

# Histopathological Evaluation of Afidopyropen Insecticide-Induced Hepatic and Renal Tissue Alterations in *Cyprinus carpio*

MAHANTESH DODAMANI AND MUNISWAMY DAVID\*

Environmental and Molecular Toxicology Research Laboratory, Department of Studies in Zoology, Karnatak University, Dharwad, 580003, Karnataka, India

E-mail: mr007dodamani@gmail.com, davidkcd@gmail.com

\*Corresponding author

## ABSTRACT

Afidopyropen, a novel insecticide with broad-spectrum efficacy, is widely used in agriculture to control pests. However, it poses ecological risks due to its persistence and potential runoff into aquatic ecosystems. Given the sensitivity of aquatic organisms to chemical pollutants, this study evaluates the histopathological effects on the liver and kidney of the freshwater fish *Cyprinus carpio* of sub-lethal concentration 0.2 mg/L of afidopyropen was given under semi-static bioassay method for the period of 1-, 10-, 20- and 30-days exposure periods. Histological analyses were conducted following sub-lethal exposure, with a control group for comparison. The results revealed significant toxic effects in both liver and kidney tissues. Liver alterations included Pyknosis, blood infiltration, hepatic vacuolisation, destructed hepatocytes, and fatty infiltrations. In the kidney, haemorrhaging, glomeruli, Bowman spaces, destructive renal tubules, and nuclear tubular cells were observed. These effects became more pronounced with prolonged exposure, suggesting a dose- and time-dependent response. These histopathological changes indicate a disruption of essential physiological processes, including metabolic regulation and excretion, leading to impaired fish health. Liver and kidney alterations serve as sensitive biomarkers of afidopyropen toxicity, offering valuable indicators for ecotoxicological biomonitoring in aquatic systems. This study underscores the potential risks posed by afidopyropen to aquatic ecosystems and highlights the need for robust environmental monitoring and regulatory measures to mitigate its adverse effects.

**Key words:** Afidopyropen, *Cyprinus carpio*, Kidney, Liver, Histopathology

## INTRODUCTION

Water contamination can significantly perturb aquatic ecosystems, destabilizing species diversity and compromising the structural integrity of habitats that rely on pristine, well-regulated hydrological conditions. The primary sources of water contamination are industrial chemicals and synthetic pesticides used in agriculture, urban areas, and the chemical industry which can directly and indirectly enter aquatic ecosystems. Direct sources include runoff from rain, agricultural irrigation, wastewater treatment plants, and industrial processes. Indirect sources involve atmospheric deposition from pesticide drift, rainfall runoff, and diffuse pollution via surface water flow. These pollutants cause toxicity, disrupt ecological functions, and harm aquatic health and biodiversity. Their persistence and bioaccumulative nature intensify their negative impact on aquatic environments (Syafudin et al. 2021, AbuQamar et al. 2024).

The success of environmental monitoring and

risk-assessment programs hinges on carefully selecting bioindicator species. *Cyprinus carpio* has been cultivated for over 8000 years, and its widespread distribution, economic importance, and advantageous traits, such as rapid growth and environmental adaptability, underscore its pivotal role in aquaculture. *C. carpio* contributes 3.4% to global fish production and is farmed in over 100 countries. Its inherent resilience to various pollutants makes it an ideal bioindicator for ecotoxicological evaluations, capable of detecting both sub-lethal and lethal effects of contaminants. Given its sensitivity to environmental stressors, *C. carpio* is also a critical organism for histopathological studies, enabling detailed assessment of pollutant-induced tissue damage and cellular alterations (da Silva Montes et al. 2020, Nakajima et al. 2019, Zhai et al. 2021, Shahi et al. 2022, Georgieva et al. 2021, Yancheva et al. 2022). Fish are commonly used as model organisms in toxicological studies due to their ecological significance, physiological sensitivity to environmental contaminants, and capacity to

integrate lethal and sub-lethal effects across multiple biological systems. Additionally, members of the Cyprinidae family, especially carps, are essential to aquaculture due to their high economic value, adaptability to a range of environmental conditions, and utility in both aquaculture production and environmental health assessments (Burgos-Aceves et al. 2018, Fiorino et al. 2018, Chromcova et al. 2015, Vajargah et al. 2018).

Histopathological studies are critical for determining fish's cellular and tissue responses to acute and chronic environmental chemical stresses. These studies shed light on the preliminary consequences of contaminated pollutant exposure, both direct and indirect. Because fish are very sensitive to differences in aquatic environments, histopathology allows for identifying minute yet crucial morphological changes that represent physiological disturbances in several organ systems, including the gills, liver, kidney, and gonads. These changes, such as cellular degeneration, necrosis, inflammation, and tissue remodelling, can act as biomarkers for exposure and environmental stress. Thus, histopathology investigations are an effective technique for monitoring environmental health and estimating the ecological dangers of chemical contaminants in aquatic environments (Iqbal et al. 2020). Fish's liver and kidneys are necessary for detoxification, waste disposal, and chemical absorption. Damage to these organs might cause physiological imbalances. The liver serves as a detoxifying organ and is crucial for the fish body's metabolism and excretion of hazardous chemicals. The kidneys, which are responsible for osmoregulation and toxicant clearance, are especially susceptible to environmental toxins.

The histopathological study of fish organs provides a good way to determine the adverse effects of xenobiotics. Thus, toxicological research in aquatic species is critical for determining the effects on organ function and general health (van der Oost et al. 2003, Rodrigues and Fanta 1998, Nkwuda et al. 2020). Another study claims that the liver is a target organ because of its abundant blood supply, which results in observable toxicant exposure (Mohame 2009). The liver has also been widely researched concerning the harmful effects of various xenobiotics because it is the principal organ for

bioaccumulation (Nunes et al. 2015, Simonato et al. 2008). The kidney is the primary organ preserving bodily fluids' equilibrium in vertebrates. The kidney's form and function have changed through time to meet various physiological needs, and fish have the biggest variety of kidney types (Hentschel and Elger 1989). The kidney contains large amounts of filtration system called Bowman's capsule and proximal and distal convoluted tubules.

The toxicity in the surrounding medium that enters the fish's body alters the kidney's pathology. The damage to the filtration unit of the body can be fatal as it loses the capacity to detoxify the toxicants entered into the body. The damage or alteration in the kidney could indicate the extent of toxicity possessed by the toxicant. Afidopyropen is an emerging insecticide formulated specifically for the targeted control of aphid populations (Horikoshi et al. 2022). It affects transient receptor potential vanilloid (TRPV) channels in insect chordotonal organs, which differs from conventional pesticides (Kandasamy et al. 2017). However, recent research by Dodamani and David (2023) highlights potential risks to aquatic species, specifically *C. carpio* (common carp), showing significant damage to internal organs and physiological disruptions. These findings emphasize the need for more comprehensive ecological risk assessments to fully elucidate the environmental consequences of afidopyropen. This study attempts to improve our knowledge of the toxicological effects of the insecticide afidopyropen on aquatic organisms. The findings are expected to provide valuable information that will direct the development of more ecologically friendly pest management strategies, minimizing negative impacts on non-target species and maintaining the integrity of the aquatic ecosystem.

## MATERIALS AND METHODS

### Experimental design

Commercial insecticide Afidopyropen was purchased from a certified agrochemical supplier in Dharwad, Karnataka. *Cyprinus carpio* (common carp) with an average body weight of  $90 \pm 4$  g and an average length of  $18 \pm 2$  cm was obtained from a local aquaculture facility. The fish were acclimated in a recirculating aquatic system for 7 days before

exposure, during which they were fed a commercial fish pellet diet. The water was maintained at  $25 \pm 2^\circ\text{C}$  with a photoperiod of 12:12 h (light/dark cycle) as per OECD guidelines (Anonymous 2019). Water quality parameters, including pH ( $7.04 \pm 0.2$ ), total hardness ( $30.2 \pm 3.2$  mg as  $\text{CaCO}_3/\text{L}$ ), calcium ( $17.28 \pm 1$  mg/L), Phosphate ( $0.031 \pm 0.003$  mg/L) and Magnesium ( $0.77 \pm 0.5$  mg/L) were monitored regularly and maintained within optimal ranges for *C. carpio*. Fish were randomly assigned to five groups: Control (no Afidopyropen), 1-day, 10-day, 20-day, and 30-day exposure to Afidopyropen at 0.2 mg/L. The water was changed every day to maintain consistent insecticide concentrations. Fish in the control group were kept in untreated water. After exposure, fish were euthanized using 2-phenoxyethanol. The liver and kidneys were immediately dissected from each fish.

### Histological preparation

The method described by Humason (1972) was followed to study the histopathology of tissues. Control, exposed groups of fish were euthanized and selected tissues, Liver, and Kidney were isolated and fixed in Bouin's fluid for 24 hours at room temperature. Tissues were repeatedly washed with 70% alcohol till all the traces of Bouin's fluid were removed. The dehydration process was carried out by washing the tissue with alcohol (90% and 100%) and alcohol-benzene in different ratios (3:1, 1:1 and 1:3), followed by pure benzene and benzene-paraffin wax (1:1). After this process, the organs were embedded in paraffin ( $58-60^\circ\text{C}$ ). Sections (5-micron thickness) were taken, stained with haematoxylin and counter-stained with eosin. All sections were mounted with DPX and histopathological changes were observed under the light microscope at  $\times 400$  magnification.

## RESULTS

### Liver

#### *Liver tissue of control group fish*

The liver tissue of the control group fish was analyzed and found to have an intact cellular structure. The cells such as hepatocytes, sinusoids, and Kupffer's cells were observed in the control tissue. The hepatic portal vein was also found to be in good condition.

The hepatocytes were found to have unique polarisation arrangements. The nuclei were prominent, and the sinusoids and hepatocytes were richly supplied with blood via the hepatic portal vein. The Kupffer's cells were found adequately in the lining of sinusoidal structures and were distinguishable. There were no signs of blood infiltration whatsoever in the control tissue of the liver (Fig. 1a).

#### *1-day treated fish liver tissue*

In the day one treated group fish liver tissues were observed to have a normal histological structure similar to that of control tissue. The hepatocytes and sinusoids were arranged all over the tissue and were supplied with blood flow through the portal vein. The Kupffer's cells were also found at the lining of the sinusoids and were intact. The nuclei were also prominent, and there was no sign of blood infiltration in the tissue (Fig. 1b).

#### *10-day treated fish liver tissue*

In 10 days of treated liver tissue, the hepatocytes were found normal. However, there was a sign of initiation of pathological conditions in the liver cells. The nuclei were prominent, and the sinusoids were arranged normally. The tissue was also found to have Pyknotic nuclei a mild frequency compared to control tissue. The sinusoidal cells infiltrated with blood cells, which was abnormal. The Kupffer's cells were normal and the overall tissue was found in good condition except for Pyknotic nuclei and blood infiltration (Fig. 1c).

#### *20-day treated fish liver tissue*

The 20-day treated liver tissues of the fish exhibited various pathological lesions compared to the control group. The hepatocytes manifested Pyknotic nuclei and hepatic vacuolisation. In addition, blood infiltration was also found to be at a moderate frequency. An elevated number of pyknotic nuclei conditions highly characterized the group tissues. However, vacuolization was also found in moderate frequencies. The pathological condition in this group seemed high compared to control groups. It was also observed that the initiation of vacuolization was all over the tissue compared to the control group (Fig. 1d).

#### *30-day treated fish liver tissue*

The liver tissues of 30 days treated fish were analyzed and observed with severe pathological conditions

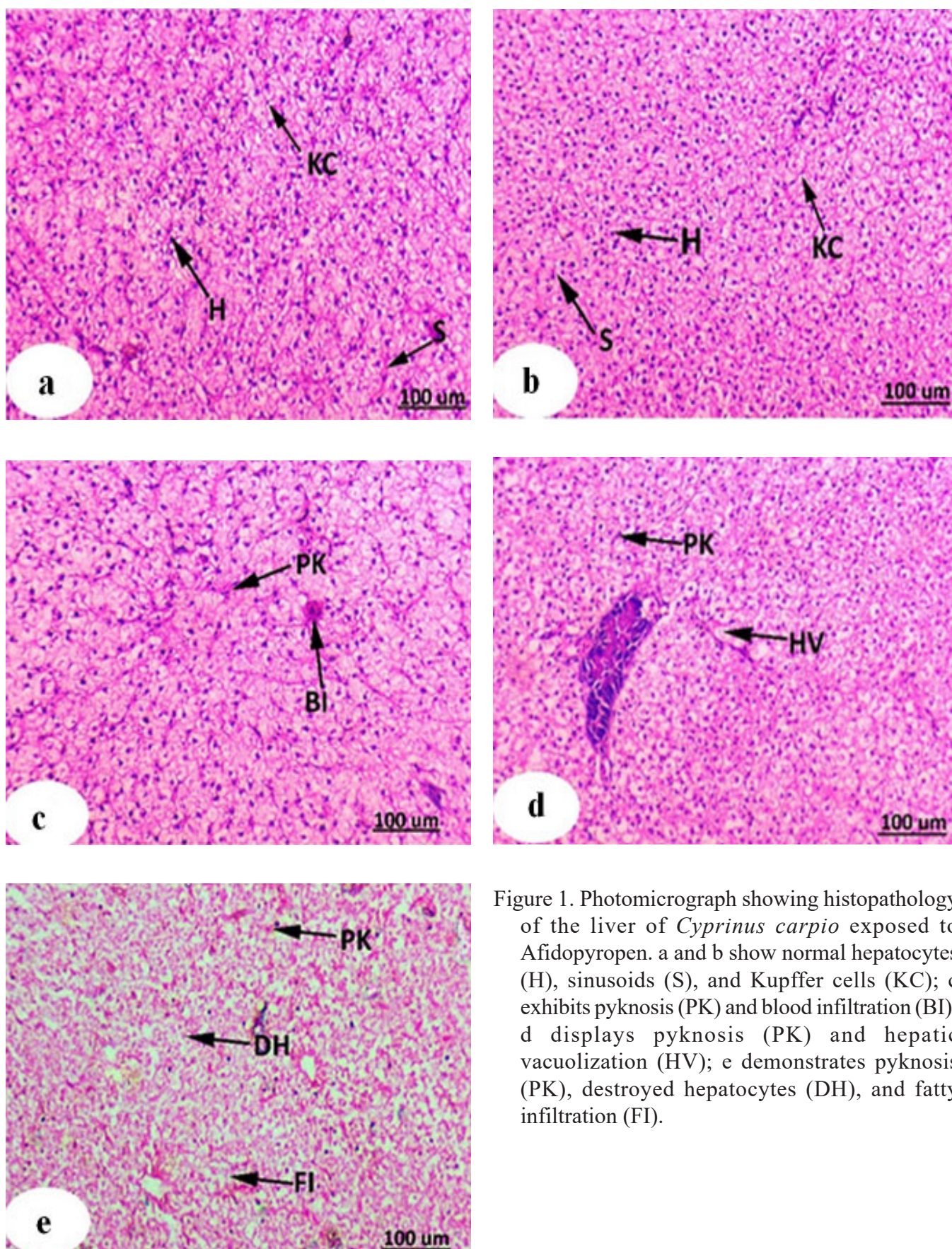


Figure 1. Photomicrograph showing histopathology of the liver of *Cyprinus carpio* exposed to Afidopyropen. a and b show normal hepatocytes (H), sinusoids (S), and Kupffer cells (KC); c exhibits pyknosis (PK) and blood infiltration (BI); d displays pyknosis (PK) and hepatic vacuolization (HV); e demonstrates pyknosis (PK), destroyed hepatocytes (DH), and fatty infiltration (FI).

compared to the control group. The hepatocytes were highly affected, and a destructed condition was found in the liver tissue. The hepatic nuclei were severely affected and were decreased. The sinusoidal cells were infiltrated with blood cells and hepatic vacuolisation. The frequency of occurrence of Pyknotic nuclei was also elevated compared to the 20-day treated and control groups. However, the tissues were observed to have a peculiar appearance of fatty infiltration. The blood infiltration was elevated compared to the control group. The overall structure of the liver tissue was altered compared to all other groups of liver tissues (Fig. 1e).

## Kidney

### *Kidney tissue of control group fish*

Histological observation of the tissues of the kidney shows all the cells with compact arrangement. The tissue was filled with distal tubule, proximal tubule, glomerulus, and Bowman's capsule. The distal and proximal tubules were found with normal structured cells with prominent nuclei. The glomerulus was covered with a Bowman's capsule and was intact. The glomerulus consisted of dense cells with capillaries. The distal tubules were composed of simple cuboidal epithelial cells, while the proximal tubules were characterized by the appearance of a ring of cells in the tissue (Fig. 2a).

### *1-day treated fish kidney tissue*

The tissues of the one-day treated fish kidney were more or less similar to the control kidney group. The nuclei of all cells were prominent and normal in structure. There was no change in the structure compared to the control group tissue. The distal and proximal tubules were found normal, and the glomerulus was covered with Bowman's capsule. The Bowman's space was also intact and the structure was not altered (Fig. 2b).

### *10-day treated fish kidney tissue*

The 10-day treated fish kidney tissues were examined for structural alterations. The tissues had damaged renal tubules (distal and proximal tubules). The glomeruli were normal in structure and the cells exhibited no change in appearance. However, mild necrosis was observable in the 10-day treated fish tissues compared to the control group. The frequent tubular damage can be seen throughout the tissue in this group (Fig. 2c). There was frequent haemorrhage

in the tissue, but not severe.

### *20-day treated fish kidney tissue*

The kidney tissues had comparatively high damage compared to the 10-day treated group. The damage was considerably severe compared to the control group. The pathological conditions include haemorrhage, necrosis, and dilated Bowman's space. The 20-day treated group was found to alter the structure of the cells moderately.

The proximal and distal tubules were highly damaged and a few were ruptured in the tissue, releasing necrotic cells. The necrosis was frequent with prominent accumulation of nuclei in the tissue. The tissue around the affected cells was also observed to have a haemorrhage. In addition, there was a condition called dilated Bowman's capsule characterized by the increased space between the glomerulus and Bowman's capsule (Fig. 2d).

### *30-day treated fish kidney tissue*

The 30-day treated fish kidney tissues were found to have severely damaged cell structures. The proximal tubule cells were altered, and the position of the nucleus in the tissue was changed. The distal tubule was damaged, and the nuclei oozed out of the cells. The overall structure of the distal and proximal tubule was drastically changed compared to the control group. The severe damage to the renal tubule also induced necrosis in the tissues. The necrosis was abundant and prominent nuclei of the cells were accumulated. The intactness of the cells was destructed compared to the control group. The degenerative glomeruli were also found frequently all over the tissue. The structure of the glomerulus was critically altered, in some parts of the tissue, it degenerated while in some parts it was observed with dilated Bowman's space. The haemorrhage in the tissue was also found in the 30-day treated group compared to the control group (Fig. 2e).

## DISCUSSION

The pathological changes in the fish can be a tool to assess toxicological implications in real-life scenarios. Every symptom or pathological condition represents either a defensive mechanism or a failure of the said mechanism. The previous study observed that pathological changes are considered an adaptive and protective mechanism from the toxicants (Mallatt

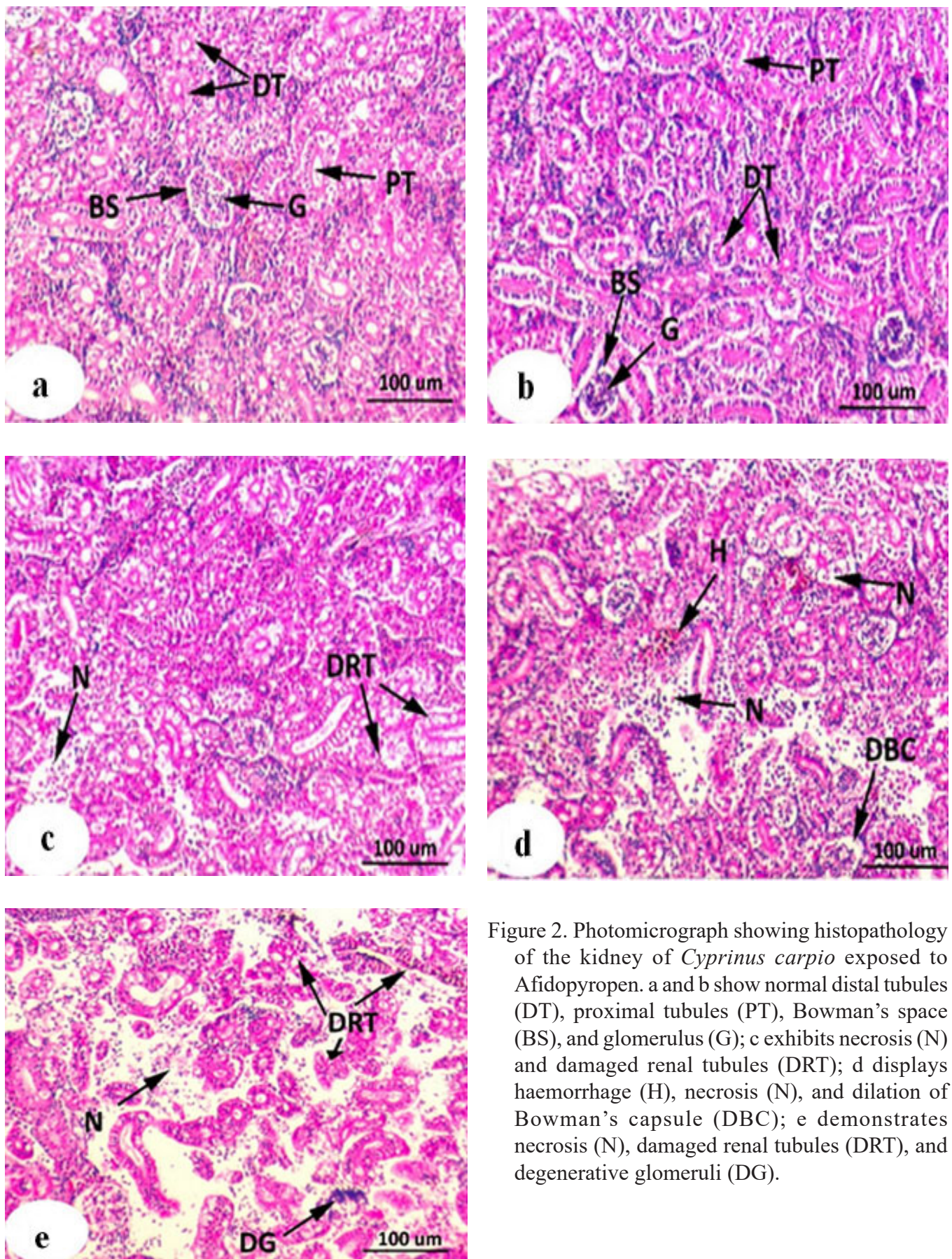


Figure 2. Photomicrograph showing histopathology of the kidney of *Cyprinus carpio* exposed to Afidopyropen. a and b show normal distal tubules (DT), proximal tubules (PT), Bowman's space (BS), and glomerulus (G); c exhibits necrosis (N) and damaged renal tubules (DRT); d displays haemorrhage (H), necrosis (N), and dilation of Bowman's capsule (DBC); e demonstrates necrosis (N), damaged renal tubules (DRT), and degenerative glomeruli (DG).

1985). The present study revealed several pathological changes in the liver and kidney of the fish *Cyprinus carpio* exposed to afidopyropen. The liver being the central organ of detoxification provides the information related to toxicity in the organism. The histological alterations in the liver indicate the organ's toxification degree (Dutta et al. 1993). The pathological changes can be used as markers of toxicity in the fish.

In the present study, the liver of the exposed groups showed Pyknosis, blood infiltration, hepatic vacuolisation, destructed hepatocytes, and fatty infiltrations. Pyknosis is the condition marked by the shrinkage of the nucleus due to the clumping of chromosomes. The previous studies observed frequent Pyknotic conditions in pesticide-exposed groups (Al-Otaibi et al. 2019, Neglur et al. 2021). The histological changes in the liver were attributed to the fish's exposure to afidopyropen. Similar pathological observations were observed in previous studies and could be interpreted as the failure of the liver system to cope with highly toxic conditions. The hepatic vacuolization was considered the initial toxicity symptom (Neglur et al. 2021). The destruction of the hepatocytes usually occurs in a highly toxic environment, and the organism usually tries to adapt to that environment. However, failure will lead to increased incidents of the above-said lesions (Al-Otaibi et al. 2019, Américo-Pinheiro et al. 2020). The present study's findings agree with those of Ghelichpour et al. (2017) and Pal et al. (2012), who observed similar histopathological alterations following exposure to pesticide formulations. These histological changes are well-established biomarkers and critical indicators in ecotoxicological assessment and monitoring (Yancheva et al. 2016).

The kidney receives a rich source of post-branchial blood and functions in the detoxification process of the blood. As a result any alteration in the kidney could lead to restricted function. Homeostasis and excretion are the two crucial kidney functions. However, afidopyropen was exposed to *Cyprinus carpio* with different exposure durations of 1, 10, 20, and 30 days. The kidney tissues showed constricted glomeruli and bowman spaces, destructive renal tubules and nuclear tubular cells were seen (Fig. 2). The control fish produced normal

glomerulus tufts, normal renal corpuscles, and normal nephrons, as well as normal Bowman's capsule encircling the glomerulus (Fig. 2a). Mammals create new nephrons only during the neonatal period; however, this process continues throughout life in fish, which is more common in young, rapidly growing fish (Reimschuessel 2001). The kidney is one of the first organs to be impacted by water pollutants, and it is the teleostean kidney (Thophon et al. 2003). The most frequent changes observed in the kidneys of fish exposed to contaminated water are tubule degeneration and changes in the corpuscle, such as damaged renal tubules, haemorrhaging, an increased bowmen space, and a glomerulus that is clogged (Takashima and Hibiya 1995). Camargo and Martinez (2007) reported on a study of *Procleodus lineatus* infestations caused by anthropogenic activities entering the Apertados stream, where possible causes were observed to be tubular epithelium necrosis due to toxicants and injury causing uncontrolled cell death. Ortiz et al. (2003) found results of Mugil and Barbus where they stated that the caused the glomerulus shrinkage and elevation of Bowman capsule space by the discharge of Lindane insecticide into the Barbate River were comparable to the present study. Mohamed (2009) described the study's findings with *Tilapia zillii* and *Solea vulgaris*, noting that the agricultural, industrial, and domestic wastes that were dumped into Lake Qarun in Egypt caused renal tubule degeneration, bleeding, necrosis in the focal area, and glomerular atrophy. However, the control and 1-day exposure were unaffected by any of these effects (Fig. 2a, b).

## CONCLUSIONS

The extended duration of afidopyropen exposure in the present study highlights its prolonged physiological impact on *Cyprinus carpio*. Interestingly, no notable histopathological alterations were detected in the one-day exposure group, indicating that healthy individuals exhibit a substantial capacity for rapid acclimatization to short-term environmental stressors. However, cumulative and severe pathological changes were observed with prolonged exposure, underscoring the compound's potential to induce chronic, progressive damage to

fish physiology. These findings highlight the need for thorough, long-term environmental monitoring and testing to understand better the lasting effects of such xenobiotics on aquatic organisms.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from Karnatak University and the Department of Zoology for providing the laboratory facilities necessary for the completion of this work at Karnatak University, Dharwad.

**Ethical statement:** All experimental procedures were conducted under the guidelines of the Institutional Animal Ethics Committee (IAEC). The care and use of animals followed the regulations established by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India

**Authors' contributions:** The first author performed the experiments, analyzed the data, prepared the figures, and drafted the manuscript. The second author developed the study, assisted with data interpretation, and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

**Conflict of interest:** The authors declare no conflict of interest

## REFERENCES

- AbuQamar, S.F., El-Saadony, M.T., Alkafaas, S.S., Elsalahaty, M.I., Elkafas, S.S., Mathew, B.T., Aljasmī, A.N., Alhammadi, H.S., Salem, H.M., Abd El-Mageed, T.A. and Zaghoul, R.A. 2024. Ecological impacts and management strategies of pesticide pollution on aquatic life and human beings. *Marine Pollution Bulletin*, 206, 116613. <https://doi.org/10.1016/j.marpolbul.2024.116613>
- Al-Otaibi, A.M., Al-Balawi, H.F.A., Ahmad, Z., Suliman, E.M. 2019. Toxicity bioassay and sub-lethal effects of diazinon on blood profile and histology of liver, gills and kidney of catfish, *Clarias gariepinus*. *Brazilian Journal of Biology*, 79, 326–336. <https://doi.org/10.1590/1519-6984.185408>
- Américo-Pinheiro, J.H.P., Machado, A.A., da Cruz, C., Aguiar, M.M., Ferreira, L.F.R., Torres, N.H., Machado-Neto, J.G. 2020. Histological changes in targeted organs of Nile Tilapia (*Oreochromis niloticus*) exposed to sublethal concentrations of the pesticide Carbofuran. *Water, Air & Soil Pollution*, 231, 228. <https://doi.org/10.1007/s11270-020-04628-5>
- Anonymous. 2019. OECD D. Test no. 203: Fish, acute toxicity test. OECD Guidelines for the Testing of Chemicals. OECD Publishing, Paris. <https://doi.org/10.1787/9789264069961-en>
- Baird, R., Eaton, A.D., Rice, E.W. and Bridgewater, L. (Eds.). 2005. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF), Washington, DC.
- Burgos-Aceves, M.A., Cohen, A., Smith, Y. and Faggio, C. 2018. MicroRNAs and their role on fish oxidative stress during xenobiotic environmental exposures. *Ecotoxicology and Environmental Safety*, 148, 995-1000. <https://doi.org/10.1016/j.ecoenv.2017.12.001>
- Camargo, M.M. and Martinez, C.B. 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical Ichthyology*, 5, 327-336. <https://doi.org/10.1590/S1679-62252007000300013>
- Chromcova, L., Blahova, J., Zivna, D., Plhalova, L., Casucelli, Di Tocco, L., & Svobodova, Z. 2015. NeemAzal T/S – toxicity to early-life stages of common carp (*Cyprinus carpio* L.). *Veterinarni Medicina*, 2015(1), 23-30. 60. <https://doi.org/10.17221/7922-VETMED>
- Da Silva Montes, C., Pantoja Ferreira, M.A., Giarrizzo, T., Amado, L.L. and Rocha, R.M. 2020. Evaluation of metal contamination effects in piranhas through biomonitoring and multi-marker approach. *Heliyon*, 6, e04666. <https://doi.org/10.1016/j.heliyon.2020.e04666>
- Dodamani, M. and David, M. 2023. Studies on histopathological alterations in the brain and gill, of *Cyprinus carpio* exposed to the insecticide Afidopyropen. *Toxicology International*, 30(4), 629-638. <https://doi.org/10.18311/ti/2023/v30i4/34393>
- Dutta, H.M., Adhikari, S., Singh, N.K., Roy, P.K. and Munshi, J.S.D. 1993. Histopathological changes induced by malathion in the liver of a freshwater catfish, *Heteropneustes fossilis* (Bloch). *Bulletin of Environmental Contamination and Toxicology*, 51, 895-900. <https://doi.org/10.1007/BF00198287>
- Fiorino, E., Schonova, P., Plhalova, L., Blahova, J., Svobodova, Z. and Faggio, C. 2018. Effect of glyphosate on early life stages: Comparison between *Cyprinus carpio* and *Danio rerio*. *Environmental Science and Pollution Research*, 25, 8542-8549. <https://doi.org/10.1007/s11356-017-1141-5>
- Forouhar Vajargah, M., Mohamadi Yalsuyi, A., Hedayati, A. and Faggio, C. 2018. Histopathological lesions and toxicity in common carp (*Cyprinus carpio* L. 1758) induced by copper nanoparticles. *Microscopy Research and Technique*, 81(7), 724-729. <https://doi.org/10.1002/jemt.23028>
- Georgieva, E., Yancheva, V., Stoyanova, S., Velcheva, I., Iliev, I., Vasileva, T., Bivolarski, V., Petkova, E., L'aszlo, B., Nyeste, K. and Antal, L. 2021. Which is more toxic? Evaluation of the short-term toxic effects of chlorpyrifos and cypermethrin on selected biomarkers in common carp

- (*Cyprinus carpio*, Linnaeus 1758). *Toxics*, 9, 9060125. <https://doi.org/10.3390/toxics9060125>
- Ghelichpour, M., Taheri Mirghaed, A., Mirzargar, S.S., Joshaghani, H. and Ebrahimzadeh Mousavi, H. 2017. Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of *Cyprinus carpio* (Linnaeus 1758) exposed to an oxadiazin pesticide, indoxacarb. *Aquaculture Research*, 48, 5666-5676. <https://doi.org/10.1111/are.13390>
- Hentschel, H. and Elger, M. 1989. Hentschel: Comparative Physiology. [https://scholar.google.com/scholar\\_lookup?hl=en&publication\\_year=1989&pages=163&author=H+Hentschel&author=M+Elger&isbn=%00null%00&title=Molecular+comparative+physiology+\(accessed+on+3.24.23\)](https://scholar.google.com/scholar_lookup?hl=en&publication_year=1989&pages=163&author=H+Hentschel&author=M+Elger&isbn=%00null%00&title=Molecular+comparative+physiology+(accessed+on+3.24.23)).
- Horikoshi, R., Goto, K., Mitomi, M., Oyama, K., Hirose, T., Sunazuka, T., Ômura, S. 2022. Afidopyropen, a novel insecticide originating from microbial secondary extracts. *Scientific Reports*, 12, 2827. <https://doi.org/10.1038/s41598-022-06729-z>
- Humason, G.L. 1972. *Animal Tissue Techniques*. 3rd ed. W.H. Freeman & Co Ltd., San Francisco & London. 492 pages.
- Iqbal, S., Atique, U., Mughal, M.S., Younus, M., Rafique, M.K., Haider, M.S., Iqbal, H.S., Sherzada, S. and Khan, T.A. 2020. Selenium-Supplemented Diet Influences Histological Features of Liver and Kidney in Tilapia (*Oreochromis niloticus*). *Jordan Journal of Biological Sciences*, 13(4).
- Kandasamy, R., London, D., Stam, L., von Deyn, W., Zhao, X., Salgado, V.L. and Nesterov, A. 2017. Afidopyropen: new and potent modulator of insect transient receptor potential channels. *Insect Biochemistry and Molecular Biology*, 84, 32-39. <https://doi.org/10.1016/j.ibmb.2017.03.005>.
- Mallatt, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fisheries and Aquatic Sciences*, 42, 630-648. <https://doi.org/10.1139/f85-08>
- Mohamed, F.A.S. 2009. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. *World Journal of Fish and Marine Sciences*, 1, 29-39. [https://www.idosi.org/wjfm/wjfm1\(1\)09/4.pdf](https://www.idosi.org/wjfm/wjfm1(1)09/4.pdf)
- Nakajima, T., Hudson, M.J., Uchiyama, J., Makibayashi, K. and Zhang, J. 2019. Common carp aquaculture in neolithic China dates back 8,000 years. *Nature Ecology & Evolution*, 3, 1415-1418. <https://doi.org/10.1038/s41559-019-0974-3>
- Neglur, S.B., Sanakal, R.D., David, M., Prakash, L. 2021. Studies on haematological and histopathological alterations induced by sublethal concentration of fenoxaprop-p-ethyl on freshwater fish *Cyprinus carpio*. *Exploratory Animal and Medical Research*, 11(1), 55-66. <https://doi.org/10.52635/EAMR/11.1.55-66>
- Nkwuda, P.J., Awoke, J.S. and Nwakpa, J.N. 2020. Histological changes in liver and kidney of *Clarias gariepinus* (Burchell 1822) juvenile exposed to sub-lethal doses of Chloramphenicol (CAP). *Aquaculture Studies*, 20, 19-28. [http://doi.org/10.4194/2618-6381-v20\\_1\\_03](http://doi.org/10.4194/2618-6381-v20_1_03)
- Nunes, B., Campos, J.C., Gomes, R., Braga, M.R., Ramos, A.S., Antunes, S.C. and Correia, A.T. 2015. Ecotoxicological effects of salicylic acid in the freshwater fish *Salmo trutta fario*: antioxidant mechanisms and histological alterations. *Environmental Science and Pollution Research*, 22, 667-678. <https://doi.org/10.1007/s11356-014-3337-2>
- Ortiz, J.B., de Canales, M.L.G. and Sarasquete, C. 2003. Histopathological changes induced by Lindane (?-HCH) in various organs of fishes. *Scientia Marina*, 67(1), 53-61. <https://doi.org/10.3989/scimar.2003.67n153>
- Pal, S., Kokushi, E., Koyama, J., Uno, S. and Ghosh, A.R. 2012. Histopathological alterations in gill, liver and kidney of common carp exposed to chlorpyrifos. *Journal of Environmental Science and Health, Part B* 47, 180-195. <https://doi.org/10.1080/03601234.2012.632285>
- Reimschuessel, R. 2001. A fish model of renal regeneration and development. *ILAR Journal*, 42, 285-291. <https://doi.org/10.1093/ilar.42.4.285>
- Rodrigues, E.L. and Fanta, E. 1998. Liver histopathology of the fish *Brachydanio rerio* after acute exposure to sublethal levels of the organophosphate Dimetoato 500. *Revista Brasileira de Zoologia*, 15, 441-450. <http://dx.doi.org/10.1590/S0101-81751998000200014>
- Shahi, N., Mallik, S.K. and Sarma, D. 2022. Muscle growth in targeted knockout common carp (*Cyprinus carpio*) mstn gene with low off-target effects. *Aquaculture*, 547, 737423. <https://doi.org/10.1016/j.aquaculture.2021.737423>
- Simonato, J.D., Guedes, C.L.B. and Martinez, C.B.R. 2008. Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. *Ecotoxicology and Environmental Safety*, 69, 112-120. <https://doi.org/10.1016/j.ecoenv.2007.01.012>
- Syafrudin, M., Kristanti, R.A., Yuniarto, A., Hadibarata, T., Rhee, J., Al-onazi, W.A., Algarni, T.S., Almarri, A.H. and Al-Mohaimed, A.H. 2021. Pesticides in drinking water-A review. *International Journal of Environmental Research and Public Health*, 18(2), 468. <https://doi.org/10.3390/ijerph18020468>
- Takashima, F. and Takashi, H. 1995. *An Atlas of Fish Histology: Normal and Pathological Features*. Kodansha Limited, Tokyo.
- Thophon, S., Kruatrachue, M., Upatham, E.S., Pokethitiyook, P., Sahaphong, S. and Jaritkuan, S. 2003. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. *Environmental Pollution*, 121, 307-320. [https://doi.org/10.1016/s0269-7491\(02\)00270-1](https://doi.org/10.1016/s0269-7491(02)00270-1)
- van der Oost, R., Beyer, J. and Vermulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology*, 13, 57-149. <https://www.ncbi.nlm.nih.gov/pubmed/21782649>
- Yancheva, V., Georgieva, E., Velcheva, I., Iliev, I., Stoyanova, S., Vasileva, T., Bivolarski, V., Todorova-Bambaldokova, D., Zulkipli, N., Antal, L. and Nyeste, K. 2022. Assessment of the exposure of two pesticides on common carp

- (*Cyprinus carpio* Linnaeus 1758): Are the prolonged biomarker responses adaptive or destructive?. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 261, 109446. <https://doi.org/10.1016/j.cbpc.2022.109446>
- Yancheva, V., Velcheva, I., Stoyanova, S. and Georgieva, E. 2016. Histological biomarkers in fish as a tool in ecological risk assessment and monitoring programs: A review. *Applied Ecology and Environmental Research*, 14, 47-75. [http://dx.doi.org/10.15666/aeer/1401\\_047075](http://dx.doi.org/10.15666/aeer/1401_047075)
- Zhai, G., Shu, T., Chen, K., Lou, Q., Jia, J., Huang, J., Shi, C., Jin, X., He, J., Jiang, D., Qian, X., Hu, W. and Yin, Z. 2021. Successful production of an all-female common carp (*Cyprinus carpio* L.) population using *cyp17a1*-deficient neomale carp. *Engineering*, 8, 181-189. <https://doi.org/10.1016/j.eng.2021.03.026>

*Received: 22nd October 2024*

*Accepted: 18th February 2025*