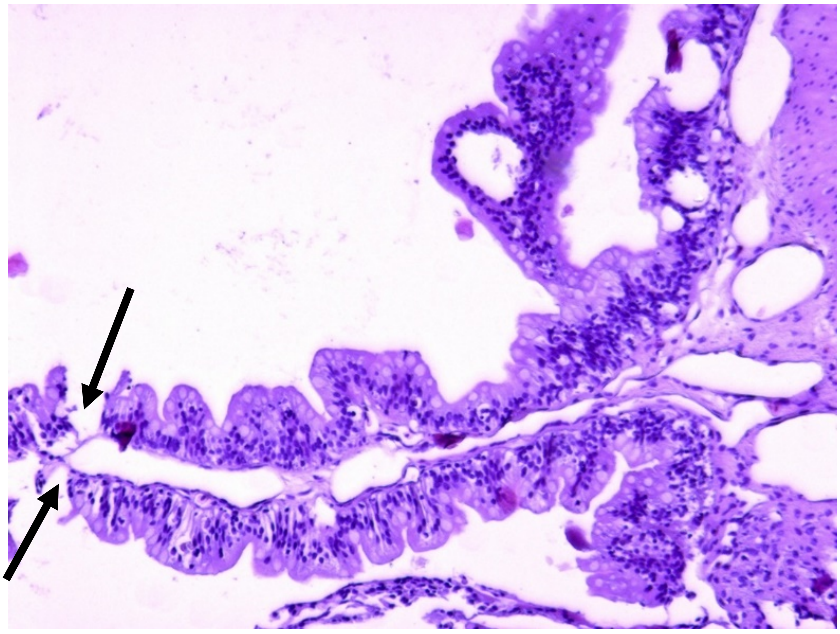

Figure 9. Duodenum of the marsh frog from the Koyandy reservoir Desquamation of the epithelium at the tip of the villus, edema. Hematoxylin-eosin, magnification ×20

Thus, a histological study of the gastrointestinal tract of the marsh frog from Lake Maibalyk, Lake Zhaltyrkol and Koyandy reservoir revealed the following changes in the organs. In specimens from Lake Zhaltyrkol, enlarged glands were found in the tongue, different heights of the epithelium, and inflammatory infiltrates were found at the end of the papilla. Edema was observed in the muscle tissue of the tongue in a sample of the marsh frog from the Koyandy reservoir. When studying the esophagus of the marsh frog from the Koyandy lake reservoir, an increase in the number of goblet cells was found, indicating the presence of external stimuli. Changes were observed in the histostructure of the stomach of the marsh frog from Lake Zhaltyrkol, which were expressed in desquamation of the integumentary epithelium with exposure of the basement membrane and inflammatory infiltration. The study of preparations of the duodenum of the marsh frogs from the Koyandy reservoir showed desquamation at the tips of the villi, edema, and desquamation of the integumentary epithelium. No pathomorphological changes were observed in the histostructure of the gastrointestinal tract of frogs from Lake Maibalyk.

Marsh frog liver. The stroma of the parenchymal lobular organ was represented by a sheath of dense fibrous connective tissue, which fuses with the visceral sheet of the peritoneum. Layers of loose fibrous unformed connective tissue divide the organ into lobules.

The study of the marsh frog liver preparation from the Koyandy reservoir and Lake Zhaltyrkol showed that in the structure of the organ there were puffiness between the plates of hepatocytes, vacuolization in the cytoplasm of hepatocytes (Figs 10 and 11).

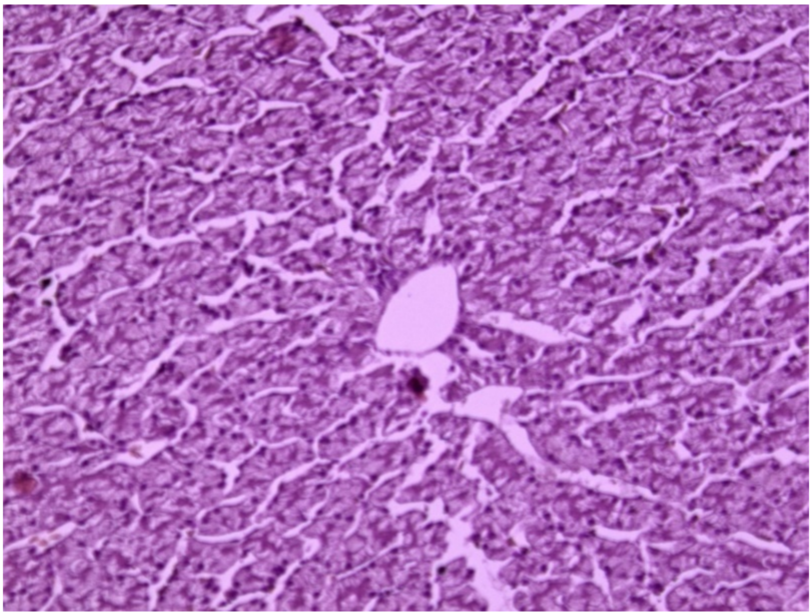

Figure 10. Liver of the marsh frog from Lake Maibalyk. Hematoxylin-eosin, magnification ×20

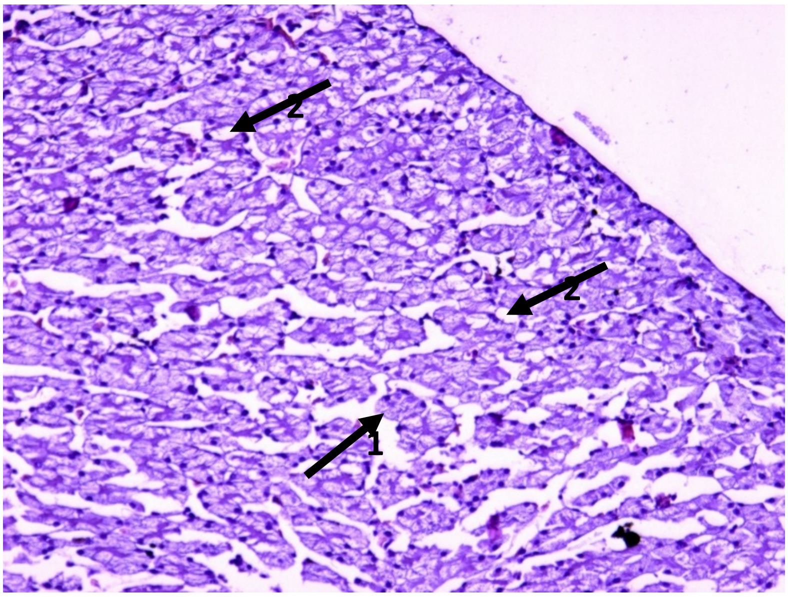

Figure 11. Liver of the marsh frog from the Koyandy reservoir. Edema of the liver tissue (1). Vacuolization of hepatocytes in the cytoplasm (2). Hematoxylin-eosin, magnification ×20

Thus, during the histological study of the marsh frog liver from the Lake Maibalyk, Lake Zhaltyrkol and Koyandy reservoir, the following results were obtained. The study of the marsh frog liver preparation from the Koyandy reservoir and Lake Zhaltyrkol showed that in the structure of the organ there were puffiness between the plates of hepatocytes, vacuolization in the cytoplasm of hepatocytes. The samples from Lake Maybalyk had no morphological changes in the histostructure of the liver.

Environmental pollution with various toxicants affects the morphofunctional state of living organisms. It is known that salts of heavy metals have the greatest negative impact on living organisms. Therefore, we performed water analyzes for the content of heavy metals such as lead, cadmium, zinc, cobalt, nickel, selenium, arsenic (Table 1).

Table 1. Results of the study of water samples.

Name of indicators of ingredients	**Detected concentrations**			**Regulatory indicators**
	Koyandy reservoir	**Lake Maibalyk**	**Lake Zhaltyrkol**	
Lead	0.0187 mg/l	0.0087 mg/l	0.0063 mg/l	not exceeding 0.03 mg/l
Cadmium	0.0006 mg/l	0.0008 mg/l	not detected	not exceeding 0.001 mg/l
Zinc	0.0118 mg/l	0.0125 mg/l	0.0221 mg/l	not exceeding 5.0 mg/l
Cobalt	not detected	0.0012 mg/l	not detected	not exceeding 0.1 mg/l
Nickel	0.0032 mg/l	not detected	0.0035 mg/l	not exceeding 0.1 mg/l
Selenium	0.0013 mg/l	0.0029 mg/l	0.0006 mg/l	not exceeding 0.01 mg/l
Arsenic	not detected	not detected	not detected	not exceeding 0.05 mg/l

According to the results of the analysis in the studied water bodies, the values of the content of heavy metals were within the permissible concentrations. However, there are many other pollution factors that have had a negative effect on the digestive organs of frogs.

4. Discussion

Lake Maibalyk is located near the city of Astana, it is endorheic lake, into which two periodically operating watercourses Karasu and Kyzylsu flow, through which the bulk of the water enters the lake in spring. The main purpose of the reservoir is the use of slightly brackish water for watering and fishing. There is no paved road to the reservoir; experienced off-road fishermen can get to the lake. Apparently, this was due to the fact that the lake turned out to be relatively clean and therefore no pathomorphological changes were found in the digestive organs of bioindicator amphibians.

Lake Zhaltyrkol is located in the Zhaltyrkol village, 12 km from the city. This area is characterized as a recreation area for citizens who come for family pastime. The Astana-Karaganda highway is adjacent to this area. Significant pathomorphological changes were found in the digestive organs of bioindicator frogs. This indicates a possible anthropogenic pollution of the area.

The Koyandy reservoir is located in the Koyandy village. In this area there are summer recreation areas, beaches, places for fishing. Anthropogenic impact on Lake Zhaltyrkol and the Koyandy reservoir can be expressed in the accumulation of household waste near the shores of reservoirs, flood runoff from nearby settlements, recreation centers, roads and parking lots.

Thus, the following results were obtained during a histological study of the gastrointestinal tract and liver of the marsh frog from the Lake Maibalyk, Lake Zhaltyrkol and the Koyandy reservoir. Of the studied organs of the gastrointestinal tract, the most sensitive to pollution were the stomach and liver. The tongue, esophagus and duodenum were found to be less sensitive to environmental changes. However, there were some histological changes, which were expressed in the expansion of the glands and inflammatory infiltrates, edema in the muscle tissues of the tongue. In the esophagus of the marsh frog, histological examination revealed an increase in the number of goblet cells, indicating the presence of external stimuli.

The following changes were observed in the stomach: desquamation of the integumentary epithelium with exposure of the basement membrane and inflammatory infiltration. Changes in the histostructure of the duodenum were observed in the lake frog in the form of desquamation of the epithelium at the tips of the villi, and edema and desquamation of the integumentary epithelium were also observed.

The following changes occurred in the liver. In the histostructure of the organ, there were puffiness between the plates of hepatocytes, vacuolization in the cytoplasm of hepatocytes.

The changes we observed may be due to the fact that in the studied water bodies, although the content of heavy metals was within acceptable limits, there were other types of pollution, such as salt ions, biogenic and organic compounds, phenols and suspended solids. Anthropogenic household waste, ingress of chemicals with ground and flood waters, precipitation, and surface runoff could have a significant impact. Pathological changes found during the histological study of animal organs indicate the need for a more detailed study of these water bodies.

5. **Conclusions**

During the histological study of the organs of the gastrointestinal tract of the marsh frog from Lake Maibalyk, no changes were found. In frogs from Lake Zhaltyrkol and the Koyandy reservoir, histological changes were found in all organs of the gastrointestinal tract, which were expressed in the appearance of inflammatory infiltrates in the papillae of the tongue, an increase in the number of goblet cells in the epithelium of the esophagus, desquamation of the integumentary epithelium of the stomach, and desquamation of the epithelium at the tips of the villi of the duodenum.

The histological structure of the marsh frog liver from Lake Maibalyk was normal. In the structure of the marsh frog liver from the Koyandy reservoir and Zhaltyrkol lake, there was swelling between the plates of hepatocytes, vacuolization of the hepatocyte cytoplasm.

Bioindication with the help of amphibians of three water bodies near the city of Astana showed that Lake Maibalyk was relatively unpolluted, since no pathomorphological changes were found in the digestive organs of frogs. In the Lake Zhaltyrkol and Koyandy reservoir pollution was present, which affected the organs with pathomorphological changes.

Histopathological changes were found in samples of frogs living in water bodies near settlements, compared to samples living in the natural environment. These results indicate that the studied species can be considered a bioindicator of early pollution of aquatic biotopes.

Declaration of interest statement

The authors declare that thee have no conflicts of interest and confirm that all procedures performed when working with animals comply with ethical standards.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Azizishirazi A., Klemish J.L. & Pyle G.G., 2021, Sensitivity of amphibians to copper. Environmental Toxicology and Chemistry 40(7): 1810-1821. Doi: 10.1002/etc.5049

Bazarbayeva Zh.M., Nurtazin S.T. & Hegai Zh.S., 2011, Comparative histological study of the stomach of the lake frog (Rana ridibunda) from some districts of the Almaty region. "Search" scientific journal of the Ministry of Education and Science 2011, No. 1, 79 pp. [in Russian]

Brady S.P., Goedert D., Frymus L.E., Zamora-Camacho F.J., Smith P.C., Zeiss C.J., Comas M., Abbott T.A., Basu S.P., DeAndressi J.C., Forgione M.E., Maloney M.J., Priester J.L., Senturk F., Szeligowski R.V., Tucker A.S., Zhang M.O. & Calsbeek R., 2022, Salted roads lead to edema and reduced locomotor function in amphibian populations. Freshwater biology 67(7): 1150-1161. Doi: 10.1111/fwb.13907

Burgos-Aceves M.A., Faggio C., Betancourt-Lozano M., Gonzalez-Mille D.J. & Ilizaliturri-Hernandez C.A., 2022, Ecotoxicological perspectives of microplastic pollution in amphibians, J Toxicol Environ Health B Crit Rev. 25(8): 405-421. Doi: 10.1080/10937404.2022.2140372

Chulenbayeva L., Ilderbayev O., Suleymeneva D., Kaliyeva A., Kabdykanov S., Nurgaziyev M., Nurgozhina A., Sergazy S., Kozhakhmetov S. & Kushugulova A., 2022, Prolonged Inhalation Exposure to Coal Dust on Irradiated Rats and Consequences. Scientific World Journal 2022, art. No. 8824275. https://doi.org/10.1155/2022/8824275

Chulenbayeva L., Ilderbayev O., Taldykbayev Z., Ilderbayeva G. & Argynbekova A, 2018, Phytocorrection of immunological and biochemical changes in the combined impact of coal dust and high dose of radiation. Georgian medical news 2018, p. 141-150. https://www.scopus.com/record/display.uri?eid=2-s2.0-85059796418&origin=resultslist&sort=plf-f

Don-Pedro K.N., Oyewo E.O. & Otitoloju A.A., 2004, Trend of heavy metal concentrations in Lagos lagoon ecosystem, Nigeria. West African Journal of Applied Ecology 5(1): 52-61.

Iztleuov M., Kaliev A., Turganbaeva A., Yesmukhanova D., Akhmetova A., Temirbayeva A., Iztleuov Y. & Iztleuova G., 2020, The effect of sodium tetraborate on chromium-induced oxidative damages in rats' lung tissue. Biomedical and Pharmacology Journal 13(1): 281-290. Doi: 10.13005/bpj/1887

Iztleuov M., Temirova G., Bashbayeva M., Komyekbay Z., Iztleuov Y., Madikhan Z. & Yemzharova G., 2019, Effect of sodium tetraborate on oxidative damages in heart tissue in chromium intoxication. Biomedical and Pharmacology Journal 12(2): 609-618. Doi: 10.13005/bpj/1681

Jayawardena U.A., Angunawela P., Wickramasinghe D.D., Ratnasooriya W.D. & Udagama P.V., 2017, Heavy Metal Induced Toxicity, [in:] The Indian Green Frog: Biochemical and Histopathological Alterations. Environ Toxicol Chem. Doi:10.1002/etc.3848

Jozwiak M.A. & Jozwiak M., 2014, Bioindication as challenge in modern environmental protection. Ecological Chemistry and Engineering 21(4): 577-591. Doi: 10.1515/eces-2014-0041

Lutsenko Ya.I., 2001, The use of bioindication and biotesting in ecology. Assembled.: Vesti. – Chelyabinsk: Ekol. valeol. Psychol. 2001, p. 67-72. [in Russian]

Lutsenko Ya.I. & Vasiliev A.V., 2007, The use of bioindication and biotesting in ecology, Ecology and life safety of industrial and transport complexes. ELPIT 2007, p. 76-79. [in Russian]

Păunescu A., Ponepal C.M., Drăghici O. & Marinescu A.G., 2010, Liver histopathologic alterations in the frog *Rana (Pelophylax) ridibunda* induce by the action of Reldan

40ec insecticide. Analele Universitatii din Oradea-Fascicula Biologie. Tom. XVII/1, p. 166-169

Polo-Cavia N., Burraco P. & Gomez-Mestre I, 2016, Low levels of chemical anthropogenic pollution may threaten amphibians by impairing predator recognition. Aquatic Toxicology 172: 30-35. https://doi.org/10.1016/j.aquatox.2015.12.019

Purucker S.T., Snyder M.N., Glinski D.A., Van Meter R.J., Garber K., Chelsvig E.A., Cyterski M.J., Sinnathamby S., Paulukonis E.A. & Henderson W.M., 2023, Estimating dermal contact soil exposure for amphibians. Integr Environ Assess Manag 19(1): 9-16. Doi: 10.1002/ieam.4619

RSE "Kazhydromet" Newsletter on the state of the environment of the Republic of Kazakhstan for 2021 (www.gov.kz). [in Russian]

Taiwo I.E. et al., 2014, Heavy metal bioaccumulation and biomarkers of oxidative stress in the wild African tiger frog, Hoplobatrachus occipitalis. African Journal of Environmental Science and Technology 8(1): 6-15.

Tapbergenov S.O., Zhetpisbaev B.A., Ilderbayev O.Z. et al., 2013, Free Radical Oxidation in Rats in the Delayed Period after Combined Exposure to Dust and Radiation. Bull Exp Biol Med 154: 747-749. https://doi.org/10.1007/s10517-013-2046-z