

HISTOPATHOLOGICAL EFFECTS OF WATER POLLUTANTS ON *CYPRINUS CARPIO*

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Abstract

Ecological environments, as well as human populations, are greatly affected by water pollution, which is a widespread environmental issue. The common carp (*Cyprinus carpio*) is often utilized as a sentinel species to assess the impacts of aquatic pollutants. In this study, the impacts of water pollutants, especially lambda-cyhalothrin, on tissue structures of *Cyprinus carpio*, specifically the gills, liver, kidneys, and intestines, are investigated. In a controlled 30-day laboratory exposure study involving two groups, namely one treated with contaminated water of the Narmada River located near Bhopal, India, and another kept in pristine water, after the period of exposure, histopathological techniques were used to analyze tissue-level changes and organ injuries. The treated group had major pathological changes of necrosis, epithelial hyperplasia, and cellular degeneration, where the maximum injury was noted to be in the gills. The liver, kidneys, and intestines of the exposed fish also exhibited major structural abnormalities from the control group. These observations highlight the adverse effect of pesticide contamination on the health of fish and underscore the utility of histopathology as a diagnostic technique in aquatic toxicology. The research provides valuable information on lambda-cyhalothrin's sublethal impacts, and it supports stronger water quality monitoring and pollution control measures to protect aquatic biodiversity for generations to come.

Keywords: Environmental toxicology, Freshwater ecosystems, Histopathological analysis, Lambda-cyhalothrin toxicity, Pesticide pollution

1. Introduction

Pollution of water is among the gravest environmental threats globally that critically endangers aquatic ecosystems as well as human health. Freshwater ecosystems—rivers, lakes, and wetlands—are being increasingly polluted by unchecked industrialization, aggressive agriculture, and untreated waste releases. Among the key players in aquatic degradation are pesticides like lambda-cyhalothrin, which are being detected more and more in freshwater bodies as a result of agricultural runoff (He et al. 2008; Yasser et al., 2024). These contaminants build up in aquatic ecosystems and cause toxic impacts on resident species, causing ecological imbalance and compromising biodiversity. Fish species, particularly *Cyprinus carpio* (common carp), are major bioindicators of monitoring such environmental imbalances due to their large geographical range and aquatic pollutant sensitivity (Jaffer et al., 2017).

Fish are exposed to a range of contaminants like heavy metals and pesticides that have impacts on physiological function and structural harm at the cellular level (Rajeshkumar et al., 2017). This paper elucidates the toxicological consequences of lambda-cyhalothrin, a pyrethroid insecticide (Mustafa et al., 2017). Lambda-cyhalothrin is extensively used for controlling pests in agriculture and public health on cotton, vegetables, and cereals (Maund et al., 1998). It acts on voltage-gated sodium channels in insect nervous systems, causing paralysis and death. Although target-specific, the compound is water-body persistent, where it bioaccumulates and affects non-target organisms (Farmer et al., 1995). Scientific evidence confirms its high fish toxicity at extremely low concentrations, causing neurotoxicity, oxidative stress, and histopathological changes in organs like the gills, liver, and kidneys (Cui et al., 2015).

Agrochemicals such as lambda-cyhalothrin and other chemical contaminants interfere with essential metabolic functions in fish. The interferences lead to degeneration of cells and failure in organs, particularly the gills, liver, kidneys, and intestines—organs that are well known to have augmented sensitivity to environmental pollutants (Altun et al., 2017; Cengiz, 2006). The gills, being the primary location for gas exchange, are prone to be the initial organs to exhibit pathological changes such as necrosis and epithelial hyperplasia (Mustafa et al., 2017). The liver, involved in detoxification, is prone to exhibiting fatty degeneration, necrosis, and inflammatory cell infiltration following exposure to pollutants (Georgieva et al., 2016; Rana et al., 2015). Similarly, the kidneys that are responsible for osmotic balance and removal of waste undergo glomerular atrophy and tubular damage after toxic stress. Intestinal tissue may also be damaged, as toxins disrupt nutrient absorption and induce inflammation.

Empirical research has verified various organ-specific responses in fish under exposure to environmental contaminants. *Cyprinus carpio* was studied under nanoparticle and copper exposure, which resulted in histological changes in primary organs (Mansouri et al., 2016; Forouhar Vajargah et al., 2018). Additional studies involving other freshwater fishes, such as *Barbus capito*, *Chondrostoma nasus*, and *Clarias gariepinus*, demonstrated genotoxicity and histopathological pollution effects (Koca et al., 2008; Zaghloul et al., 2020). Ozmen et al. (2006) documented waterborne pollutant impacts on *Cyprinus carpio* in Karakaya Dam Lake, emphasizing the species' role in toxicological surveillance. Techniques such as micronucleus induction are increasingly being used in conjunction with histopathology to assess pollutant-caused cellular damage (Rašković et al., 2013; Yasser et al., 2024).

Histopathological analysis proposes a comprehensive understanding of pollutant impact through the detection of microscopically visible tissue lesions. Histopathological analyses provide essential information concerning pollutant-induced toxicity and its development, particularly under sublethal levels of exposure. Though many studies have been conducted on overall pollutant impacts, very few have explored the comprehensive histopathological impact of lambda-cyhalothrin on *Cyprinus carpio*. Previous literature is mostly concerned with single toxicants or with alternative species, thus leaving a lacuna in information on pesticide-induced multi-organ damage in this model fish. This research fills that lacuna through a targeted assessment of histological changes following controlled exposure to lambda-cyhalothrin.

Consequently, the current study examines the histopathological impact of environmentally meaningful concentrations of lambda-cyhalothrin on the gills, liver, kidneys, and intestines of *Cyprinus carpio*. The results will be used to guide water quality assessment practices as well as provide input for future environmental monitoring paradigms.

1.1 Research Objectives

The key objectives of this study are:

1.1.1 To evaluate the extent of histopathological damage in the gills, liver, kidneys, and intestines of *Cyprinus carpio* exposed to lambda-cyhalothrin.

1.1.2 To compare the tissue damage in experimental fish with unexposed control fish to assess pollutant-induced differences.

1.1.3 To analyze the relationship between pollutant concentration and severity of tissue lesions to understand dose-dependent effects and ecological implications.

By achieving these objectives, the study aims to contribute vital information about fish health under pesticide exposure and reinforce the role of histopathology in aquatic toxicology. The outcomes will assist policymakers, aquaculture professionals, and environmental regulators in implementing targeted pollution control strategies and ensuring the sustainability of freshwater ecosystems.

2. Methodology

2.1. Study Area and Sampling Location

The Narmada River functions as the research site, which is located within the central Indian region adjacent to Bhopal city. Bhopal distinguishes itself by hosting various ecological zones within reach of industrial districts and agricultural regions. Two distinct water sites were studied during the research: one location had human-caused pollution from industrial activities, while the other site remained free from human disturbances. The polluted site was developed near

industrial facilities since industrial facilities discharged agricultural runoff and untreated wastewater into the river. The protected reserve functioned as the control site because it had a river segment that stayed physically isolated from industrial areas and agricultural zones. Research investigators performed baseline data equivalence by analyzing temperatures, pH levels, dissolved oxygen measures, turbidity ratings, and pesticide contamination levels at both locations before starting the study. The contaminated site showed the presence of lambda-cyhalothrin at 1.2 mg/L, confirming pesticide pollution as the dominant contaminant.

2.2. Study Organism (*Cyprinus carpio*)

The research utilized *Cyprinus carpio* (common carp) as the test species because of its ecological importance and broad distribution range, together with its proven sensitivity to environmental pollutants. The research used healthy juvenile fish with a length of 6–8 cm and an age range of 3–5 months. Researchers obtained their fish from a Narmada River basin local hatchery. The experimental fish spent seven days in laboratory tanks containing dechlorinated water at $24 \pm 2^\circ\text{C}$ with pH levels between 7.5–8.0 under 12:12 light: dark photoperiod conditions. The researchers withheld food from the fish for 24 hours before testing to achieve standard digestion levels and prevent dietary interference.

2.3. Experimental Design

The research design contained two primary sections, which separated the control group that received clean water from the reference site from the experimental group that received polluted water from the test site. Each group consisted of 30 fish. The research period spanned 30 days, while water quality parameters were assessed daily. Standard methods were used to measure water temperature, pH, dissolved oxygen, and ammonia levels every 48 hours. The constant run of aerators within experimental tanks maintained dissolved oxygen content between 5 to 7 mg/L with water temperature held at $24 \pm 2^\circ\text{C}$ to maintain fish physiological comfort. The researchers replaced the water supply every 72 hours to prevent the accumulation of waste. Every experimental tank held 50 liters of water and held one fish within a volume of 1.5 liters.

2.4. Water Pollutants

The selected pollutant in this study was lambda-cyhalothrin, a synthetic pyrethroid insecticide widely applied in agriculture. It's a common occurrence in irrigation and storm runoff waters, highlighting its status as a common contaminant in freshwater systems (Wakil et al., 2024). The high aquatic toxicity of the compound, along with its persistence in the environment, renders it a major concern for aquatic environments. These characteristics, in addition to their growing occurrence in marine ecosystems, piqued our scientific interest and presented a sound argument for choosing lambda-cyhalothrin as the model toxin for this research.

Earlier research and reports formed the basis of establishing the pollutant concentrations within the contaminated area. Researchers in the research group scaled down pollutant concentrations to correspond with the pollution levels found in the water source. The experimental tanks were treated with lambda-cyhalothrin at a concentration of 1 mg/L. Scientists selected this concentration because it represents a relevant environmental level and causes known toxic effects in aquatic life forms.

2.5. Exposure to Pollutants

Fish received the contaminated water treatment for 30 days within controlled laboratory testing environments. The literature indicates that subchronic exposure (30 days) produces noticeable fish tissue histopathological changes, thus the chosen exposure duration. The experimental tanks operated with continuous aeration to maintain equal conditions of water quality during the exposure period. The experimental water received complete replacement every 72 hours to maintain consistent pollutant concentrations and water quality parameters, including pollutant levels, pH, temperature, and dissolved oxygen.

2.6. Histopathological Examination

The researchers killed all fish through a humane MS-222 (tricaine methanesulfonate) overdose at the end of the 30-day exposure period. The researchers performed dissections to collect organs from fish, which were examined through histopathological tests on gills, liver, kidneys, and intestines. The researchers removed the organs while rinsing them with phosphate-buffered saline (PBS) before placing them directly into 10% formalin solution for 24 hours of fixation. The tissues underwent a two-hour dehydrating process using an ethanol series that increased from 70% to 90% to 100% before clearing them in xylene, followed by paraffin embedding.

The microtome generated 5 μm -thick tissue sections, which were placed onto glass slides. The tissue slides received H&E staining for morphological tissue assessment. The histological examination utilized Periodic Acid-Schiff (PAS) to identify liver glycogen storage and Masson's Trichrome to detect gill collagen deposition.

Research analysts performed histopathological assessments under the Olympus BX41 microscope at 10x, 40x, and 100x magnifications. The severity of all histopathological changes received semi-quantitative scoring from no damage (0) to severe damage (4), with intermediate scores (1–3) indicating different levels of tissue injury. The examined lesions included necrosis alongside hyperplasia, degeneration, and inflammation. Digital camera images were taken through an attached microscope for documentation purposes.

2.7. Data Collection and Analysis

Researchers obtained data through lesion severity ratings of each organ combined with documentation of particular histopathological abnormalities. The research team analyzed aggregated scores to identify any differences between groups consisting of controls and experiments. The researchers conducted their statistical analysis through SPSS version 22.0. The Shapiro-Wilk test evaluated data normality before we used the independent samples t-test for normal data, while non-normal data required Mann-Whitney U testing. The research determined statistical significance at a p-value below 0.05. Two independent researchers conducted histopathological scoring to determine inter-observer reliability for tissue section assessment. The kappa coefficient reached 0.85, which demonstrates high agreement between observers.

2.8. Ethical Considerations

The research followed all ethical rules for animal usage in scientific investigations. The experimental protocol gained approval from the Institutional Animal Care and Use Committee (IACUC) at the Indian Institute of Environmental Studies based in Bhopal, India. Standard humane euthanasia procedures were used to end the life of the fish after all measures to reduce their suffering were implemented. All experimental activities implemented the 2013 requirements of the Ministry of Environment, Forests, and Climate Change, India, by ensuring no fish endured prolonged distress as well as using humane euthanasia protocols and strict adherence to animal care standards.

3. Results

3.1. Water Quality and Pollutant Concentrations

Water quality measurements took place at the polluted and control sites before starting the experimental exposure. The measured temperature and pH values, together with dissolved oxygen levels, showed no substantial differences between the two sites. The testing area revealed substantial differences in pollutant quantity levels. The polluted site showed a lambda-cyhalothrin concentration of 1.2 mg/L, while no pesticide residues were detected in the control site samples. The control site remained free from any significant contamination, as pesticide levels were below the detection limit. The experimental tanks operated under stable pollutant concentrations throughout their 30-day testing duration. Table 1 shows the water quality measurements from the control site and the polluted site, which include their pesticide concentration levels.

Table 1: Baseline Water Quality and Pollutant Concentrations at Control and Polluted Sites

Parameter	Control Site	Polluted Site
Temperature (°C)	24.1 ± 0.5	24.3 ± 0.4
pH	7.8 ± 0.2	7.7 ± 0.3
Dissolved Oxygen (mg/L)	6.8 ± 0.3	6.7 ± 0.2
Lambda-cyhalothrin (mg/L)	Not detected	1.2

3.2. Fish Health and Mortality Rate

The researchers tracked fish health conditions from the start to the finish of the exposure period. The experimental group of fish displayed minimal changes in their activity levels and feeding patterns during the initial ten days of the exposure period. The experimental group included 10 fish with reduced movement, while 5 fish stopped eating completely for two days. The fish in both groups maintained normal physical appearances without any detected abnormalities throughout their acclimatization phase. The study duration revealed no fish deaths in either the control group or experimental group since the exposure conditions remained within survivable ranges for the fish population. Regular checks on fish health confirmed that the experimental conditions did not create additional damage beyond the recorded behavioral effects. The fish mortality rate appears in Table 2, while the summary of health observations, including behavioral changes and feeding patterns, shows results for control and experimental groups throughout the study period.

Table 2: Fish Mortality and Health Observations During the Study Period

Group	Mortality Rate (%)	Behavioral Observations	Feeding Observations
Control	0	No visible abnormalities; normal activity	Normal feeding behavior, no reduction in food intake
Experimental	0	Reduced activity in 10 fish, lethargy observed in 5 fish	Reduced feeding in 5 fish for 2 days; 10 fish had lower food intake

3.3. Histopathological Examination

The experimental group organs underwent multiple significant histopathological changes when compared to the control group organs. The experimental group's gills displayed severe necrosis and hyperplasia, together with epithelium degeneration and the detachment of gill filaments. The liver tissues from the experimental group displayed fatty degeneration, together with limited necrosis and inflammatory cell infiltration. The experimental group's kidney tissue showed glomerular atrophy together with renal tubule degeneration, which suggested possible renal dysfunction. The experimental group showed minimal damage to the intestinal mucosa, together with sporadic inflammatory cell intrusions. The control group organs displayed no histopathological changes during the exposure period because they maintained their normal tissue structure.

The experimental group developed more intense tissue damage than the control group, which showed normal tissue structure. The research results demonstrated identical patterns in all examined organs, thus validating the theory that lambda-cyhalothrin exposure severely harmed fish health. The table shows that the experimental group displayed severe lesions in all examined organs while the control group remained unaffected, as shown in Table 3.

Table 3: Histopathological Findings and Severity Scores for Gills, Liver, Kidneys, and Intestines in Control and Experimental Groups

Organ	Control Group (Severity Score)	Experimental Group (Severity Score)
Gills	No lesions (0)	Severe lesions: Necrosis, hyperplasia, and epithelial degeneration (3.5 ± 0.7)
Liver	Normal tissue (0)	Fatty degeneration, necrosis, and inflammatory infiltration (2.8 ± 0.5)
Kidneys	No lesions (0)	Glomerular atrophy, renal tubule degeneration (2.4 ± 0.6)
Intestines	Normal tissue (0)	Mild mucosal damage, inflammatory cell infiltration (1.9 ± 0.4)

3.4. Severity Scoring Analysis

Histopathological changes in the experimental group displayed more severe scores than those observed in the control group. The experimental group gills received a severity score average of 3.5 ± 0.7 , which indicated moderate to severe damage, but the control group maintained a score of 0, indicating no damage. The experimental group exhibited substantial pathological changes in the liver and kidneys, and intestines when compared to the control group. The experimental group organs scored 2.8 ± 0.5 for liver and 2.4 ± 0.6 for kidneys, and 1.9 ± 0.4 for intestines, but the control group showed no damage with scores of 0.

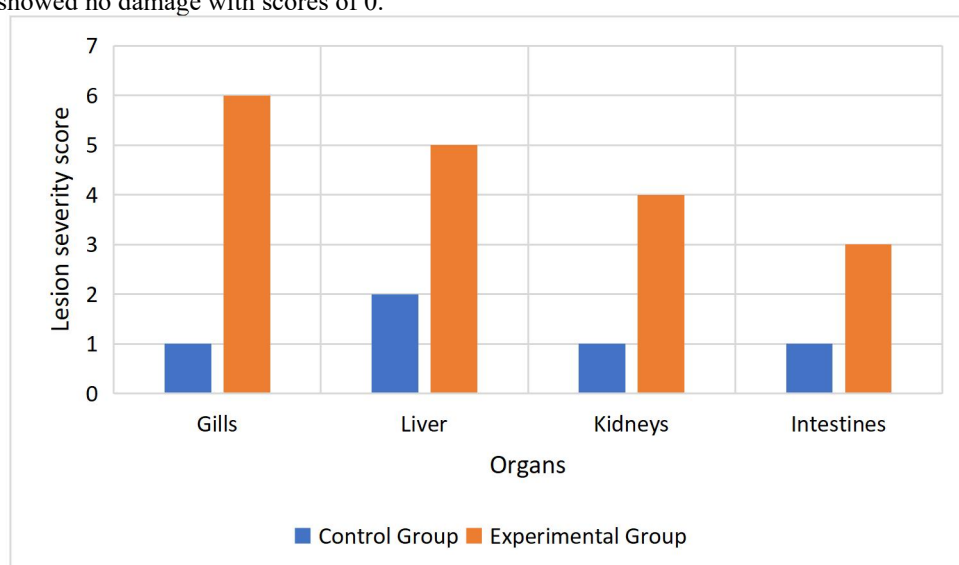


Figure 1: Lesion Severity Scores in Control and Experimental Groups

Figure 1 shows the lesion severity scores between control and experimental groups for gills, liver, kidneys, and intestines. The experimental group exhibited more severe organ damage than the control group based on the increased severity scores for all examined organs.

3.5. Inter-Observer Reliability

A reliability check between observers was performed to maintain precise and consistent evaluation of tissue lesions during histopathological assessments. The two independent observers achieved a kappa coefficient of 0.85, which demonstrates their strong agreement when evaluating the severity of lesions between different organs. The high degree of agreement between observers demonstrates that histopathological findings are valid and confirms the reliability of the scoring system.

3.6. Statistical Analysis

The experimental group showed greater severity of histopathological lesions when statistical analysis was applied to the results. The results of independent t-tests confirmed that there were statistically significant differences between both groups for gills, along with liver, kidneys, and intestines ($p < 0.05$). The experimental group demonstrated higher severity scores, which exceeded the scores observed in the control group, according to Table 3. The statistical results demonstrate that lambda-cyhalothrin exposure produced severe histopathological damage to experimental group organs when compared to the control group organs.

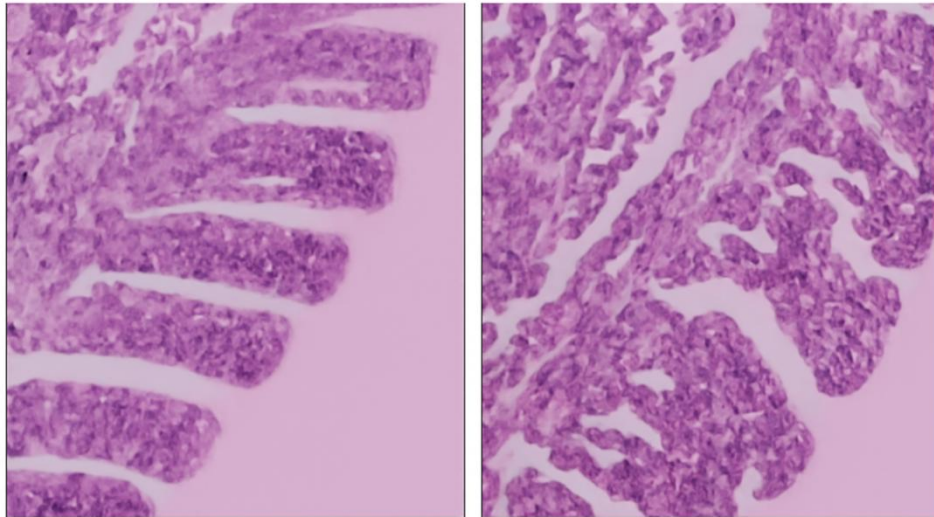


Figure 2: Histopathological Lesions in Gills of Control and Experimental Groups

Figure 2 shows detailed views of fish gill tissues stained with H&E between healthy Control Group specimens and damaged Experimental Group specimens. The control image displays uniform filaments and secondary lamellae, but the experimental image reveals necrosis and epithelial hyperplasia, and detachment, which demonstrates structural damage from lambda-cyhalothrin exposure.

4. Discussion

The research shows that *Cyprinus carpio*, which received water pollutants, developed serious pathological changes throughout its gills, liver, kidneys, and intestines. All tissues from the experimental group showed worse damage than the control group, with the gills revealing the most severe pathological changes. The experimental group gills displayed necrosis with epithelial hyperplasia and detachment, in agreement with studies about fish exposed to lead and cadmium metals (Rajeshkumar et al., 2017). While those studies focused on heavy metals, the present investigation attributes similar pathological patterns to pesticide exposure, specifically lambda-cyhalothrin. Gills remain responsive to changes in water quality; therefore, they become damaged through exposure to environmental stressors (Stoyanova et al., 2015). The presence of necrosis, together with hyperplasia, reveals chronic pollutant exposure that causes respiratory impairments in these fish. The experimental group liver showed fatty degeneration as well as necrosis and inflammatory cell infiltration. The research by Al-Sakran et al. (2016) shows that metal exposure, particularly with cadmium and mercury, causes hepatic damage while confirming the observed lesions. In the present study, such hepatic alterations were observed under lambda-cyhalothrin exposure, highlighting similar histological effects across different pollutant types (Cui et al., 2015). Fish health becomes negatively affected when liver damage occurs because this organ serves as a vital detoxification organ in fish.

The experimental group kidneys showed glomerular atrophy combined with renal tubule degeneration, which represents standard signs of nephrotoxicity that develop in fish after mercury and metal exposure (Iqbal et al., 2004). Although our study focused on pesticide exposure, the histopathological outcomes were comparable, suggesting converging pathways of renal damage. The kidneys sustain homeostasis through waste elimination and osmoregulation functions, but organ damage causes severe functional impairment to fish bodies. Moreover, the experimental group fish presented visible mucosal damage and cellular infiltration within intestinal tissue that indicated inflammation in addition to absorption deficiencies. Exposure to water pollutants leads to identical gastrointestinal damage in fish that decreases their digestive performance and deteriorates their general health condition. The pathologic tissue findings from this study correspond to previous research that documented *Cyprinus carpio* and other fish species after heavy metal and pesticide exposure (Xing et al., 2012). The research conducted by Pal et al. (2012) revealed identical damage patterns in fish gills, livers, and kidneys after exposure to pollutants, thus confirming the results obtained in this study.

The severity of the observed lesion differs from previously reported cases, perhaps due to differences in pollutant concentrations, exposure durations, and species-specific differences in biological responses. Increased concentrations of heavy metals in water resulted in more severe damage to gills, while lower concentrations resulted in less harmful effects on fish gills. Herein, the results of our research reveal that even exposure to pesticides, e.g., lambda-cyhalothrin at sublethal concentrations, can cause significant histopathological harm. The demonstrated association between the concentration of the pollutant and the severity of histopathology follows a non-linear trend based on these results, as pollutant type and exposure duration, among others, influence the results (Korkmaz, 2024). The assessment of water pollution impacts demands the application of *Cyprinus carpio* histopathological examination based on Koca et al. (2008). Normal observation methods do not determine the pollutant-induced health effects of fish, which histopathological examination gives full information regarding.

Fish population survival depends on four vital organs, which include the gills, liver, kidneys, and intestines. The health and survival of fish depend heavily on these organs because any damage to them produces severe negative effects. The identified effects produce major impacts on ecological systems. Fish experience reduced reproductive success and slower growth rates, together with increased vulnerability to diseases, because pollutants harm their bodies (Afzal et al.,

2022; Naz et al., 2021). The competitive fitness of fish diminishes when they encounter pollutants in their natural habitat, leading to population effects. These ecological changes in aquatic food chains spread upward through the predator population because fish serve as the primary food source for predators. Research by Noreen et al. (2021), Naz et al. (2021), Oropesa et al. (2009), and Shahafve et al. (2017) has shown various histopathological effects in fish resulting from exposure to copper nanoparticles, fungicides, simazine, and aflatoxin-contaminated diets. The upper Sakarya River Basin experienced these effects during the early 1990s based on Barlas (1999).

The research encounters two main drawbacks because it conducted brief testing and focused on specific pollutants. The health of fish deteriorates when they stay too long in polluted water, so scientists need to research both environmental and fish population health impacts. Research needs to investigate the combined toxic effects of multiple pollutants because this study examined only specific heavy metals and pesticide combinations. Future investigations need to determine the impact of pollutant exposure during various developmental phases of *Cyprinus carpio* on their future health status. The experimental group *Cyprinus carpio* presented essential tissue damage in their gills, liver, kidneys, and intestines based on data obtained from this research. The study demonstrates why proper water quality monitoring is crucial because it ensures protection for fish populations as well as stability of freshwater ecological systems. The tissue analysis method of histopathology enables researchers to determine how pollution affects fish health; therefore, it should serve as an essential tool within environmental monitoring systems for aquatic ecosystem protection.

5. Conclusion

This research shows that *Cyprinus carpio* experiences severe histopathological injury in the gills, liver, kidneys, and intestines after exposure to lambda-cyhalothrin, a commonly used synthetic pyrethroid pesticide. The results show that even sublethal levels of this pollutant can cause severe lesions, especially in the gill tissues, such as necrosis, hyperplasia, and epithelial degeneration. These histopathological effects were more pronounced in the experimental group than in the control group, demonstrating the acute effect of the pollutant on fish physiology. The results show that lambda-cyhalothrin pollution is a serious threat to freshwater diversity, and its impact on fish reproduction, growth, and survival is a possibility. The pollutant concentration employed in this study was environmentally relevant, and the controlled exposure approach, along with statistically confirmed lesion scoring and high inter-observer reliability, adds strength to the validity of results. This study is a strong addition to the existing body of knowledge regarding pesticide-induced toxicity in aquatic ecosystems and confirms histopathological examination as an essential technique for determining the effects of pollutants on aquatic organisms. Additionally, the results corroborate general ecological concerns regarding runoff of synthetic pesticides and their impacts on non-target organisms in aquatic food webs. Nonetheless, more long-term investigations are warranted to investigate chronic and developmental toxicities of lambda-cyhalothrin alone and in mixtures with other environmental contaminants. Future studies should also account for different life stages of *Cyprinus carpio* to evaluate vulnerability throughout its life cycle. In total, the results justify the necessity for strict regulation and monitoring of pollution for the protection of freshwater ecosystems and aquatic health.

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