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Research Article

Histopathological Alterations in *Rutilus rutilus* and *Carassius gibelio* as Indicators of Aquatic Pollution

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Abstract | Reliable biological indicators are crucial for environmental monitoring, as freshwater ecosystems are under serious threat from water pollution. Histopathological alterations in Rutilus rutilus and Carassius gibelio were investigated as bioindicators of aquatic pollution in the Sitnica River. Fish from the Sitnica River near Obiliq showed significantly more severe degenerative and inflammatory changes in muscle, liver and gills, and also showed increased micronuclei formation, indicating increased genotoxic stress associated with polluted conditions. Because these organs can accumulate environmental contaminants, histopathological examination may provide a sensitive indicator of tissue injury associated with aquatic pollution. These results support the use of micronuclei analysis and histopathological changes as practical indicators for monitoring environmental pollution in freshwater environments.

Keywords | Histopathology, Bioindicators, Aquatic pollution, Heavy metals, *Rutilus rutilus*, *Carassius gibelio*, Sitnica River

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INTRODUCTION

Researchers have extensively investigated the hazardous effects of heavy metals and other environmental contaminants on aquatic organisms, particularly fish, due to their ecological relevance and sensitivity to pollution (Authman *et al*., 2015; Javed and Usmani, 2013). Organs such as the liver, gills, and muscles are among the most frequently examined in such studies, as they are directly exposed to environmental stressors and play essential roles in physiological and metabolic processes (Au, 2004).

The liver is a central organ involved in metabolic regulation, detoxification, and the maintenance of biochemical homeostasis. Environmental pollutants, including polychlorinated biphenyls (PCBs), pesticides, and heavy metals such as mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As), tend to accumulate in hepatic tissue (Javed and Usmani, 2013; Topić-Popović *et al*., 2023). Their presence may disrupt detoxification pathways and induce oxidative stress, inflammation, and structural damage, ultimately impairing normal liver function (Topić-Popović *et al*., 2023; Jamil-Emon *et al*., 2023).

Gills are vital respiratory organs in fish, directly interfacing with the aquatic environment and facilitating gas exchange, ionic regulation, and osmoregulation. Due to their large surface area and continuous exposure to water, gills are highly susceptible to contaminant uptake (Au, 2004; Marinović *et al*., 2021). Toxic substances may alter gill morphology and function, thereby compromising respiration and physiological balance, which can adversely affect overall organismal health and survival (Marinović *et al*., 2021; Mustafa *et al*., 2024).

Muscle tissue can accumulate heavy metals either directly from the surrounding water or indirectly through trophic transfer within the food chain (Jamil-Emon *et al*., 2023; Korkmaz *et al*., 2019). This accumulation is of particular concern due to its implications for food safety and human health. Toxic effects in muscle tissue may include cellular degeneration, altered protein synthesis, and progressive bioaccumulation at higher concentrations (Korkmaz *et al*., 2019; Shahid *et al*., 2022). In recent years, increasing anthropogenic pressure on aquatic ecosystems has intensified the need for comprehensive studies assessing the impact of pollution on resident species (Authman *et al*., 2015). Continuous exposure of fish to contaminated environments can result in a spectrum of alterations, ranging from subcellular biochemical disturbances to pronounced morphological and histopathological changes (Javed and Usmani, 2013; Jamil Emon *et al*., 2023). The accumulation of heavy metals in fish tissues is widely utilized as a biomarker in environmental monitoring programs, providing valuable insight into ambient ecological conditions (van der Oost *et al*., 2003). Notably, histopathological alterations are considered early and sensitive indicators of environmental stress, offering a reliable assessment of organism health and pollutant impact (Au, 2004; Yancheva *et al*., 2016). Such alterations may arise from multiple stressors, including toxic substances, microbial infections, nutritional imbalances, and adverse environmental conditions (Mustafa *et al*., 2024; Yancheva *et al*., 2016). The Sitnica River has increasingly functioned as a recipient of untreated industrial and urban wastewater, particularly from energy production facilities in Obiliq and municipal sewage systems, due to insufficient treatment infrastructure. Previous investigations have demonstrated that the water quality of this river is significantly compromised by elevated concentrations of heavy metals (Pb, Cd, Cu, and Zn) and organic pollutants such as phenols (Morina *et al*., 2013; Buçinca *et al*., 2024). In certain locations, particularly near Mitrovica, the concentrations of these elements have exceeded permissible limits for surface water quality, leading to pronounced toxic effects in both water and sediment (Morina et al., 2013). Earlier studies, including those by regional authors, have reported increased levels of biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and total proteins in fish collected from the Sitnica River, indicating physiological stress and hepatic damage (Morina *et al*., 2013; Buçinca *et al*., 2024). Among these, AST exhibited a more pronounced increase compared to ALT, suggesting severe tissue impairment. ALT, as a key enzyme released during hepatocellular injury, serves as an important indicator of liver dysfunction.

The objective of the present study was to evaluate histopathological alterations in the liver, gills, and muscle tissues of *Rutilus rutilus* and *Carassius gibelio* collected from the Sitnica River.

MATERIALS AND METHODS

Fish sampling and histopathological preparation

A total of 30 fish specimens belonging to the species *Rutilus rutilus* and *Carassius gibelio* were collected from the Sitnica river in Kosovo. Liver, gill, and muscle tissues were collected from each specimen for histopathological examination.

Immediately after collection, tissue samples were carefully excised and fixed in 10% neutral buffered formalin for 24 hours at 4°C to preserve tissue architecture. The samples were subsequently dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax using an automated tissue processor (Suvarna *et al*., 2018).

Paraffin-embedded tissues were sectioned at a thickness of 3–5 μm using a microtome. The sections were mounted on glass slides, deparaffinized in xylene, rehydrated through descending concentrations of ethanol, and stained with hematoxylin and eosin according to standard histological procedures (Suvarna et al., 2020; Wolf *et al*., 2015).

Following staining, the sections were dehydrated, cleared, and permanently mounted using a synthetic mounting medium. Microscopic examination was performed using a Zeiss optical microscope at 100× total magnification, and representative images were captured for documentation.

Histopathological alterations in the liver, gills, and muscle tissues were evaluated qualitatively, with particular attention to tissue integrity, cellular architecture, degeneration, necrosis, inflammatory alterations, and other visible morphological abnormalities (Wolf *et al*., 2015; Authman et al., 2015). Micronuclei were identified as small, round chromatin bodies separated from the main nucleus and showing similar staining intensity.

RESULTS AND DISCUSSION

Histopathological analysis of fish organs

Liver histopathology

The liver performs essential metabolic and detoxification functions. Samples from the Sitnica River showed hepatocellular degeneration, inflammatory cell infiltration, vascular congestion, focal hemorrhage, and disruption of hepatic architecture (Figure 2). These lesions are compatible with tissue injury associated with environmental stress;

however, the histological findings alone do not identify a specific contaminant.

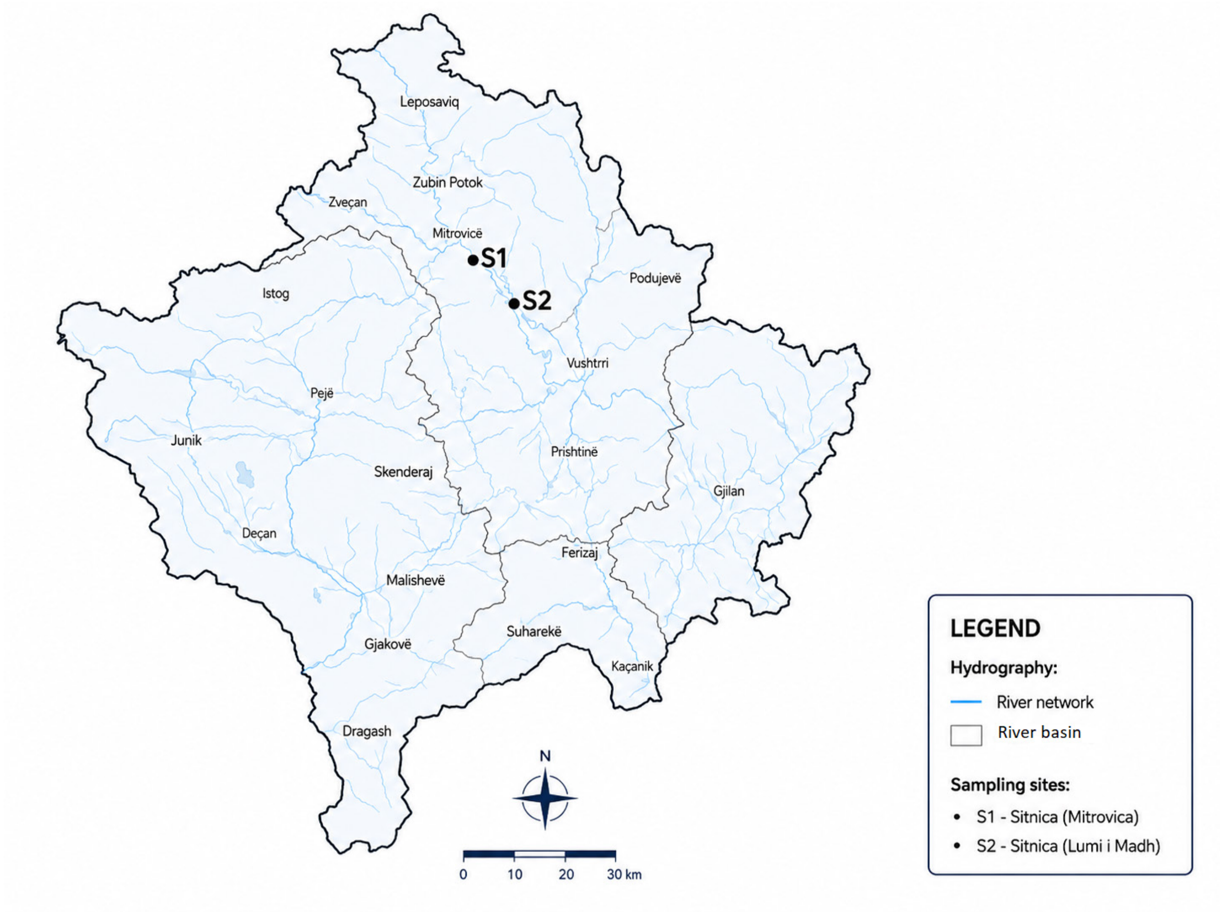

Figure 1: Study area and fish sampling sites.

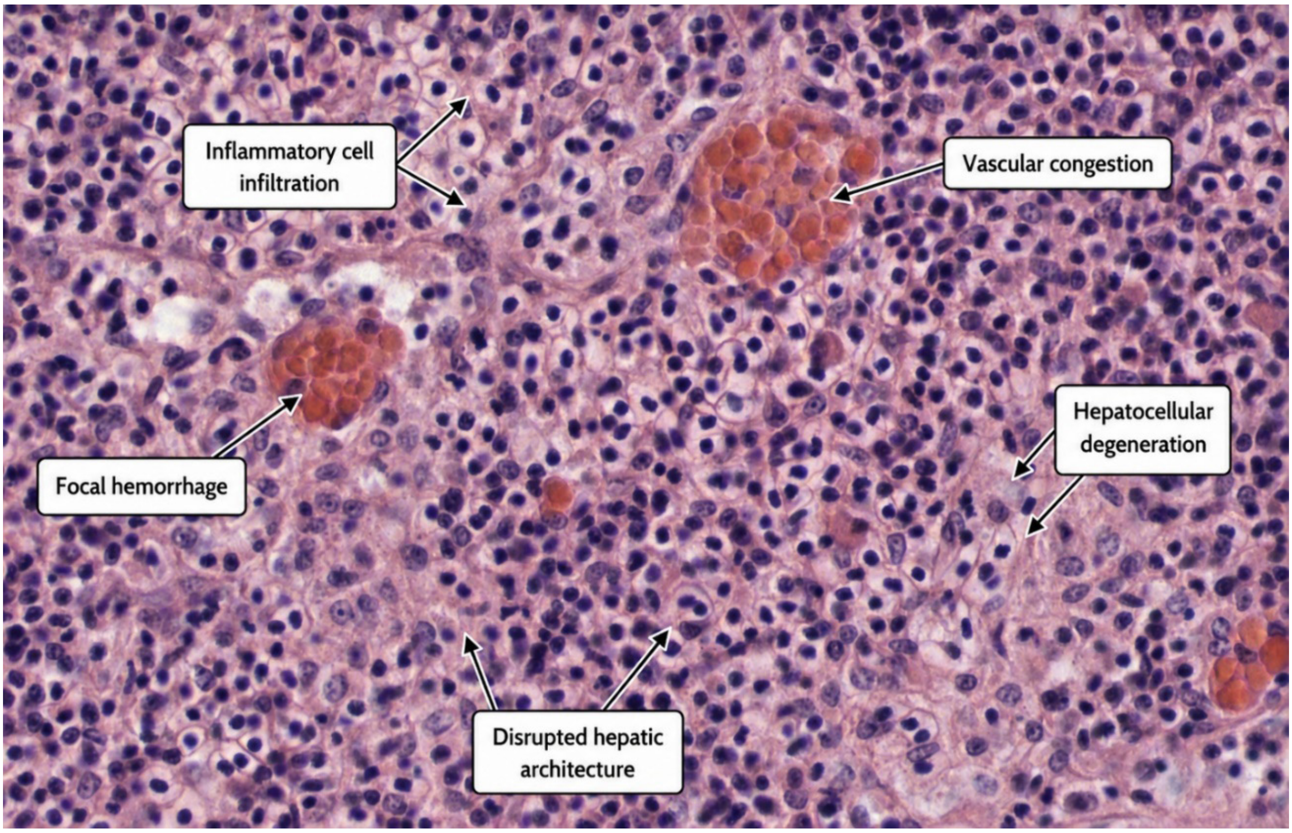

Figure 2: Hepatocellular degeneration and inflammatory alterations in liver tissue from the Sitnica River.

Muscle tissue histopathology

Figure 3 show degeneration, separation, loss of striations, necrosis, and inflammatory infiltration. Muscle tissue from control fish showed intact, regularly arranged fibers, preserved striations, minimal inter-fiber spaces, and no evident inflammatory infiltration. However, for a definitive assessment of muscle fiber degeneration, necrosis, loss of striations, or inflammatory infiltration, further and more comprehensive analyses should be performed.

Gills histopathology

Gill tissue from the Sitnica River showed epithelial lifting, lamellar fusion, focal necrosis, inflammatory cell infiltration, and vascular congestion (Figure 4), indicating marked tissue injury associated with environmental stress.

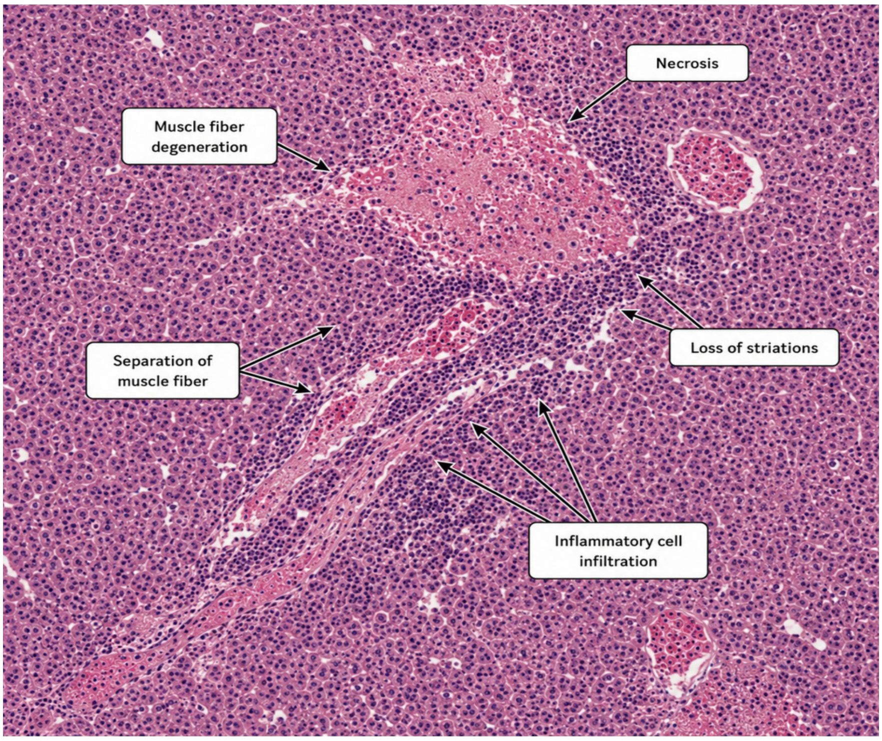

Figure 3: Muscle degeneration and necrosis in fish from the Sitnica River.

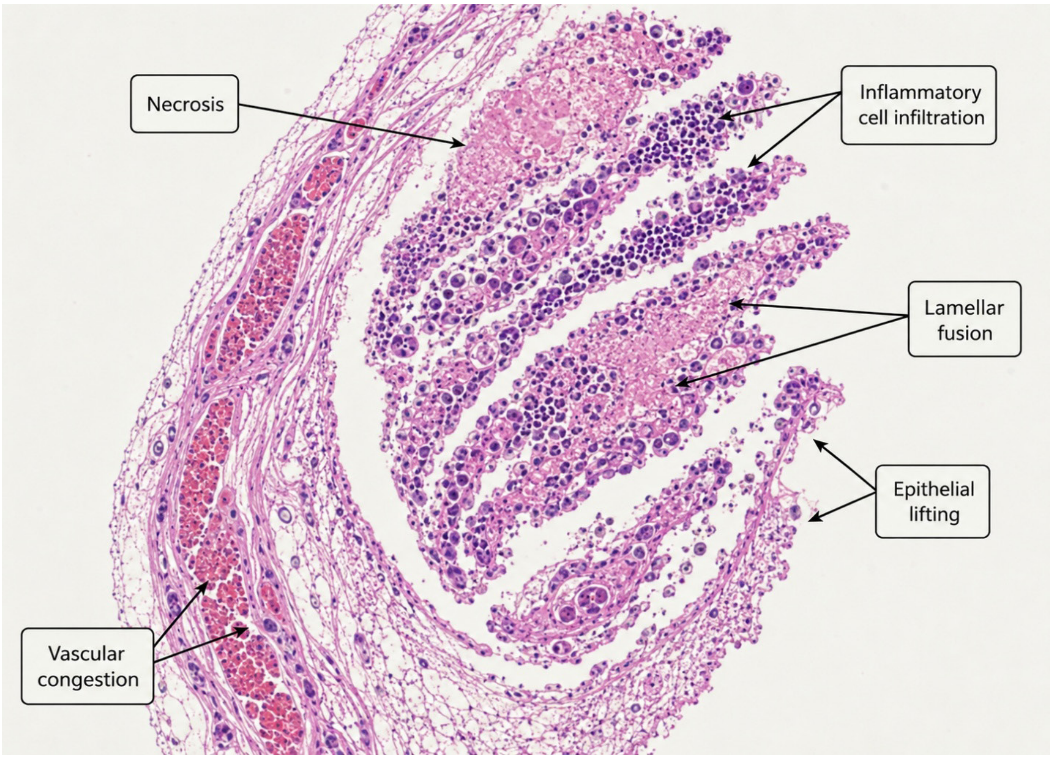

Figure 4: Necrosis and inflammatory alterations in gill tissue from the Sitnica River.

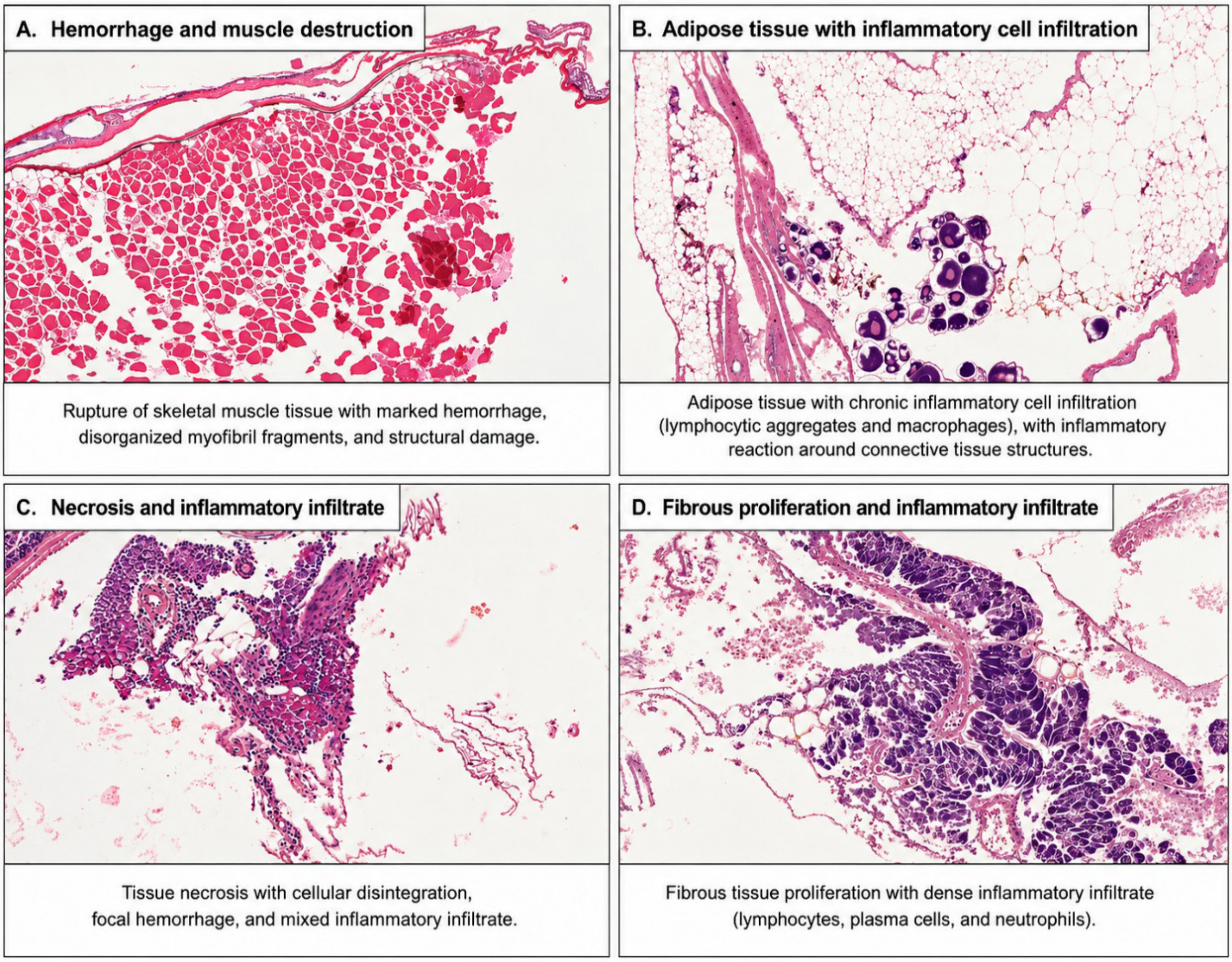

Figure 5: Histopathological alterations in tissues of fish from the Sitnica River. (A) Muscle hemorrhage and fiber disruption. (B) Inflammatory infiltration in adipose tissue. (C) Necrosis with hemorrhage and inflammatory cells. (D) Fibrous proliferation with dense inflammatory infiltration.

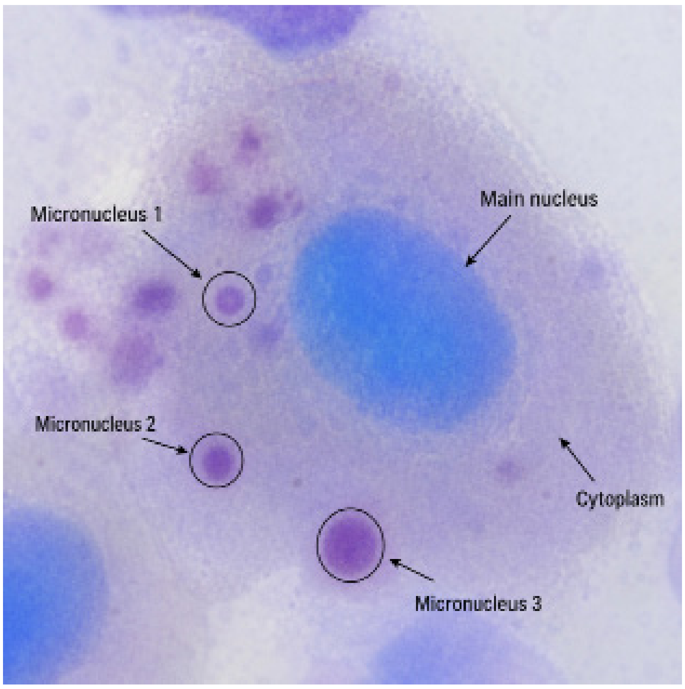

Figure 6: Micronucleus frequency in peripheral blood samples from the Sitnica River.

Micronucleus assay

Micronucleus frequency was evaluated to determine genotoxic impacts. Representative micronucleated cells were observed in peripheral blood smears from fish collected from the investigated rivers (Figure 6). The presence of micronuclei was considered an indicator of cellular and genotoxic stress, in accordance with previously described applications of the micronucleus assay in aquatic organisms (Bolognesi and Hayashi, 2011). The findings corroborate prior studies. For example, Khidr *et al.* (2012) observed histological changes in hepatocytes of *Oreochromis niloticus* after exposure to lead nitrate at 2.5, 5, and 10 ppm for 1–4 weeks, including necrosis and degeneration. Similarly, Al-Balawi *et al.* (2013) reported sublethal heavy metal concentrations caused pronounced liver, gill, and muscle damage, confirming our observations.

Overall, histopathological examination of liver, gill, and muscle tissues provides useful evidence of tissue injury associated with environmental stress. These observations can support environmental risk assessment when interpreted together with verified chemical analyses, appropriate controls, and quantitative or semi-quantitative lesion data (Yancheva *et al*., 2016; Wolf *et al*., 2015).

In fish, the liver, gills, and muscle are vital organs that perform a range of physiological functions. The following figures present representative histological findings from fish collected from the Sitnica River. The observed lesions are consistent with environmental stress, but the micrographs alone cannot establish the identity or concentration of the causative pollutants.

Fish from the Sitnica River exhibited hepatocellular degeneration, inflammatory cell infiltration, vascular congestion, focal hemorrhage, and disruption of hepatic architecture (Figure 5).

Areas of hepatocellular necrosis, inflammatory cell infiltration, vascular congestion, focal hemorrhage, and disruption of hepatic architecture were observed. These alterations are compatible with chronic toxic stress.

CONCLUSION

Histopathological examination identified degenerative, necrotic, inflammatory, vascular, and architectural alterations in the examined tissues. The liver and gills appeared sensitive to environmental stress and may serve as useful bioindicator organs. Conclusions regarding muscle pathology, fibrosis, micronucleus frequency, or specific causative pollutants require also additional research to reflect pollution elements accumulate in this tissue.

These observations are broadly consistent with previous reports of organ-specific tissue injury in fish exposed to contaminated environments.

In conclusion, histopathological examination is useful for detecting tissue-level responses in fish and can contribute to aquatic environmental monitoring. It should be interpreted as evidence of biological effect and not only rather than as a stand-alone method for identifying a specific chemical pollutant. Future analyses will be included additional verified organ-specific micrographs, immunohistochemical analyses of lesion scoring, chemical of pollutants measurements, and statistical comparisons among sites and sampling periods.

The more pronounced tissue alterations observed in fish from the Sitnica River suggest poorer environmental conditions.

Effective treatment of industrial and municipal wastewater before discharge and continued environmental monitoring are recommended.

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AUTHOR'S CONTRIBUTION

RF conceived and designed the study. QM and RF were responsible for field sampling, laboratory analyses, data acquisition, and interpretation of histopathological findings. MFR contributed to scientific interpretation, critical revision of the manuscript, and intellectual content improvement. All authors have read and approved the final version of the manuscript.

Ethical approval

All procedures involving fish specimens were conducted with consideration of animal welfare principles and in accordance with internationally accepted ethical guidelines for the use of animals in environmental and biological research. Efforts were made to minimize stress, harm, and disturbance during sampling and handling.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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