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Antibiotic toxicity under climate-change stressors in fish – A liver histopathology assessment

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Abstract

Background: Antibiotics in aquatic systems, combined with temperature and pH changes, threaten non-target organisms. Histopathological analysis provides integrative evidence of sublethal toxicity, reflecting cumulative physiological disruptions of several stressors on fish health [1]. **Objective:** This study evaluated liver histopathological alterations in *Danio rerio* after chronic exposure to environmentally relevant concentrations of sulfamethoxazole (150µg SMX/L), trimethoprim (30µg TRIM/L), and their mixture (MIX: 150µg SMX/L + 30µg TRIM/L) under different environmental conditions [2]. **Methods:** Three independent assays were conducted to assess the effects of temperature (26, 28, and 32 °C), pH (6.5, 7.5, and 9.0), and a combined climate-change scenario (28 °C + pH 9.0) in zebrafish liver. Liver alterations were analyzed using qualitative and semi-quantitative methods, and a total liver histopathological index (LI) was calculated. An Independent Action (IA) model was applied to integrate the effects of temperature and pH with antibiotic exposure and to evaluate the interactive impacts on liver histopathology [3]. **Results:** Qualitative analysis revealed circulatory (e.g., sinusoidal dilation), regressive (e.g., necrosis and hepatocellular degeneration), and progressive (e.g., hepatocyte nuclear hypertrophy) alterations across all tested scenarios. Semi-quantitative analysis showed that increasing temperature intensified the LI, even in the absence of antibiotics. At 32 °C, SMX induced severe lesions (e.g., hepatocellular degeneration, necrosis), indicating a temperature-dependent increase in LI. LI values increased for all antibiotic treatments, at pH 7.5 and for TRIM and MIX at pH 9.0. Although no significant alterations in LI values were detected under combined scenario, IA model revealed synergistic interactions for SMX and TRIM under combined temperature and pH, with liver damage exceeding predicted effects, whereas MIX exhibited antagonistic interactions, resulting in lower-than-expected damage. In general, histological lesions were observed across all antibiotic treatments and scenarios, indicating persistent adverse effects. **Conclusions:** Overall, these results highlight liver histopathology as a sensitive biomarker of toxicity and show that environmental stressors strongly modulate antibiotic effects. This underscores the need to adopt multi-stressor approaches in ecological risk assessment, particularly under future climate-change scenarios.

Keywords: Sulfamethoxazole, Trimethoprim, Mixtures, Temperature, pH, Zebrafish, Histology

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References

1. Rodrigues, S. et al. Histopathological effects of the antibiotic erythromycin on the freshwater fish species *Oncorhynchus mykiss*. *Ecotoxicol Environ Saf* **2019**, *181*, 1–10, doi:10.1016/J.ECOENV.2019.05.067.
2. Diogo, B.S. et al. How climate stressors intensify antibiotic toxicity: A histopathological study in zebrafish. *Environ Toxicol Pharmacol* **2026**, *123*, 104972, doi:10.1016/j.etap.2026.104972.

3. Diogo, B.S. et al. Mixture matters: Exploring the overlooked toxicity of sulfamethoxazole and trimethoprim in aquatic environments. *Environ Toxicol* **2025**, *40(11)*, 1277-1293, doi:10.1002/tox.24528.