Transdisciplinary Insights in Life Sciences

ISSN: 2584-2161(Online)
Journal Homepage: www.panainool.com

RESEARCH ARTICLE

Influence of Cypermethrin and Chelating Properties of 2r, 4c-Bis (p-Methoxyphenyl)-7c-(t-Butyl)-3-Azabicyclo (3.3.1) Nonan-9-One Oxime on ultrastructure of Gill and Liver

Kaliyaperumal Jayachandran[1*], Munusamy Susiladevi[2], & Bharathanithi Jeevarasika[1]

*[*1]Department of Zoology, Annamalai University, PG Extension Centre, Villupuram, Tamilnadu, India.*

[2]Department of Zoology, Immaculate College for Women, Cuddalore, Tamilnadu, India.

ARTICLE HISTROY

Received 07 November 2023

Revised 11 November 2023

Accepted 22 November 2023

Keywords

Cypermethrin

Labeo rohita

Tissues

Histopathological analysis

ABSTRACT

The toxic effects of cypermethrin on the ultrastructure of the gills and liver of the freshwater fish *Labeo rohita* gills and liver were studied. The purpose was to see if pesticide concentrations and exposure time influenced the degree and nature of ultrascture alterations in exposed fish's gills and liver. Selected fish were exposed to a mixture of 5% concentrations of the LC_{50} of cypermethrin for both short and long periods of time. The gills and liver of specimens subjected to 120 hours experienced similar hyper structural alterations. This clearly indicates a harmful response to both pesticide doses. These ultra structural changes included lesions such as oedema in the primary and secondary lamellae, fusion of neighbouring secondary lamellae, and epithelial layer lifting, hyalinization, hepatocyte vacuolation, cellular swelling, and blood vessel congestion. The amount of the exposure period, however, had an effect on the degree of these hyper structural alterations.

✉ Jayachandran
drjaichandran@gmail.com

Introduction

Fish are often used to assess the effects of environmental pollution on aquatic ecosystems (Mohan et al., 2018). The freshwater teleost, Labeo rohita, is a common cyprinoid found in rivers, ponds, reservoirs, and lakes (Masood et al., 2022). Fish are a popular polyculture species and are easily produced in labs (Mansour et al., 2021). Cypermethrin is also widely used as an insecticide for a variety of crops in this region (Bej et al., 2021), and the literature does not provide enough information on histopathological and oxidative stress responses to determine this pyrethroid's integrative effect on teleosts (Farag et al., 2021). The study will look at changes in the ultrastructure of the liver and certain oxidative stress responses to see if they can be used in biomonitoring programs (Guo et al., 2021; Tenji et al., 2020). Furthermore, biomarkers have demonstrated their sensitivity and ability to serve as short-term indicators of environmental contamination. These

biomarkers exhibit minimal temporal volatility and effectively capture the cumulative impacts of many stressors, including environmental toxins (Arumugam et al., 2021).

Research conducted on the modifications in the surface ultrastructure can provide insights into the overall well-being and physiological condition of fish (Arumugam & Ramaiah, 2018). Histological inquiry is a crucial component of post-mortem examination as it serves to understand the cause and mechanism of death and injury (Wolf & Wheeler, 2018), the post-mortem autolytic process is influenced by several parameters, including temperature, air humidity, and environmental conditions (Tavares et al., 2021). Therefore, studying the ultrastructure of fish gills has been used to learn more about the branchial physiological mechanisms and behavioral traits shown by different types of fish (Foyle et al., 2020). The gill arches possess gill rakers on their pharyngeal aspect, which are widely recognized for their significant contribution to the process of feeding (Shih et al., 2022). In a recent study, Mohammed and Mahmood conducted an analysis of the surface ultrastructure of gill arches and gill rakers in the context of the feeding ecology of *Rita rita*, a carnivorous catfish (Mohammed & Mahmood, 2021). The liver is very important for fish because it is a target organ that does important things like biotransformation and getting rid of xenobiotics (Bharti & Rasool, 2021). Consequentially, the liver possesses considerable potential for investigation in the realm of environmental monitoring owing to its heightened susceptibility to various pollutants (Almunadiya et al., 2023). Modifications in the liver system have the potential to serve as indicators that signify previous encounters with environmental stresses.

Therefore, an endeavour has been undertaken to examine the ultra structural changes that occur in Cypermethrin (group-2), Cypermethrin along with 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime group-3 and 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime alone (group-4) exposed to fingerlings of *Labeo rohita* fish sublethal concentration for 24 - 120 hours.

Materials and Methods

Sample collection and Maintenance

Labeo rohita, a kind of freshwater fish, was captured at a fish farm located in Pinnaloor, within the Cuddalore district, specifically at the Navarathna farm. The fish specimens were transported to the laboratory and subsequently relocated to rectangular cement tanks measuring 100 by 175 centimetres, each with a capacity of 500 litres. The tanks were filled with well water that had been treated to remove chlorine and were aerated to ensure optimal oxygen levels. For the studies, fish of equivalent size and weight were employed, irrespective of their gender (Waiho et al., 2021). The execution of this investigation did not necessitate a permit. The experiment did not include any ethical considerations.

Preparation of 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime

It is prepared by the reaction of 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one with the hydroxylamine hydrochloride and sodium acetate in 1:1:3 ratio in ethanol.

Experimental design

Group 1, referred to as the untreated control group, consisted of fish that were subjected to tap water and thereafter monitored for a duration ranging from 24 to 120 hours.

In Group 2, fish were subjected to a sublethal concentration of Cypermethrin at a dosage of 30 g/l for a duration ranging from 24 to 120 hours.

In this study, we investigated the effects of Cypermethrin at a concentration of 30 g/l, in combination with 2r, 4c-Bis (p-methoxyphenyl), on fish belonging to Group 3 (CYP+ Oxime). The chemical compound -7c-(t-butyl) is of interest due to its uniqueness. The compound -3azabicyclo (3.3.1) nonan-9-one oxime was subjected to a

reaction for a duration of 24 to 120 hours, using a quantity of 2g.

The subject of discussion is Group 4, namely the oxime compound. The fish were subjected to the compound 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl). The compound -3azabicyclo (3.3.1) nonan-9-one oxime was subjected to a solitary dosage of 2g for a duration ranging from 24 to 120 hours.

Scanning Electron Microscopic (SEM) Studies

The Gill and Liver tissues of fish were extracted and subsequently preserved in a karnovsky solution, consisting of a 2% paraformaldehyde and 2.5% glutaaldehyde mixture in a 0.2M sodium cacodylate buffer, for the purpose of Scanning Electron Microscopy (SEM). The tissues underwent dehydration in a solution consisting of graded concentrations of ethanol and acetone (1%), followed by four subsequent washes in pure acetone. Following the completion of the aforementioned process, the samples were meticulously arranged on aluminum stubs, subsequently coated with a layer of gold. These prepared samples were then subjected to examination and photographic documentation utilizing the Joel Tsm-P15 Scanning Electron Microscope, as described by (Samanta et al., 2018). The experiment was conducted in accordance with the Regulations of Animal Experimentation of C.M.C. Institute Lab Vellore, which adheres to the Guidelines established by the International Committee on Laboratory Animals.

Result and Discussion

On either side of the buccal cavity, there are a total of four gill arches. Each arch is made up of several gill filaments, and each filament has two rows of secondary lamellae that are angled at right angles to it. The lamella structure consists of two epithelial sheets separated by numerous contractile pillar cells that serve to demarcate the capillary channels. Typically, one or two red blood cells are observed within the lumen of each capillary (Hadi & Alwan, 2012). According to Pisam et al., (1988) chloride cells have an enormous size, are epithelial in nature, and have a light cytoplasm. These cells are typically found in the basal region of lamellae. The epithelium of the filament and the base of lamellae contain mucus cells and pavement cells, which have a smaller size and lack bright cytoplasm compared to chloride cells (Sardella et al., 2004).

The gills of the control fish exhibited typical morphology during the whole observation period. The gills of the experimental fish exhibited significant edema in the epithelial cells and blood congestion, characterized by aneurism, in multiple regions of the secondary lamellae. Additionally, the pillar cell system experienced structural degradation. The scanning electron microscopy (SEM) analysis revealed the presence of edema in the secondary lamellae, as depicted in Figure 1a and 1b.

The gills exhibited significant aneurysm formation, characterized by the presence of ruptures in several secondary lamellae, as well as the observed deterioration of the pillar cell system. Furthermore, the scanning electron microscopy (SEM) analysis also verified the presence of pronounced aneurysms, edema, and hypertrophy in several secondary lamellae. Ramudu et al., (2020) and Sharma et al., (2021) observed significant hypertrophy and hyperplasia in the chloride cells and mucus cells located at the base of the gill filaments and secondary lamellae in various fish species. The gill epithelial cells of fish subjected to cypermethrin in combination with 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime (group 3) exhibit hypertrophy and hyperplasia, resulting in noticeable swelling when compared to the control fish. The presence of fusion of secondary lamellae, edema, necrosis, and desquamation of lamellar epithelium is evident. The central cartilaginous rod within the major lamellae exhibits disruption in multiple regions throughout the recovery or therapy of the gill. The fish was subjected to the compound 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime (group 4), and no observable alterations in the gill were seen when compared to group 3. The fish in group 4 exhibited a pattern of near normality in

their ultrastructure, as observed using scanning electron microscopy (SEM).

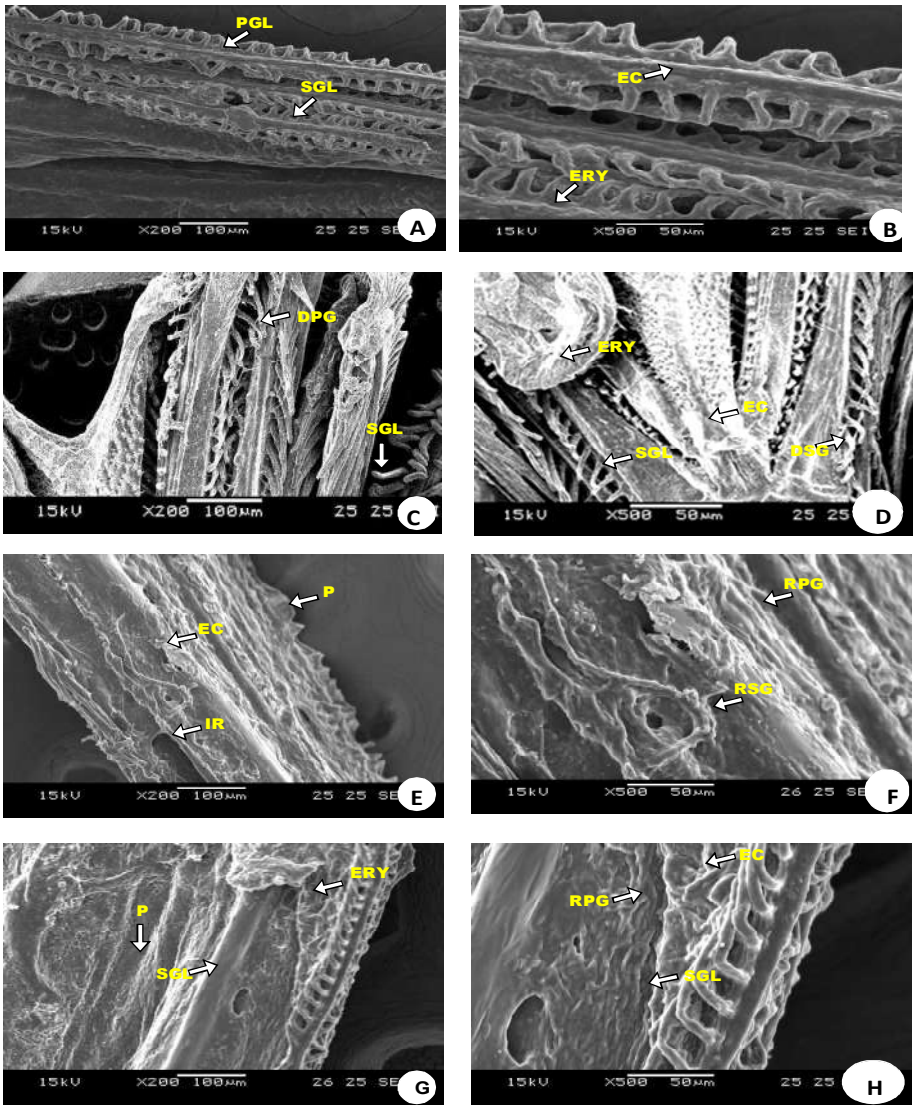

Fig. 1. Scanning electron microscope images of gill tissues of *Labeo rohita*

The parenchyma is predominantly comprised of polyhedral hepatocytes, which commonly exhibit central nuclei characterized by heavily stained chromatin borders and a conspicuous nucleolus (Stoyanova et al., 2020). The liver receives venous blood from the gut through the hepatic portal veins, which enters the liver in a caudal direction. Within the liver, this blood further divides into capillaries referred to as sinusoids. The sinusoids of the liver are composed of reticular-endothelial cells, which are enveloped by hepatocytes. The liver ultra structural scans indicated the presence of thin cell layers that enveloped dense and irregular connective tissue capsules, commonly referred to as Glisson's capsules, as illustrated in Figure 2.

The cellular cohesiveness in dispute is preserved by the existence of desmosomes and peripheral hepatocytes, together with the inclusion of fibroblasts. The hepatocytes observed in the control fish exhibited the presence of many organelles, such as mitochondria, rough endoplasmic reticulum (RER), golgi complex, and a spherical nucleus with distinct perinuclear space.

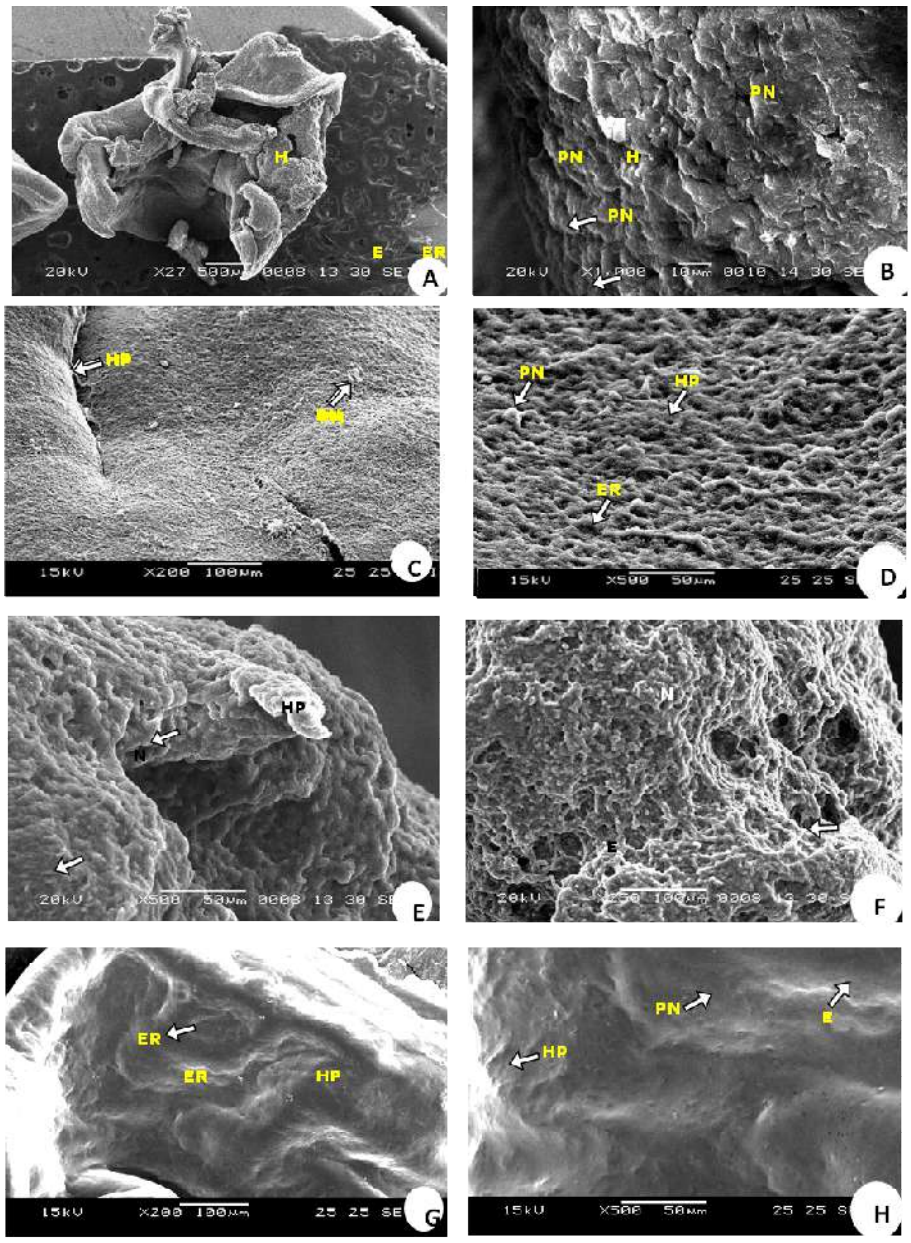

Fig. 2. Scanning electron microscope images of liver tissues of *Labeo rohita*

The endoplasmic reticulum (ER) of *Labeo rohita*, on the other hand, is arranged parallel to and near the plasma membrane. This arrangement leads to the creation of a thin layer with a high electron density that separates the hepatocytes. Lysosomes and lipid bodies are sporadically present inside the cytoplasm of hepatocytes. The nucleus displays a significant proportion of euchromatin, accompanied by a restricted presence of condensed heterochromatin situated at the nuclear periphery. Additionally, a prominent nucleolus is observed at the center of the nucleus (Fig. 2c & d). Group 3 has a notable occurrence of cytoplasmic vacuolization, as well as the lateralization and condensation of nuclei and blood. The fish is subjected to the compound 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl). The compound 3azabicyclo (3.3.1) nonan-9-one oxime was seen to induce ultra structural abnormalities in the liver cells of fish. These modifications were compared to the normal liver cells in the control group, as depicted in Figure 2e, f, g, and h.

In the current study, fish that were exposed to cypermethrin exhibited significant edema in the epithelial cells of their gills, along with numerous areas of secondary lamellae characterized by the breakdown of pillar cells and the presence of mucus protein. The gills of fish are particularly susceptible to waterborne pollutants, such as pesticides, due to their direct and continuous exposure to the surrounding environment (Venkateswarlu & Venkatrayulu, 2020). The rapid binding and accumulation of toxic substances in the gills prompt the secretion of mucoproteins on the epithelium (Lemonnier et al., 2021). This secretion can serve as a protective layer on the gill surface, preventing direct contact with contaminated water and thereby restricting the entry of pesticides. This protective mechanism increases the barrier distance for the influx of toxic substances (Rohani, 2023). Alternatively, the accumulation of mucoproteins may interfere with respiratory processes, potentially leading to suffocation.

Histopathological anomalies in the gills of several species exposed to pesticides and heavy metals in the environment have been documented by a number of researchers (Ahmad et al., 2021; Alesci et al., 2022; El-Garawani et al., 2022; Shah et al., 2020). In a study conducted by Liu et al., (2022) it was observed that *Carassius auratus* exposed to cadmium exhibited the presence of empty mucus cells and a substantial accumulation of mucus on the gill surface. Additionally, noticeable separation between the respiratory epithelium and the capillary epithelium was observed. In the case of *Aequidens portalegrensis* exposed to cadmium, hypertrophy and hyperplasia, as well as filament and lamellar fusion, were observed in the gills. The researchers also noted that the morphological alterations, with the exception of epithelial hyperplasia, were reversible after a depuration period. The fish exhibited notable pathological alterations in their gills, including oedema in the primary and secondary lamellae, fusion of adjacent secondary lamellae, and lifting of the epithelial layer. These observed effects are expected to have a negative impact on respiratory efficiency, and the extent of damage will determine the potential for reversibility of these changes. In the present study, the compound 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl) is being investigated. In the experimental group 3, the exposure to -3azabicyclo (3.3.1) nonan-9-one oxime resulted in a partial restoration of the gill structure, characterized by the presence of moderate lesions including lamellar hypertrophy and hyperplasia.

The liver is recognized as a significant organ for the accumulation of pesticides. The liver serves as both a storage organ and a key location for detoxification processes (Lushchak et al., 2018). Toxic substances are transported to the liver and kidneys by circulation after being absorbed through the gills (Topić Popović et al., 2023). In a comparable manner, the liver of fish exposed to cypermethrin has exhibited hepatocyte ultrastructural changes, including mitochondrial condensation of the rough endoplasmic reticulum (RER) and the presence of numerous large lipoid droplets. Ultimately, when administered at dosages that are ecologically significant, the herbicides cypermethrin (classified as group-2) and 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime (classified as group-3) exhibited an impact on the ultra structural characteristics of both fish gill and liver. Although not necessarily lethal, these alterations have the potential to hinder the gills and liver's capacity to effectively manage xenobiotics and pathogenic pathogens. In various researches, *Labeo rohita* has been employed as a model organism in biomarker investigations pertaining to organic contaminants.

Conclusion

We demonstrated that it could be used to investigate the effects of a pesticide mixture at low concentrations. The administration of oxime (group-4) results in the maximal normalisation of cypermethrin's toxic effect, emphasising oxime's protective activity. An impact on the ultra structural properties of both the fish gill and liver has been observed. While not inherently fatal, these modifications possess the capability to impede the gills and liver's ability to efficiently

regulate xenobiotics and pathogenic microorganisms.

Acknowledgement

The authors' thankful to the Department of Zoology, Annamalai University for providing necessary facilities for carried out of this work. We are also grateful to the experiment was carried out following the Regulations of Animal Experimentation of C.M.C. Institute Lab Vellore.

References

Ahmad, W., Sultan, A., Jan, S. M. H., Ali, A., Rahman, I. U., Usman, H., Sher, A., Khan, N., S., Khan, Z., & Khan, A. (2021). Environmental Risk of Atrazine (Herbicide) to Brain, Gills and Liver Tissues of Fish <i>Ctenopharyngodon idella</i> (Valenciennes, 1844). *Journal of Geoscience and Environment Protection*, *09*(02), 1–16. https://doi.org/10.4236/gep.2021.92001

Alesci, A., Cicero, N., Fumia, A., Petrarca, C., Mangifesta, R., Nava, V., Lo Cascio, P., Gangemi, S., Di Gioacchino, M., & Lauriano, E. R. (2022). Histological and Chemical Analysis of Heavy Metals in Kidney and Gills of Boops boops: Melanomacrophages Centers and Rodlet Cells as Environmental Biomarkers. *Toxics*, *10*(5). https://doi.org/10.3390/toxics10050218

Almunadiya, S., Muarif, M., Sabar, M., Akmal, Y., Irfannur, I., Muliari, M., & Ruzanna, A. (2023). Comparative anatomical study of gill fish of Tor tambra, Tor soro, and Hampala macrolepidota as an environmental bioindicator. *IOP Conference Series: Earth and Environmental Science*, *1221*(1), 012004. https://doi.org/10.1088/1755-1315/1221/1/012004

Arumugam, S., Abul Asan Sathali, M. S., Ramaiah, S., & Krishnan, G. (2021). Diversification of Dawkinsia filamentosa (Valenciennes, 1844) and their growth conditions by the impact of toxic metals in the river Tamiraparani. *Ecotoxicology*, 1043–1055. https://doi.org/10.1007/s10646-021-02427-0

Arumugam, S., & Ramaiah, S. (2018). Concentrations of toxic metals (Pb, Cd, Cr) in the tissues and their effects on diversification of Devario aequipinnatus populations. *International Journal of Environmental Health Research*, *28*(4), 379–390. https://doi.org/10.1080/09603123.2018.1479516

Bej, S., Ghosh, K., Chatterjee, A., & Saha, N. C. (2021). Assessment of biochemical, hematological and behavioral biomarkers of Cyprinus carpio on exposure to a type-II pyrethroid insecticide Alpha-cypermethrin. *Environmental Toxicology and Pharmacology*, *87*, 103717. https://doi.org/https://doi.org/10.1016/j.etap.2021.103717

Bharti, S., & Rasool, F. (2021). Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on Channa punctatus (Bloch) fish. *Toxicology Reports*, *8*, 443–455. https://doi.org/10.1016/j.toxrep.2021.02.018

El-Garawani, I. M., Khallaf, E. A., Alne-na-ei, A. A., Elgendy, R. G., Sobhy, H. M., Khairallah, A., Hathout, H. M. R., Malhat, F., & Nofal, A. E. (2022). The Effect of Neonicotinoids Exposure on Oreochromis niloticus Histopathological Alterations and Genotoxicity. *Bulletin of Environmental Contamination and Toxicology*, *109*(6), 1001–1009. https://doi.org/10.1007/s00128-022-03611-6

Farag, M. R., Alagawany, M., Bilal, R. M., Gewida, A. G. A., Dhama, K., Abdel-Latif, H. M. R., Amer, M. S., Rivero-Perez, N., Zaragoza-Bastida, A., Binnaser, Y. S., Batiha, G. E.-S., & Naiel, M. A. E. (2021). An Overview on the Potential Hazards of Pyrethroid Insecticides in Fish, with Special Emphasis on Cypermethrin Toxicity. *Animals*, *11*(7). https://doi.org/10.3390/ani11071880

Foyle, K. L., Hess, S., Powell, M. D., & Herbert, N. A.

(2020). What Is Gill Health and What Is Its Role in Marine Finfish Aquaculture in the Face of a Changing Climate? *Frontiers in Marine Science*, *7*(June), 1–16. https://doi.org/10.3389/fmars.2020.00400

Guo, B., Feng, D., Xu, Z., Qi, P., & Yan, X. (2021). Acute benzo[a]pyrene exposure induced oxidative stress, neurotoxicity and epigenetic change in blood clam Tegillarca granosa. *Scientific Reports*, *11*(1), 18744. https://doi.org/10.1038/s41598-021-98354-5

Hadi, A. A., & Alwan, S. F. (2012). Histopathological changes in gills , liver and kidney of fresh water fish, Tilapia zillii, exposed to aluminum. *International Journal of Pharmacy & Life Sciences*, *3*(11), 2071–2081.

Lemonnier, H., Royer, F., Caradec, F., Lopez, E., Hubert, C., Rabiller, É., Desclaux, T., Fernandez, J. M., & Andrieux-Loyer, F. (2021). Diagenetic Processes in Aquaculture Ponds Showing Metal Accumulation on Shrimp Gills. *Frontiers in Marine Science*, *8*(February), 1–14. https://doi.org/10.3389/fmars.2021.625789

Liu, Y., Chen, Q., Li, Y., Bi, L., Jin, L., & Peng, R. (2022). Toxic Effects of Cadmium on Fish. *Toxics*, *10*(10). https://doi.org/10.3390/toxics10100622

Lushchak, V. I., Matviishyn, T. M., Husak, V. V, Storey, J. M., & Storey, K. B. (2018). Pesticide toxicity: a mechanistic approach. *EXCLI Journal*, *17*, 1101–1136. https://doi.org/10.17179/excli2018-1710

Mansour, A. T., Allam, B. W., Srour, T. M., Omar, E. A., Nour, A. A. M., & Khalil, H. S. (2021). The feasibility of monoculture and polyculture of striped catfish and nile tilapia in different proportions and their effects on growth performance, productivity, and financial revenue. *Journal of Marine Science and Engineering*, *9*(6). https://doi.org/10.3390/jmse9060586

Masood, Z., Gul, G. N., Khan, T., Khan, W., Kabir, M., Hamidullah, Iqbal, M. A., & Swelum, A. A. (2022). Comparison of growth performance and morphological variation among three carp species (Cyprinus carpio, Hypophthalmichthys molitrix, and Labeo rohita). *Journal of King Saud University - Science*, *34*(8), 102326. https://doi.org/https://doi.org/10.1016/j.jksus.2022.102326

Mohammed, Y., & Mahmood, S. K. (2021). *Comparative Morphometrical Study of Gills of Carp Fish (Cyprinus Carpio L .) and Catfish (Silurus Triostegus (Heckel)) In Tigris River Water*. *25*(6), 12653–12662.

Mohan, D. S., Arumugam, S., & Ramaiah, S. (2018). Diversification and microscopic structure of tissues in endemic and endangered species of Dawkinsia tambraparniei from the river Tamiraparani, Tamil Nadu, India. *Environmental Science and Pollution Research International*, *25*(7), 6570–6583. https://doi.org/10.1007/s11356-017-0896-z

Pisam, M., Prunet, P., Boeuf, G., & Jrambourg, A. (1988). Ultrastructural features of chloride cells in the gill epithelium of the atlantic salmon, Salmo salar, and their modifications during smoltification. *American Journal of Anatomy*, *183*(3), 235–244. https://doi.org/10.1002/aja.1001830306

Ramudu, K. R., Shivam, S., Sanil, N. K., Krupesha Sharma, S. R., Vijayagopal, P., Babu, P. P. S., Anuraj, A., Navanath, K., Rao, B. M., & Loka, J. (2020). Histopathological Changes in the Gills Associated with Dactylogyrus spp. Infestation in Orange-spotted Grouper, Epinephelus coioides Reared in Sea Cages. *International Journal of Current Microbiology and Applied Sciences*, *9*(8), 1–8. https://doi.org/10.20546/ijcmas.2020.908.001

Rohani, M. F. (2023). Pesticides toxicity in fish: Histopathological and hemato-biochemical aspects – A review. *Emerging Contaminants*, *9*(3), 100234. https://doi.org/https://doi.org/10.1016/j.emcon.2023.100234

Samanta, P., Kumari, P., Pal, S., Mukherjee, A. K., &

Ghosh, A. R. (2018). Histopathological and Ultrastructural Alterations in Some Organs of Oreochromis niloticus Exposed to Glyphosate-based Herbicide, Excel Mera 71. *Journal of Microscopy and Ultrastructure*, *6*(1), 35–43. https://doi.org/10.4103/JMAU.JMAU_8_18

Sardella, B. A., Matey, V., Cooper, J., Gonzalez, R. J., & Brauner, C. J. (2004). Physiological, biochemical and morphological indicators of osmoregulatory stress in "California" Mozambique tilapia (Oreochromis mossambicus x O. urolepis hornorum) exposed to hypersaline water. *Journal of Experimental Biology*, *207*(8), 1399–1413. https://doi.org/10.1242/jeb.00895

Shah, N., Khan, A., Ali, R., Marimuthu, K., Uddin, M. N., Rizwan, M., Rahman, K. U., Alam, M., Adnan, M., Muhammad, Jawad, S. M., Hussain, S., & Khisroon, M. (2020). Monitoring Bioaccumulation (in Gills and Muscle Tissues), Hematology, and Genotoxic Alteration in Ctenopharyngodon idella Exposed to Selected Heavy Metals. *BioMed Research International*, *2020*, 6185231. https://doi.org/10.1155/2020/6185231

Sharma, D., Gandotra, R., Dhiman, S. K., Arif, M., Dogra, A., Lone, A., Choudhary, N., & Choudhary, P. (2021). Ultrastructural biomarker responses in gill tissues of Cirrhinus mrigala (Hamilton, 1822) through SEM after exposure to zinc sulphate. *The Egyptian Journal of Aquatic Research*, *47*(2), 157–162. https://doi.org/https://doi.org/10.1016/j.ejar.2021.03.003

Shih, S.-W., Yan, J.-J., Tsou, Y.-L., Lu, S.-W., Wang, M.-C., Chou, M.-Y., & Hwang, P.-P. (2022). In Vivo Functional Assay in Fish Gills: Exploring Branchial Acid-Excreting Mechanisms in Zebrafish. *International Journal of Molecular Sciences*, *23*(8). https://doi.org/10.3390/ijms23084419

Stoyanova, S., Georgieva, E., Velcheva, I., Iliev, I., Vasileva, T., Bivolarski, V., Tomov, S., Nyeste, K., Antal, L., & Yancheva, V. (2020). Multi-Biomarker Assessment in Common Carp (Cyprinus carpio, Linnaeus 1758) Liver after Acute Chlorpyrifos Exposure. *Water*, *12*(6). https://doi.org/10.3390/w12061837

Tavares, J., Martins, A., Fidalgo, L. G., Lima, V., Amaral, R. A., Pinto, C. A., Silva, A. M., & Saraiva, J. A. (2021). Fresh Fish Degradation and Advances in Preservation Using Physical Emerging Technologies. *Foods*, *10*(4). https://doi.org/10.3390/foods10040780

Tenji, D., Micic, B., Sipos, S., Miljanovic, B., Teodorovic, I., & Kaisarevic, S. (2020). Fish biomarkers from a different perspective: evidence of adaptive strategy of Abramis brama (L.) to chemical stress. *Environmental Sciences Europe*, *32*(1), 47. https://doi.org/10.1186/s12302-020-00316-7

Topić Popović, N., Čižmek, L., Babić, S., Strunjak-Perović, I., & Čož-Rakovac, R. (2023). Fish liver damage related to the wastewater treatment plant effluents. *Environmental Science and Pollution Research*, *30*(17), 48739–48768. https://doi.org/10.1007/s11356-023-26187-y

Venkateswarlu, V., & Venkatrayulu, C. (2020). Bioaccumulation of heavy metal lead (Pb) in different tissues of brackish water fish Mugil cephalus (Linnaeus, 1758). *Journal of Applied Biology and Biotechnology*, *8*(2), 1–5. https://doi.org/10.7324/JABB.2020.80201

Waiho, K., Fazhan, H., Shu-Chien, A. C., Abualreesh, M. H., Ma, H., Syahnon, M., Azmie, G., Razman, N. J., & Ikhwanuddin, M. (2021). Size Distribution, Length-Weight Relationship, and Size at Morphometric Maturity of the Mud Spiny Lobster Panulirus polyphagus (Herbst, 1793) in the Johor Strait. *Frontiers in Marine Science*, *8*(November), 1–8. https://doi.org/10.3389/fmars.2021.766038

Wolf, J. C., & Wheeler, J. R. (2018). A critical review of histopathological findings associated with endocrine and non-

endocrine hepatic toxicity in fish models. *Aquatic Toxicology*, *197*, 60–78. https://doi.org/https://doi.org/10.1016/j.aquatox.2018.01.013