SAKARYA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ DERGİSİ

Sakarya University Journal of Science
SAUJS

ISSN 1301-4048 e-ISSN 2147-835X Period Bimonthly Founded 1997 Publisher Sakarya University
http://www.saujs.sakarya.edu.tr/

Title: Effects of Single and Combined Exposure to Environmentally Relevant Concentrations of Cyfluthrin and Copper on Digestive Gland Histology of Lymnaea stagnalis

Authors: Sezgi ARMAN

Recieved: 2022-09-01 00:00:00

Accepted: 2022-11-08 00:00:00

Article Type: Research Article

Volume: 27
Issue: 1
Month: February
Year: 2023
Pages: 38-47

How to cite
Sezgi ARMAN; (2023), Effects of Single and Combined Exposure to Environmentally Relevant Concentrations of Cyfluthrin and Copper on Digestive Gland Histology of Lymnaea stagnalis. Sakarya University Journal of Science, 27(1), 38-47, DOI: 10.16984/saufenbilder.1169843
Access link
https://dergipark.org.tr/en/pub/saufenbilder/issue/75859/1169843

Sakarya University Journal of Science 27(1), 38-47, 2023

Effects of Single and Combined Exposure to Environmentally Relevant Concentrations of Cyfluthrin and Copper on Digestive Gland Histology of *Lymnaea stagnalis*

Sezgi ARMAN*[1]

Abstract

Pyrethroid insecticides and heavy metals frequently co-exist in aquatic systems, due to intensive anthropogenic activities, and their effects on aquatic organisms are needed to be investigated. In the present work, single and combined effects of environmentally realistic concentrations of cyfluthrin and copper on the digestive gland histology of the freshwater pond snail (*Lymnaea stagnalis*) were evaluated. Snails were exposed to 1 µg/L cyfluthrin, 10 µg/L copper, and 1 µg/L cyfluthrin + 10 µg/L copper mixture for 96 h. Cyfluthrin-exposed samples showed apparent vacuolization, basal lamina separations, and disrupted digestive cells. Exposure to copper alone gave rise to enlargements of the tubule lumens and the intertubular area, degenerated tubules, atrophied basophilic cells, prominently disrupted and ruptured digestive cells, and nuclear enlargements in some basophilic cells. Cyfluthrin and copper mixture caused more severe histopathological changes in the digestive gland. General tissue appearance was altered by prominently degenerated, and fused tubules lacking cellular structure; tubule lumens filled with cellular content were noted. Increased intertubular regions were filled with connective tissue formations. Progressive disruption of digestive cells; and basophilic cell atrophy were also observed. The findings of the current study highlighted that cyfluthrin and copper at environmentally relevant concentrations caused a biological response in *L. stagnalis*; however, this response was more intense following their co-exposure.

Keywords: Pyrethroid, heavy metal, toxicity, histopathology, snail

1. INTRODUCTION

Surface waters are contaminated by various environmental toxicants via anthropogenic activities, such as agricultural and industrial processes, and water pollution is a global concern directly affecting aquatic life. Aquatic organisms are generally exposed to multiple chemical stressors from various sources found in water simultaneously [1, 2]. Pesticides and heavy metals are two of the common pollutants detected in water bodies and negatively impact aquatic organisms [3].

Copper is a trace element needed as a micronutrient in the metabolic processes of organisms. However, excessive copper concentrations cause toxic effects on

*Corresponding author: sezgiarman@gmail.com (S ARMAN)
[1] Sakarya University, Faculty of Science, Department of Biology
ORCID: https://orcid.org/0000-0002-4247-0639

aquatic animals [4]. Laboratory works have revealed the adverse effects of copper exposure on various aquatic species including mollusks [5], crustaceans [6], and fish [7]. Although copper naturally occurs in water sources, anthropogenic release due to mining, industrial discharges, waste management, antifouling feature, and pest control efficiency leads to increased copper concentrations in water [8, 9]. Copper concentrations in pristine waters are noted ranging between 0.3- 3 µg/L [8]; however, copper was detected at concentrations of 4-2,500 µg/L in mined areas, 0.4-15 µg/L in urban watersheds, and 0-12.30 µg/L in agricultural watersheds [10].

Pyrethroid insecticides have a wide range of uses in agricultural, domestic, and veterinary fields [11]. Pyrethroids show lower toxicity in birds and mammals and are considered easily degradable insecticides; however, excessive application of these chemicals causes residual contamination in the environment [12] and they are very toxic to fish and aquatic invertebrates [11-13]. Cyfluthrin is a broad-spectrum pyrethroid insecticide widely used in agriculture and urban environments against aphids, cabbage stem flea beetles, mosquitoes, houseflies, cockroaches, etc. [14]. Frequent cyfluthrin application has enabled the detection of this insecticide in aquatic environments. In Sacramento-San Joaquin Delta water, cyfluthrin concentration was measured at 5 ng/L [15]. 1388 ng/L cyfluthrin was measured in water samples from the Jiulong River in China [16]. Several reports revealed cyfluthrin toxicity via different aspects in various aquatic specimens including; mollusks [17], crustaceans, and fish [11, 18].

Lymnaea stagnalis, also known as the pond snail, is a pulmonate gastropod widely distributed in freshwater bodies. This species can be easily housed in the laboratory. *L. stagnalis* has been recognized as a beneficial model organism and a bioindicator to evaluate the potential impacts of environmental contaminants [19, 20]. Adult pond snails are generally 2-6 cm in shell length allowing researchers to dissect sufficient sizes of tissue and perform histopathological analysis [19, 21].

The digestive gland, also known as hepatopancreas, is responsible for digestion, metabolism, and detoxification in gastropods [22]. Digestive gland tissue is considered a useful bioindicator to evaluate the histopathological alterations caused by environmental toxicants [21, 23-25].

This study aimed to investigate the histopathological alterations in the digestive gland of *L. stagnalis* in response to single and combined exposure to environmentally realistic concentrations of cyfluthrin and copper.

2. MATERIALS AND METHODS

2.1. *L. stagnalis* Maintenance

Adult *L. stagnalis* for the starter culture was obtained from Vrije Universiteit Amsterdam, and they have been reared and reproduced at Sakarya University, Department of Biology. Snails were held in 20-L glass aquaria filled with dechlorinated and aerated Sakarya City tap water at 20±2 °C, under a photoperiod of 12 h light:12 h dark. They were fed with lettuce and commercial fish flakes (Tetraphyll, Tetra GmbH). Snails were also supplied with cuttlebones to support shell growth.

2.2. Test Chemicals

Cyfluthrin (CAS No: 68359-37-5) was purchased in a commercial wettable powder formulation (Solfac ® WP 10, registered trademark of Bayer). The copper stock solution was prepared from hydrated copper sulfate ($CuSO_4.5H_2O$) (Merck, Germany).

2.3. Experimental Setup

Four different experimental groups including chemical-free control and three exposure tanks (1 µg (a.i.) /L cyfluthrin alone, 10 µg/L copper alone, 1 µg/L cyfluthrin + 10 µg/L copper mixture) were prepared. Six snails (2.31 ± 0.05 g in weight, 2.72 ± 0.048 cm in shell height, 1.73 ± 0.034 cm in shell width) were used in each tank filled with 3 L test solution. Test containers were continuously aerated with air pumps during the experiment, and the snails were not fed. The static test method was conducted for 96 h. Test solutions were not renewed during the experiment. Faeces were removed daily by pipetting.

2.4. Histology

Following exposure, the snails were anaesthetized with 25% Listerine ® solution [26]. They were removed from their shells, and the digestive gland tissues were dissected. Samples were fixed in Bouin's fluid for 24 h at room temperature. They were dehydrated in ascending series of ethanol concentrations, cleared with xylene, and embedded in paraffin. 5 µm-thick serial sections were prepared with Leica microtome (RM2125RT). Sections were stained with Harris' hematoxylin and eosin and investigated by Leica DM500 light microscope. Images were captured with a Leica MC170 HD camera.

3. RESULTS

No mortality was observed during the experiment in the control and 1 µg/L cyfluthrin exposure groups. At the 48th hour, one dead individual was observed in 10 µg/L copper and 1 µg/L cyfluthrin + 10 µg/L copper exposure groups each. Dead snails were immediately removed from the tanks. No behavioural alteration was noticed in the experimental groups.

The digestive gland of *L. stagnalis* was greenish brown. It consisted of unequal lobes. The tissue preserved its shape during the removal process of the body from the shell. The exposure groups did not exhibit any colour or texture difference compared to the control.

The digestive gland of the control specimens was composed of many tubules and intertubular areas (Figure 1a). The tubule (Figure 1b) epithelium had two major types of cells, including digestive cells and basophilic cells. Digestive cells were more prevalent than basophilic cells, and they were mainly columnar, while basophilic cells were triangular and relatively shorter than digestive cells (Figure 1c). Digestive cells contained vacuoles and many endocytotic vesicles in their cytoplasm. Their nuclei were located in the basal region of the cell. Microvilli were also distinguishable on the apical surface of the cells, which border the tubule lumen (Figure 1d).

Control specimens did not exhibit any histopathological lesions. The histopathological alterations in the exposure groups were summarized in Table 1.

1 µg/L cyfluthrin exposed samples showed apparent vacuolization. Vacuole numbers were distinctly increased compared to the control tissue (Figure 2a,b). Basal lamina separations from the tubule cells were partly noticed (Figure 2c). Furthermore, some of the digestive cells were disrupted and microvilli were not observed (Figure 2d).

Table 1 Digestive gland tissue lesions of *Lymnaea stagnalis* exposed to cyfluthrin and copper

Experimental groups	Lesions	Severity
Control	-	-
1 µg/L cyfluthrin	Vacuolization, basal lamina separations, disrupted digestive cells	+
10 µg/L copper	Enlargements of the tubule lumens and the intertubular area, degenerated tubules, atrophied basophilic cells, disrupted and ruptured digestive cells, nuclear enlargements	++
1 µg/L cyfluthrin + 10 µg/L copper	Altered appearance of the tissue, degenerated and fused tubules, enlarged intertubular regions filled with connective tissue formations, disrupted digestive cells, basophilic cell atrophy	+++

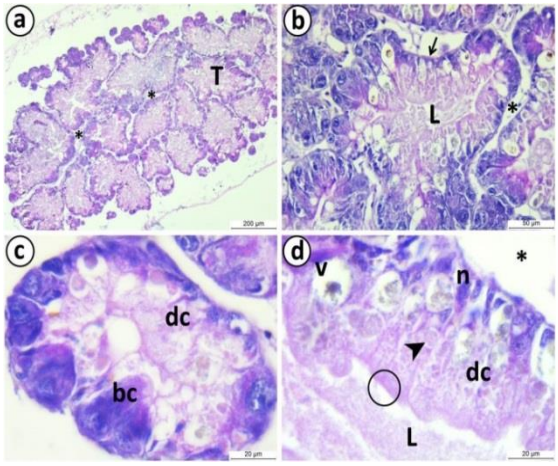

Figure 1 Normal digestive gland histology of the control samples of *L. stagnalis* a) General view of the digestive gland histology at lower magnification. T: Tubule; asterisks: Intertubular area b) A digestive gland tubule. L: Tubule lumen, arrow: Basal lamina, asterisk: Intertubular area c) Epithelial cells compose a digestive gland tubule. dc: Digestive cells, bc: Basophilic cells d) Digestive cells in detail. L: Tubule lumen, asterisk: Intertubular area, dc: Digestive cells, n: Nucleus, v: Vacuole, arrowhead: Endocytotic vesicle, circle: Microvilli

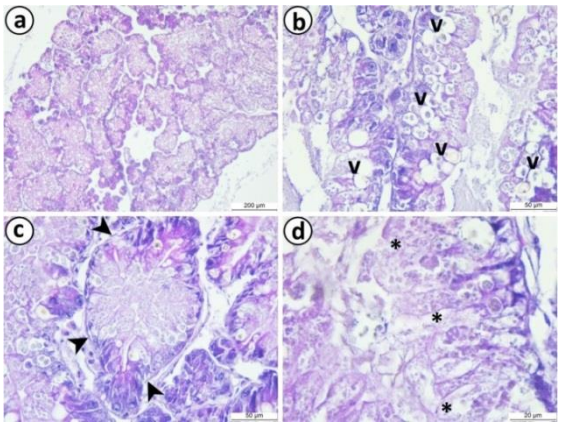

Figure 2 The digestive gland histology of *L. stagnalis* exposed to 1 µg/L cyfluthrin a) General view of the gland histology at lower magnification b) Increased vacuolization. v: Vacuoles c) Separations of the basal lamina (arrowheads) from the tubule epithelium d) Disrupted digestive cells (asterisks)

The digestive gland histology of 10 µg/L copper-treated snails exhibited distinct alterations. The general appearance of the tissue was altered by enlargements of the tubule lumens and the intertubular area (Figure 3a). Some of the tubules prominently degenerated, and atrophied basophilic cells were also noticed (Figure 3b). Digestive cells were more disrupted than the cyfluthrin-exposed samples, and cell borders were not distinguishable (Figure 3c). Ruptured digestive cells; and

nuclear enlargements in some basophilic cells were also observed (Figure 3d).

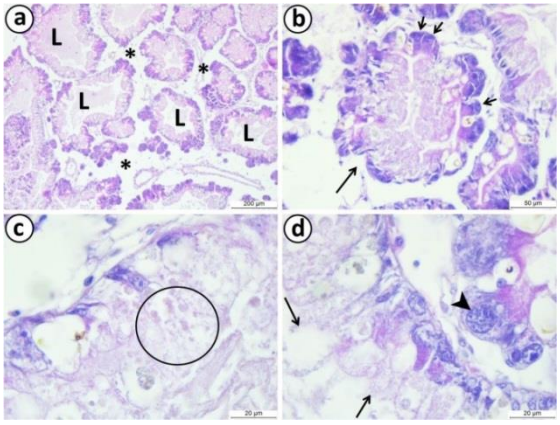

Figure 3 The digestive gland histology of *L. stagnalis* exposed to 10 µg/L copper a) General view of the gland histology at lower magnification. Note the tubule lumen enlargements (L) and increased gaps between the tubules (asterisks) b) A degenerative digestive tubule (long arrow) consisting of atrophied basophilic cells (short arrows) c) Disrupted digestive cells lacking cell borders (encircled) d) Ruptured digestive cells (arrows) and nuclear enlargement (arrowhead)

Cyfluthrin and copper together gave rise to more serious histopathological changes in the digestive glands of the exposed snails. General tissue appearance altered, the tubules severely degenerated, and neighbour tubules were fused. Enlarged intertubular regions were filled with connective tissue formations (Figure 4a). Progressive disruption of digestive cells and basophilic cell atrophy were observed (Figure 4b). Some of the tubules were strikingly degenerated, lost their cellular structure, and their lumens were filled with cellular content (Figure 4c). Increased connective tissue formation with adipocytes was notable in the intertubular region (Figure 4d).

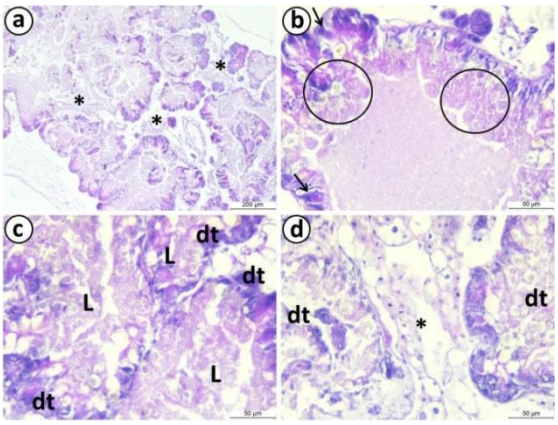

Figure 4 The digestive gland histology of *L. stagnalis* co-exposed to 1 µg/L cyfluthrin and 10 µg/L copper a) General view of the gland histology at lower magnification. Note increased connective tissue formation (asterisks) between the progressively degenerated tubules b) Severely disrupted digestive cells (encircled) and atrophied basophilic cells (arrows) c) Prominent degeneration of the tubules (dt) and the lumens filled with cellular content (L) d) Increased connective tissue formation with adipocytes in the intertubular region (asterisk) between the degenerated tubules (dt)

4. DISCUSSION

The increasing growth of the human population brings along intensive domestic, industrial, and agricultural activities. These activities lead to threatening environmental health issues by releasing toxic chemicals into nature [1]. Water sources are considered a major compartment for receiving a variety of chemicals raised from anthropological processes [27]. Pesticides and heavy metals are among the widely known water contaminants causing adverse effects on aquatic organisms [3]. These organisms are rarely exposed to a single type of pollutant; rather, they are exposed to a chemical cocktail due to their co-occurrence [2]. The mixture toxicity of two or more chemicals involves additive, synergistic, or antagonist effects [28, 29]. The coexistence of pyrethroids and heavy metals in the environment was previously reported [30]. In the current work, single and combined effects of environmentally relevant

concentrations of cyfluthrin and copper on the digestive gland of *L. stagnalis* were investigated.

Only cyfluthrin-treated samples showed relatively slight histopathological alterations, including increased vacuolization, separation of the basal lamina from the tubule epithelium, and disrupted digestive cells. Previous studies highlighted that the digestive gland tissues of molluscs were sensitive to pyrethroid exposure. For instance, bifenthrin brought about distinct histopathological alterations in *Corbicula fluminea*, such as vacuolization and degeneration in the digestive tubules [31]. Cypermethrin-induced hemocyte infiltration, increased number of basophilic cells, epithelial atrophy, and necrosis were observed in the digestive gland tissue of *Pomacea canaliculata* [32].

The current work showed that copper alone caused enlarged tubule lumens and intertubular areas, degenerated tubules, atrophied basophilic cells, disrupted digestive cells lacking cell boundaries, ruptured digestive cells, and nuclear enlargements in some basophilic cells. These alterations were more striking than those induced by cyfluthrin. Previous studies proved copper toxicity in *L. stagnalis*. 96-h median lethal concentration (LC_{50}) of copper was reported as 31 µg/L for juvenile pond snails [33]. 28-d exposure to 7.5 µg/L copper gave rise to a decrease in egg number per clutches [34]. Enzymatic antioxidant responses were observed in hepatopancreas following 48-h copper treatments at concentrations between 2 µg/L to 90 µg/L [35]. Digestive gland histopathology in *L. stagnalis* caused by other heavy metals also exists in the literature. Karakaş and Otludil [21] examined the histopathological alterations induced by 7 to 28 days of cadmium exposure in *L. stagnalis*. The authors noted vacuolization in the digestive cells, deteriorated connective tissue, increased amoebocytes, swelling of basophilic cells, expanded lymphatic areas, increased lipid vacuoles between the tubules, connective tissue atrophy, pyknotic cells, and necrosis [21].

Microscopic observations revealed that the samples of cyfluthrin + copper exposed groups showed more severe histopathological changes than those induced by individual chemicals. Prominently degenerated and fused tubules lacking their cellular structure, increased intertubular regions filled with connective tissue formations, progressive disruption of digestive cells and basophilic cell atrophy were noted. Several reports evaluated the effects of exposure to mixtures of pyrethroids and heavy metals via various biological aspects [36-38]. These effects might be antagonistic or synergistic (reviewed in [30]). This study presents preliminary results suggesting that the coexistence of cyfluthrin and copper might have additive or synergistic effects in the digestive gland of *L. stagnalis*; however, further studies are required to fully understand the *in vivo* cyfluthrin-copper interactions.

Acknowledgement
The author gratefully thanks Dr. Y. Nakadera for providing the snails for the starter culture and the expert advice on snail husbandry.

Funding
The author has not received any financial support for the research, authorship, or publication of this study.

Authors' Contribution
SA designed the study, conducted the laboratory work, and wrote the manuscript.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the author.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

The author of the paper declares that she complies with the scientific, ethical, and quotation rules of SAUJS in all processes of the paper and that he does not make any falsification on the data collected. In addition, he declares that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

REFERENCES

[1] Ö. Fırat, H. Y. Cogun, T. A. Yüzereroğlu, G. Gök, Ö. Fırat, F. Kargin, Y. Kötemen, "A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*," Fish Physiology and Biochemistry, vol. 37, no. 3, pp. 657-666, 2011.

[2] H. Mai, J. Cachot, C. Clérandeau, C. Martin, N. Mazzela, P. Gonzalez, B. Morin, "An environmentally realistic pesticide and copper mixture impacts embryonic development and DNA integrity of the Pacific oyster, *Crassostrea gigas*," Environmental Science and Pollution Research, vol. 27, no. 4, pp. 3600-3611, 2020.

[3] P. Amoatey, M. S. Baawain, "Effects of pollution on freshwater aquatic organisms," Water Environment Research, vol. 91, no. 10, pp 1272-1287, 2019.

[4] C. A. Flemming, J. T. Trevors, "Copper toxicity and chemistry in the environment: a review," Water, Air, and Soil Pollution, vol. 44, no. 1, pp. 143-158, 1989.

[5] M. B. Jorge, V. L. Loro, A. Bianchini, C. M. Wood, P. L. Gillis, "Mortality, bioaccumulation and physiological responses in juvenile freshwater mussels (*Lampsilis siliquoidea*) chronically exposed to copper," Aquatic Toxicology, vol. 126, pp. 137-147, 2013.

[6] J. Bao, Y. Xing, C. Feng, S. Kou, H. Jiang, X. Li, "Acute and sub-chronic effects of copper on survival, respiratory metabolism, and metal accumulation in *Cambaroides dauricus*," Scientific Reports, vol. 10, pp. 16700, 2020.

[7] M. E. Tesser, A. A. de Paula, W. E. Risso, R. A. Monteiro, A. D. E. Santo Pereira, L. F. Fraceto, C. B. dos Reis Martinez, "Sublethal effects of waterborne copper and copper nanoparticles on the freshwater Neotropical teleost *Prochilodus lineatus*: A comparative approach," Science of The Total Environment, vol. 704, pp. 135332, 2020.

[8] J. L. Stauber, C. M. Davies, "Use and limitations of microbial bioassays for assessing copper bioavailability in the aquatic environment," Environmental Reviews, vol. 8, no. 4, pp. 255-301, 2000.

[9] M. D. Baldissera, C. F. Souza, D. C. Barroso, R. S. Pereira, K. O. Alessio, C. Bizzi, B. Baldisserotto, A. L. Val, "Acute exposure to environmentally relevant concentrations of copper affects branchial and hepatic phosphoryl transfer network of *Cichlasoma amazonarum*: Impacts on bioenergetics homeostasis," Comparative Biochemistry and

Physiology Part C: Toxicology & Pharmacology, vol. 238, pp. 108846, 2020.

[10] B. Das, R. Nordin, A. Mazumder, "Watershed land use as a determinant of metal concentrations in freshwater systems," Environmental Geochemistry and Health, vol. 31, no. 6, pp. 595-607, 2009.

[11] A. Sepici-Dinçel, A. Ç. K. Benli, M. Selvi, R. Sarıkaya, D. Şahin, I. A. Özkul, F. Erkoç, "Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: biochemical, hematological, histopathological alterations," Ecotoxicology and Environmental Safety, vol. 72, no. 5, pp. 1433-1439, 2009.

[12] L. Li, D. Yang, Y. Song, Y. Shi, B. Huang, A. Bitsch, J. Yan, "The potential acute and chronic toxicity of cyfluthrin on the soil model organism, *Eisenia fetida*," Ecotoxicology and Environmental Safety, vol. 144, pp. 456-463, 2017.

[13] E. G. Günde, S. V. Yerli, "A comparative study on the acute toxicity of cyfluthrin and tetramethrin on carp (*Cyprinus carpio* L. 1758)," Journal of the Black Sea/Mediterranean Environment, vol. 17, pp. 260-268, 2011.

[14] A. J. Thatheyus, A. G. Selvam, "Synthetic pyrethroids: toxicity and biodegradation," Applied Ecology and Environmental Sciences, vol. 1, no. 3, pp. 33-36, 2013.

[15] S. M. Brander, I. Werner, J. W. White, L. A. Deanovic, "Toxicity of a dissolved pyrethroid mixture to *Hyalella azteca* at environmentally relevant concentrations," Environmental Toxicology and Chemistry: An International Journal, vol. 28, no. 7, pp. 1493-1499, 2009.

[16] S. Zheng, B. Chen, X. Qiu, M. Chen, Z. Ma, X. Yu, "Distribution and risk assessment of 82 pesticides in Jiulong River and estuary in South China," Chemosphere, vol. 144, pp. 1177-1192, 2016.

[17] P. Arslan, "Determinations of the effects of cyfluthrin on the hemocytes parameters of freshwater mussel (*Unio delicatus*)," Ege Journal of Fisheries and Aquatic Sciences, vol. 39, no. 1, 39-45, 2022.

[18] M. Lanteigne, S. A. Whiting, M. J. Lydy, "Mixture toxicity of imidacloprid and cyfluthrin to two non-target species, the fathead minnow *Pimephales promelas* and the amphipod *Hyalella azteca*," Archives of Environmental Contamination and Toxicology, vol. 68, no. 2, pp. 354-361, 2015.

[19] J. Amorim, I. Abreu, P. Rodrigues, D. Peixoto, C. Pinheiro, A. Saraiva, A. P. Carvalho, L. Guimarães, L. Oliva-Teles, "*Lymnaea stagnalis* as a freshwater model invertebrate for ecotoxicological studies," Science of the Total Environment, vol. 669, pp. 11-28, 2019.

[20] R. Kuroda, M. Abe, "The pond snail *Lymnaea stagnalis*," EvoDevo, vol. 11, no. 1, pp 1-10, 2020.

[21] S. B. Karakaş, B. Otludil, "Accumulation and histopathological effects of cadmium on the great pond snail *Lymnaea stagnalis* Linnaeus, 1758 (Gastropoda: Pulmonata)," Environmental Toxicology and Pharmacology, vol. 78, pp. 103403, 2020.

[22] R. W. Zurawell, J. I. Goldberg, C. F. Holmes, E. E. Prepas, "Tissue distribution and oral dose effects of

microcystin in the freshwater pulmonate snail *Lymnaea stagnalis jugularis* (Say)," Journal of Toxicology and Environmental Health, Part A, vol. 70, no. 7, pp. 620-626, 2007.

[23] J. Lajtner, R. Erben, G. I. Klobucar, "Histopathological effects of phenol on the digestive gland of *Amphimelania holandri* Fér. (Gastropoda, Prosobranchia)," Bulletin of Environmental Contamination and Toxicology, vol. 57, no. 3, pp. 458-464 1996.

[24] B. Otludil, E. I. Cengiz, M. Z. Yildirim, Ö. Ünver, E. Ünlü, "The effects of endosulfan on the great ramshorn snail *Planorbarius corneus* (Gastropoda, Pulmonata): a histopathological study," Chemosphere, vol. 56, no. 7, pp. 707-716, 2004.

[25] E. Ünlü, E. I. Cengiz, M. Z. Yildirim, B. Otludil, Ö. Ünver, "Histopathological effects in tissues of snail *Lymnaea stagnalis* (Gastropoda, Pulmonata) exposed to sublethal concentration of Thiodan® and recovery after exposure," Journal of Applied Toxicology, vol. 25, no. 6, pp. 459-463, 2005.

[26] J. Murakami, R. Okada, H. Sadamoto, S. Kobayashi, K. Mita, Y. Sakamoto, M. Yamagishi, D. Hatakeyama, E. Otsuka, A. Okuta, H. Sunada, S. Takigami, M. Sakakibara, Y. Fujito, M. Awaji, S. Moriyama, K. Lukowiak, E. Ito, "Involvement of insulin-like peptide in long-term synaptic plasticity and long-term memory of the pond snail *Lymnaea stagnalis*,".Journal of Neuroscience, vol. 33, no. 1, pp. 371-383, 2013.

[27] N. Akhtar, M. I. Syakir Ishak, S. A. Bhawani, K. Umar, "Various natural and anthropogenic factors responsible for water quality degradation: A review," Water, vol. 13, no. 19, pp. 2660, 2021.

[28] C. de Perre, T. M. Murphy, M. J. Lydy, "Mixture toxicity of phostebupirim and cyfluthrin: Species-specific responses," Environmental Toxicology and Chemistry, vol. 36, no. 7, pp. 1947-1954, 2017.

[29] I. H. Baek, Y. Kim, S. Baik, J. Kim, "Investigation of the synergistic toxicity of binary mixtures of pesticides and pharmaceuticals on *Aliivibrio fischeri* in major river basins in South Korea," International journal of Environmental Research and Public Health, vol. 16, no. 2, pp. 208, 2019.

[30] C. Wang, Y. Yang, N. Wu, M. Gao, Y. Tan, "Combined toxicity of pyrethroid insecticides and heavy metals: a review," Environmental Chemistry Letters, vol. 17, no. 4, pp. 1693-1706, 2019.

[31] H. Zhang, X. Hong, S. Yan, J. Zha, J. Qin, "Environmentally relevant concentrations of bifenthrin induce changes in behaviour, biomarkers, histological characteristics, and the transcriptome in *Corbicula fluminea*," Science of The Total Environment, vol. 728, pp. 138821, 2020.

[32] F. Arrighetti, E. Ambrosio, M. Astiz, A. R. Capítulo, S. Lavarías, "Differential response between histological and biochemical biomarkers in the apple snail *Pomacea canaliculata* (Gasteropoda: Amullariidae) exposed to cypermethrin," Aquatic Toxicology, vol. 194, pp. 140-151, 2018.

[33] K. V. Brix, A. J. Esbaugh, M. Grosell, "The toxicity and physiological effects of copper on

the freshwater pulmonate snail, *Lymnaea stagnalis*," Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, vol. 154, no. 3, pp. 261-267, 2011.

[34] A. Weber, M. von Randow, A. L. Voigt, M. von der Au, E. Fischer, B. Meermann, M. Wagner, "Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: No microplastic-induced effects alone or in combination with copper," Chemosphere, vol. 263, pp. 128040, 2021.

[35] G. Atli, M. Grosell, "Characterization and response of antioxidant systems in the tissues of the freshwater pond snail (*Lymnaea stagnalis*) during acute copper exposure," Aquatic Toxicology, vol. 176, pp. 38-44, 2016.

[36] J. A. Adeyemi, O. O. Adewale, A. Y. Oguma, "Mortality, oxidative stress and hepatotoxicity in juvenile African catfish, *Clarias gariepinus* Burchell, exposed to lead and cypermethrin," Bulletin of Environmental Contamination and Toxicology, vol. 92, no. 5, pp. 529-533, 2014.

[37] Y. Yang, X. Ye, B. He, J. Liu, "Cadmium potentiates toxicity of cypermethrin in zebrafish," Environmental Toxicology and Chemistry, vol. 35, no. 2, pp. 435-445, 2016.

[38] H. Rehman, A. T. Aziz, S. Saggu, A. L. VanWert, N. Zidan, S. Saggu, "Additive toxic effect of deltamethrin and cadmium on hepatic, hematological, and immunological parameters in mice," Toxicology and Industrial Health, vol. 33, no. 6, pp. 495-502, 2017.