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HISTOPATHOLOGICAL EXAMINATION OF ZEBRAFISH ORGANS UNDER THE EXPOSURE TO MICROPLASTICS

The article was written as part of the Ministry of Science and Higher Education of the Republic of Kazakhstan's project AP 23486220, titled "Study of the important organ systems reactivity in the main commercial fish species of the Ile-Balkhash basin under anthropogenic load." (2024-2026). The article represents the study investigating the long-term effects of polyethylene terephthalate (PET) microplastics on zebrafish (Danio rerio). Zebrafish exposed to PET microplastics at concentrations of 1.9 mg/l and 5.64 mg/l exhibited significant kidney and gill damage. This included necrosis, circulatory disorders, and tissue damage. Histological analysis revealed specific pathological changes such as vacuolization and hyaline-droplet degeneration in kidneys, and destruction of epithelium and lamellae in gills. Notably, no compensatory-adaptive reactions in gills were observed, indicating a lack of adaptation to prolonged exposure. These findings suggest that microplastics may migrate through the bloodstream, disrupting normal kidney and gill structures. The study highlights the severe impact of microplastic pollution on fish organs, emphasizing the urgent need for further research to develop strategies for mitigating microplastic pollution in aquatic ecosystems. Additionally, the observed lack of adaptive responses underscores the potential for significant ecological consequences, urging immediate attention to address this critical environmental issue.

Key words: pollution, microplastic, Polyethylene terephthalate (PET), histology, toxicology, zebrafish (*Danio rerio*).

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Микропластиктің әсеріне ұшыраған Данио рерио балықтарының мүшелерін гистопатологиялық зерттеу

Мақала Қазақстан Республикасы Ғылым және жоғары білім министрлігінің AP23486220 «Антропогендік жүктеме жағдайында Іле-Балқаш бассейнінің негізгі кәсіпшілік балық түрлерінің маңызды орган жүйелерінің реактивтілігін зерттеу» жобасы (2024-2026 жж.) аясында жазылған. Мақалада зерттеу барысында полиэтилентерефталат (ПЕТ) микропластиктің Данио рерио балықтарына (Danio rerio) ұзақ мерзімді әсері зерттелді. 1,9 мг/л және 5,64 мг/л концентрациясындағы микропластиктің әсеріне ұшыраған Данио рерио балықтарының (Danio rerio) бүйрегі мен желбезектеріне айтарлықтай зақым келді. Нәтижелер бойынша келесі патологиялық процестер анықталды: некроз, қан айналымының бұзылуы және тіндердің зақымдануы. Гистологиялық талдау кезінде бүйректің вакуолизациясы және гиалинді-тамшылы дистрофиясы, эпителий мен желбезек тақталарының жойылуы түріндегі ерекше патологиялық өзгерістер анықталды. Айта кетерлігі, желбезектерде компенсаторлық бейімделу реакциялары байқалмады, бұл ұзақ әсер етуге бейімделудің жоқтығын көрсетеді. Бұл дәлелдер микропластиктің бүйрек пен желбезектің қалыпты құрылымдарын бұза отырып, қан айналымы арқылы қозғалу қасиетіне ие болуы мүмкін екенін көрсетеді. Зерттеу балық мүшелеріне микропластикалық ластанудың ауыр әсерін көрсетеді, бұл су экожүйелеріндегі микропластикалық ластануды азайту стратегияларын әзірлеу үшін қосымша зерттеулердің шұғыл қажеттілігін көрсетеді. Сонымен қатар, адаптивті реакциялардың байқалмауы осы маңызды экологиялық проблеманы шешуге жедел назар аударуды қажет ететін елеулі экологиялық зардаптардың ықтималдығын көрсетеді.

Түйін сөздер: ластану, полиэтилентерефталат (ПЭТ), микропластик, гистология, токсикология, (Danio rerio)

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Гистопатологическое исследование органов рыбок Данио при воздействии микропластика

Статья написана в рамках Проекта Министерства науки и высшего образования РК AP23486220 «Изучение реактивности важнейших систем органов основных промысловых видов рыб Иле-Балхашского бассейна в условиях антропогенной нагрузки.» (2024-2026 годы). В статье представлено исследование долгосрочного воздействия микропластика полиэтилентерефталата (ПЭТ) на рыбок данио *(Danio rerio)*. У рыбок данио *(Danio rerio)*, подвергшихся воздействию микропластика ПЭТ в концентрациях 1,9 мг/л и 5,64 мг/л, наблюдалось значительное повреждение почек и жабр. Полученные результаты включали в себя такие патологические процессы: как: некроз, нарушения кровообращения и повреждение тканей. При гистологическом анализе были выявлены специфические патологические изменения в виде вакуолизации и гиалиново-капельной дистрофии почек, деструкции эпителия и пластинок жабр. Примечательно, что компенсаторно-приспособительных реакций в жабрах не наблюдалось, что свидетельствует об отсутствии адаптации к длительному воздействию. Эти данные свидетельствуют о том, что микропластик может обладать свойством мигрировать через кровоток, нарушая нормальные структуры почек и жабр. В исследовании подчеркивается серьезное воздействие загрязнения микропластиком на органы рыб, подчеркивая острую необходимость дальнейших исследований для разработки стратегий по смягчению загрязнения микропластиком в водных экосистемах. Кроме того, наблюдаемое отсутствие адаптивных реакций подчеркивает возможность серьезных экологических последствий, требуя немедленного внимания к решению этой критической экологической проблемы.

Ключевые слова: загрязнение, микропластик, полиэтилентерефталат (ПЭТ), гистология, токсикология, Danio rerio.

Introduction

Over the past few decades, environmental pollution by plastic and its wastes has become urgent and global in the environmental community. Since the 1960s, global plastic production has increased rapidly, reaching 300 million tons annually [1, 2, 4]. Plastic waste poses a great threat to wildlife, especially to organisms living in the aquatic environment, as it accumulates in reservoirs due to poor disposal and recycling. In modern classification, plastics are diverse. The most common is polyethylene terephthalate (PET), as it is used in food production and beverage packages like plastic water bottles [6]. Getting into the aquatic ecosystems, microplastic particles enter the fish's body through ingestion of water and food particles, and further accumulate in the bloodstream and various organs. Damage caused by stress resulted from exposure to PET particles can directly affect the immune system, leading to inflammatory processes in tissues.

The toxic effect of microplastics leads to a slowdown in metabolic processes in cells and tissues, which suppresses the immune response. The immune system includes external barriers, such as mechanical barriers to prevent the penetration of pathogens and other foreign particles [14, 15, 16, 17]. Structural changes in cells and tissues of immunocompetent organs can lead to various reactions in organs, including inflammatory processes, in response to external impact [18-19]. However, the specific effects of microplastics have not been sufficiently studied and require detailed study under experimental conditions.

In this case, zebrafish (*Danio rerio*) is an ideal model organism for studying basic biological processes and mechanisms due to numerous advantages that render them convenient and economical for research [20-21]. Conducting experiments with these fish species enables us to generalize the results obtained to a wider range of organisms [22-25].

Therefore, the aim of this study was to investigate the effects of polyethylene terephthalate (PET) microplastics on the gills and kidneys of zebrafish (*Danio rerio*) under a chronic experiment.

Materials and methods

Gills and kidneys of zebrafish *Danio rerio* (*Cyprinidae* family) were the target of experimental research. A stock of adult zebrafish (*Danio rerio*) was obtained and maintained in a 40L aquarium in

the Aquarium Room of al-Farabi KazNU (Almaty, Kazakhstan). Adult healthy fish of both sexes were divided into a control group and two experimental groups. Each experimental group consisted of 15 individuals maintained in 17L aquaria throughout the experiment, with daily monitoring of water quality. The duration of the experiment was one month. Polyethylene terephthalate (PET) microplastic particles ranging in size from 1.34 to 239 μm were obtained by abrasion with sandpaper (Fig. 1).

In the first experimental group, the daily concentration of PET microplastics was 1900 μg/l (1.9 mg/l) (lethal concentration – 13,300 μg/l) [8], which accounted for 10% of the total feed mass (0.16 g) per serving. In the second experimental group, the daily concentration of PET microplastics was 5640 μg/l (5.64 mg/l), representing 30% of the feed weight. Microplastics were introduced into the fish's body during feeding with food twice a day.

At the end of each exposure period, fish were euthanized in accordance with guidelines for the use of laboratory animals in preclinical studies [9]. Subsequently, they were dissected under a stereomicroscope, and the gills and kidneys were immediately fixed in 10% buffered formaldehyde. Further tissue processing followed the routine histological technique [10, 11]. Slides were stained with hematoxylin and eosin. Hemosiderin was identified using the Perls histochemical method [13]. For scanning electron microscope (SEM) analysis, the tissue after formaldehyde fixation was washed under running water for 2 hours, followed by dehydration in alcohols and acetone [13]. Stained histological preparations were photographed using an Optix 600 camera mounted on MX300T microscope, while polyethylene terephthalate microplastic particles and fish organs were photographed using a Quantum 3D 200i scanning electron microscope.

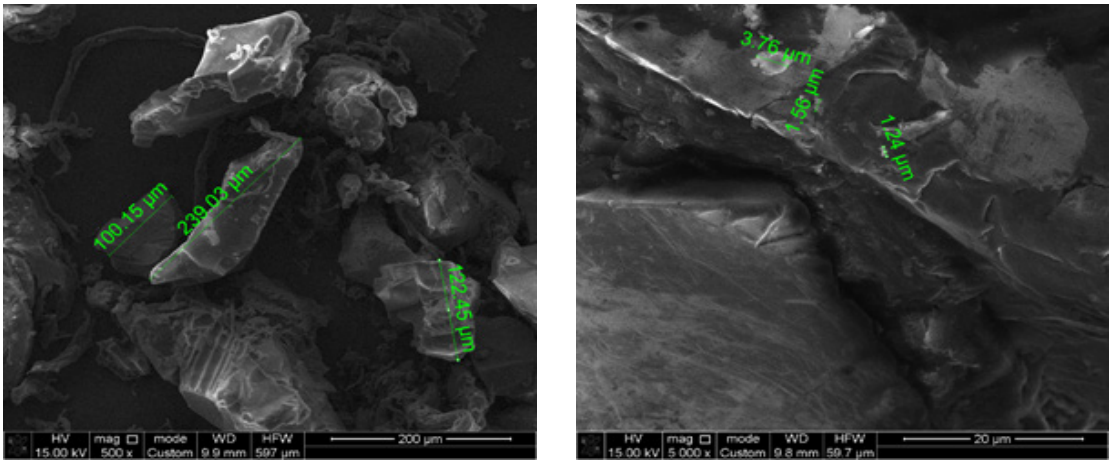

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Figure 1 – Photomicrograph of PET microplastic particles by Scanning Electron Microscope Quantum 3D 200i. A – magnification 500x; B – magnification 5000x.

Results and discussion

SEM examination of gills and kidneys samples of both experimental groups revealed the presence of microplastic particles within the tissues. The observed particles ranged in size from 3.36 to 4.77 μm and from 5.99 to 7.73 μm.(Fig. 2).

Histopathological examination of the zebrafish kidneys from the experimental group, exposed to a 1.9 mg/l daily concentration of microplastic particles, revealed several notable observations. These included abundant infiltration of the organ stroma with mononuclear leukocytes (Fig. 3-5), blood stasis in large vessels, and the presence of edema (Fig. 3). Additionally, diapedesis and hemorrhage into the interstitial tissue of the organ were observed (Fig. 3-5). Swelling of the epithelium was evident in the kidney tubules, with the cytoplasm of some epithelial cells containing hyaline-like inclusions stained bright pink (Fig. 3, 4 B). Moreover, the cell boundaries appeared unclear, lumens narrowed or filled with protein masses (Fig. 4A, 4B). Some tubules exhibited signs of necrosis. A slight narrowing of Bowman's space and blood stasis in the capillaries (Fig. 3), with no significant alterations were noted in kidneys glomeruli.

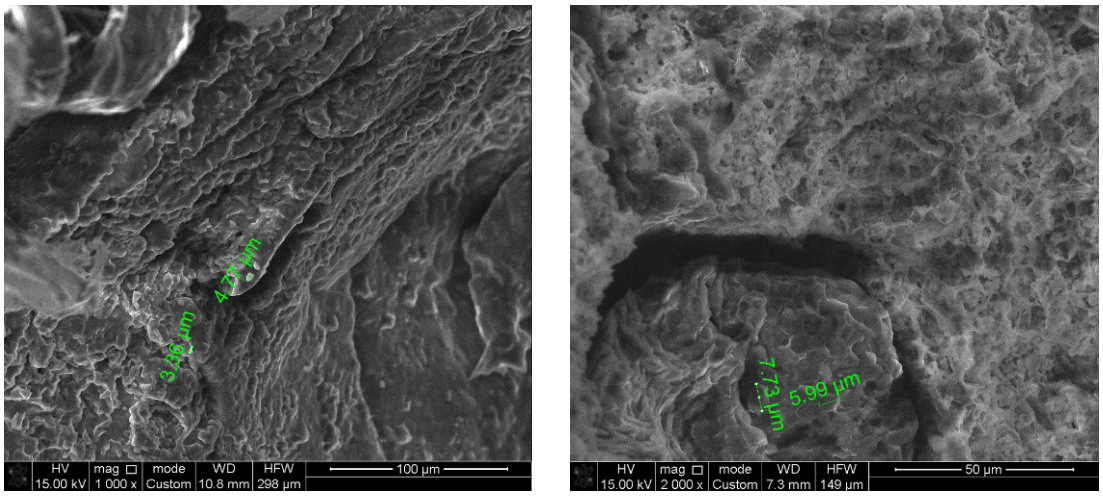

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Figure 2 – Photomicrograph of kidney (A) and liver (B) of zebrafish (Danio rerio) showing the comparison of PET microplastics particle sizes in kidney (A) and red blood cells in the lumen of liver hepatic vein (blood stasis) (B). Scanning Electron microscope Quantum 3D 200i. Magnification (A) 1000x; (B) 2000x.

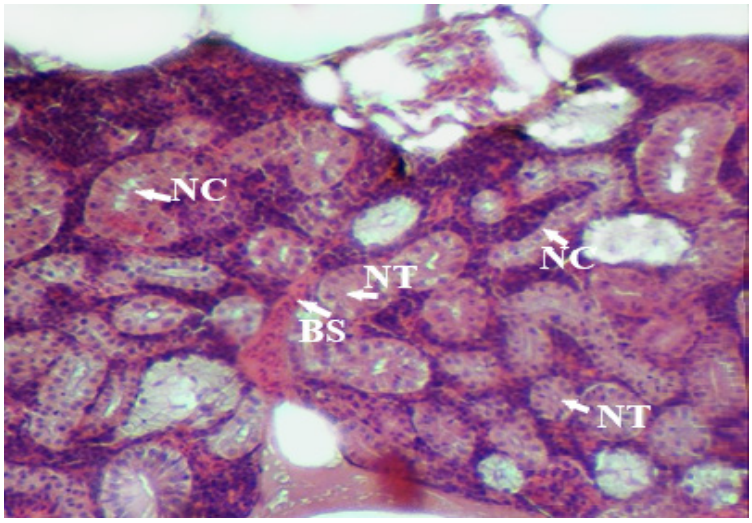

Figure 3 – Photomicrograph of zebrafish **(***Danio rerio)* kidney after exposure to 1.9 mg/L of PET microplastic particles showing increased infiltration of tissue with mononuclear leukocytes, blood stasis in vein (BS), and narrowing of the lumen of some proximal and distal tubules (NT). The necrotic processes are followed by epithelial desquamation in some tubules (NC). H&E staining. Magnification 200x

The exposed fish displayed severe edema of the renal interstitium, accompanied by diapedesis and hemorrhage. Additionally, numerous foci of necrosis were observed in the epithelial cells of both proximal and distal tubules (Fig. 4, 5), resulting in a narrowing of their lumens.

A histological examination of zebrafish (*Danio rerio)* kidneys exposed to polyethylene terephthalate (PET) particles at a daily concentration of 5.64 mg/l (5640 µg/l) revealed similar histopathological changes to those observed in the first experimental group. These changes included abundant infiltration of the stroma by mononuclear leukocytes, bleeding, and narrowing of the lumens of renal tubules, some of which were filled with protein masses (Fig. 7, 8). Additionally, cases of epithelial cell necrosis were noted (Fig. 7, 8).

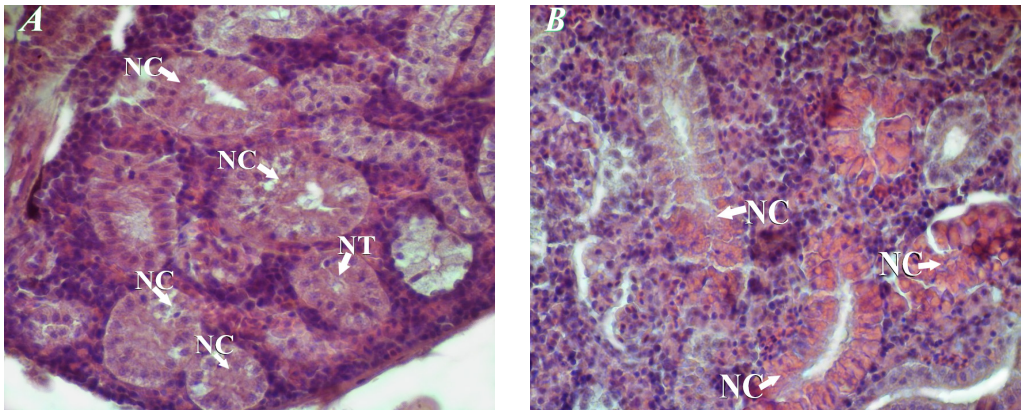

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Figure 4 – Photomicrograph of zebrafish (*Danio rerio)* kidney after exposure to 1.9 mg/L of PET microplastic particles showing (A) necrosis of epithelial cells (NC) and narrowing of lumen (NT) in proximal tubules, diffuse blood filling of the kidney interstitium; (B) complete and partial vacuolization of tubule epithelial cells and necrosis (NC). H&E staining. Magnification 400x

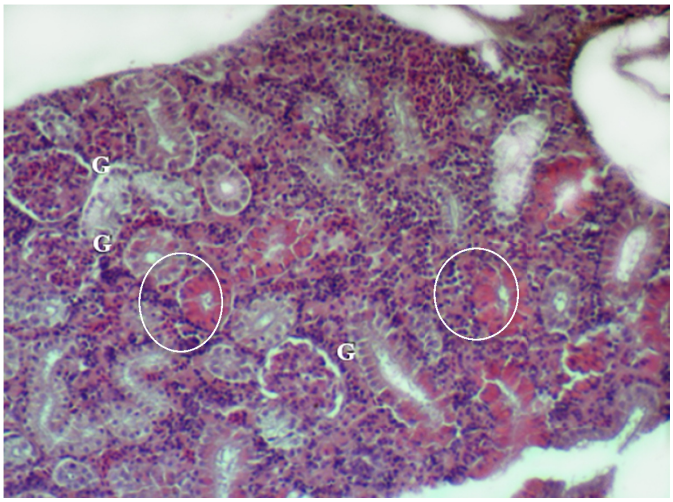

Figure 5 – Photomicrograph of zebrafish (*Danio rerio)* kidney after exposure to 1.9 mg/L of PET microplastic particles showing epithelial cells vacuolization. Tubules and their hyaline degradation (incircle). Narrowing of Boumen's lumen and blood stasis in the capillaries of renal glomeruli (G). H&E staining. Magnification 100x

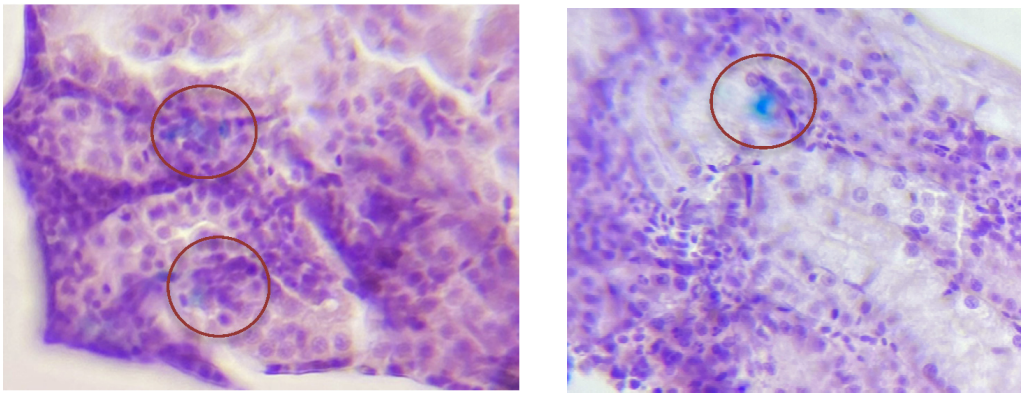

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Figure 6 – Photomicrograph of zebrafish (*Danio rerio)* kidney after exposure to 5.64 mg/L of PET microplastic particles showing hemosiderin granules (A, B) Perl's staining. Magnification 400x

Figure 7 – Photomicrograph of zebrafish *(Danio rerio)* kidney after exposure to 5.64 mg/L of PET microplastic particles showing hemorrhage in the organ stroma, orange-brown vacuoles in the epithelial cells of the renal tubules (V); necrosis of epithelial cells (NC) in renal tubules and narrowing of their lumen (NT). H&E staining. Magnification 400x

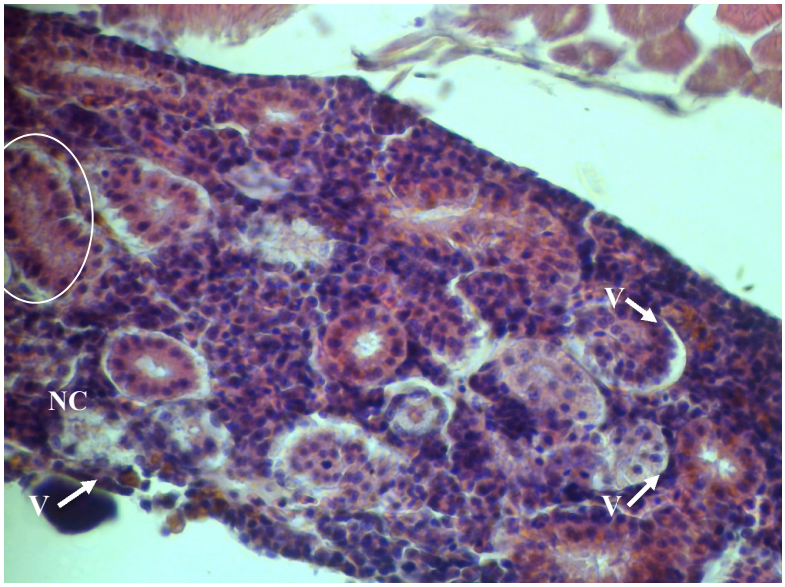

Figure 8 – Photomicrograph of zebrafish *(Danio rerio)* kidney after exposure to 5.64 mg/L of PET microplastic particles showing orange-brown vacuoles in the interstitium of the kidney and epithelial cells of the renal tubules (V); necrosis of renal tubules epithelial cells (NC); the presence of protein masses in the lumen of the renal tubule (in circle). H&E staining. Magnification 400x

Compared to the first experimental group, the higher concentration of PET microplastics resulted in the presence of hemosiderin granules in the interstitium of fish kidneys, confirmed by a positive Perls test (Fig. 6A, B). The cytoplasm of tubule epithelium contained large vacuoles filled with orange-brown secretion (Fig. 7, 8). Furthermore, an increase in the number of cells exhibiting pyknotic nuclei (karyopyknosis), indicative of the onset of apoptosis, was visually observed in the epithelial cells of the tubules (Fig. 7, 8).

When exposed to microplastic particles (PET) at a concentration of 1.9 mg/l (1900 µg/l) in the gills, several histopathological changes were observed. These included swelling and destruction of the primary gill epithelium, alteration in the shape of the secondary lamellae to hook-shaped, their shortening, destruction of columnar cells, and hemorrhage. Additionally, degeneration and necrosis of cartilaginous tissue were observed in the gills of some individuals (Fig. 9 A, B).

Similar histopathological changes were noted in fish gill tissue when the concentration of microplastic particles increased to 5.64 mg/l (5640 µg/l) (Fig. 10A, B).

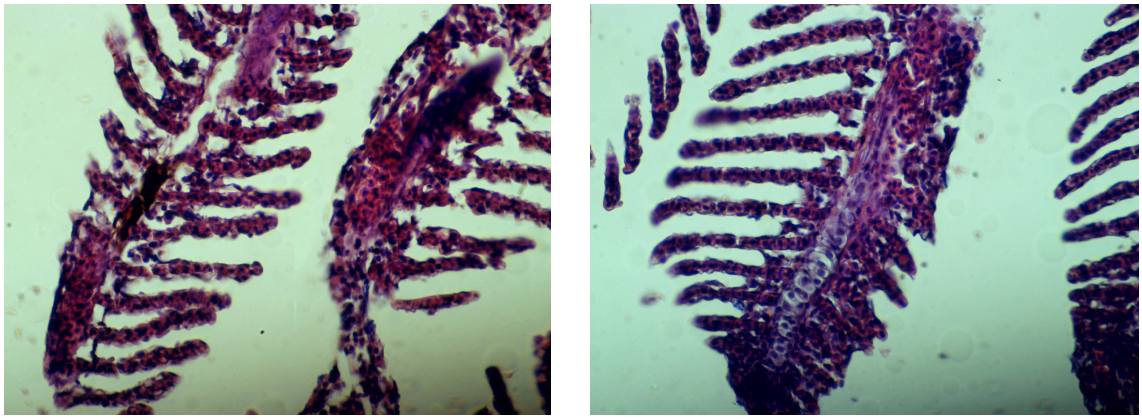

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Figure 9 – Photomicrograph of zebrafish *(Danio rerio)* gills after exposure to 1.9 mg/L of PET microplastic particles showing destruction of primary and secondary gill lamellae with the blood stasis in vessel and hemorrhage (A, B); destruction (A, B) and shortening of secondary gill epithelium (B). | Degeneration and necrosis of cartilage tissue (A). Magnification 400x. H & E staining

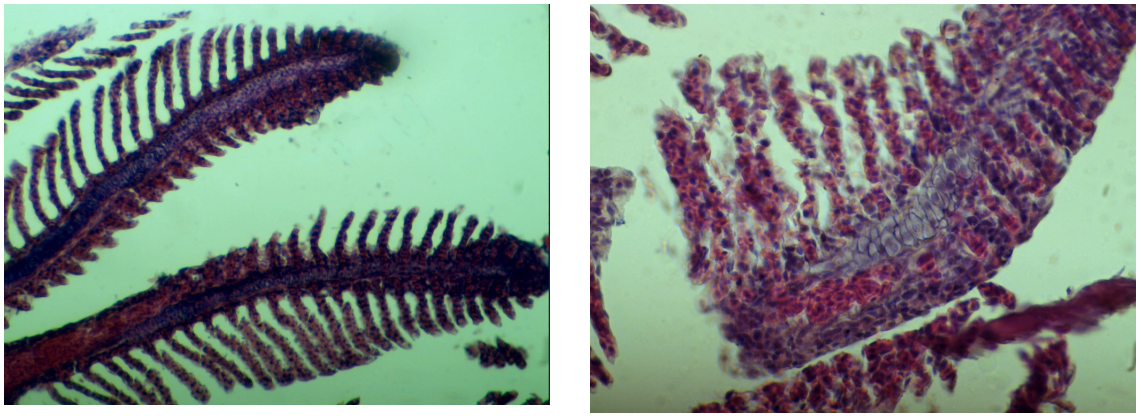

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Figure 10 – Photomicrograph of zebrafish *(Danio rerio)* gills after exposure to 5.64 mg/L of PET microplastic particles showing shortening of secondary gill epithelium (black arrow), hemorrhage, and blood stasis in the vessel (white arrow). Magnification x200 (A); x400 (B). H & E staining

Conclusion

A pathological study of zebrafish (*Danio rerio*) exposed to microplastic polyethylene terephthalate (PET) particles revealed significant negative impact on the organs. Exposure to PET microplastic particles at a concentration of 1.9 mg/l resulted in necrotic processes in tissue, generalized circulatory disorders, tissue edema, destruction of blood vessels, impaired permeability, and hemorrhages in both kidneys and gills.

In the kidneys of the first experimental group, vacuolization and hyaline-droplet degeneration of epithelial cells of the renal tubules were recorded, suggesting a toxic effect of microplastics to the organ. At a higher concentration of microplastic particles (5.64 mg/l) in the kidneys, similar pathological processes in the microvasculature were observed, although hyaline-droplet degeneration was absent. Additionally, a small amount of hemosiderin in the organ's stroma and orange-brown vacuoles were observed, requiring further investigation.

In the gills, exposure to microplastics at both concentrations resulted in characteristic changes in the histological structure of the organ, including destruction of the primary and secondary gill epithelium, curvature, and shortening of the secondary gill lamellae. Despite the chronic exposure to microplastics, no compensatory-adaptive reactions were observed in the organs, indicating a lack of adaptation processes in the fish's body under prolonged exposure to microplastic particles, posing a greater threat to marine organisms.

Based on the results of the study, it can be inferred that smaller microplastic particles may not only accumulate in the gastrointestinal tract during food intake, as suggested by some authors [5], and mechanically damage organ tissue, but also migrate through the bloodstream. This migration can lead to a toxic effect and induce characteristic pathological processes in the kidneys and gills of fish.

These results highlight the potentially serious impact of microplastics on zebrafish (*Danio rerio*), including kidney and gill dysfunction, as they have consequences such as the deterioration of vital functions in fish and increased mortality rates, as well as reflect the harm to the ecosystem as a whole. Considering the role of this fish species as a model research object, the results obtained can have wide practical applications in ecology and environmental protection.

Thus, further research in this area is needed to better understand the mechanisms and consequences of microplastics impact on the physiology of aquatic organisms and to develop effective strategies to control and reduce plastic pollution in aquatic ecosystems.

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