

Tissue- and Species-Specific Distribution of Esterases in *Catla catla* and *Labeo rohita* of the Order Cypriniformes

V. Rajaiah¹

¹Department of Zoology, Kakatiya Government College, Hanamkonda, India

Publication Date: 2026/10/07

Abstract: Esterase distribution was investigated in six tissues—gill, liver, intestine, muscle, brain, and eye—of two cypriniform fishes, *Catla catla* and *Labeo rohita*. Esterase patterns were examined by electrophoresis and the individual zones were characterized on the basis of their relative mobility (R_m) and their sensitivity to specific inhibitors. In *Catla catla*, esterase zones showed marked tissue-specific variation. Esterases resistant to inhibitors (ER) were widely distributed, while cholinesterase-like enzymes (CHsp), carboxylesterases (CE), cholinesterases (ChE), and arylesterases (ArE) occurred in particular tissues. In *Labeo rohita*, CE and ER esterases were prominent, with additional ChE, CHsp, ArE, and Esdp zones occurring in specific tissues. The results demonstrate that esterase profiles differ among tissues and between the two species, and that zones with similar electrophoretic mobility may differ in inhibitor sensitivity depending on the tissue.

Keywords: Electrophoresis; Esterases; Gill; Liver; Intestine; Muscle; Brain; Eye; *Catla catla*; *Labeo rohita*.

How to Cite: V. Rajaiah (2026) Tissue- and Species-Specific Distribution of Esterases in *Catla catla* and *Labeo rohita* of the Order Cypriniformes. *International Journal of Innovative Science and Research Technology*, 11(10), 44-49.
<https://doi.org/10.38124/ijisrt/26oct145>

I. INTRODUCTION

Esterases are hydrolytic enzymes that catalyse the cleavage of ester bonds to produce an acid and an alcohol. Lov(en)hart (1906) distinguished esterases that hydrolyse esters of short-chain fatty acids (C₂–C₄) from lipases that act primarily on esters of long-chain fatty acids (>C₈), as cited by Seligman and Nachlas (1950).

Esterase enzymes participate in several physiological processes, including nervous impulse regulation, reproduction, development, detoxification, and tolerance to xenobiotic compounds. Because their activities can respond to environmental contaminants, esterases have also been used as biomarkers for assessing environmental pollution and as genetic markers in a wide variety of organisms.

During recent decades, esterases have attracted considerable industrial interest because of their applications in food processing, detergents, fine-chemical production, wastewater treatment, biodiesel production, pharmaceutical processes, and bioremediation (Rao et al., 1998; Sharma et al., 2001; Bornscheuer, 2002; Jaeger and Eggert, 2002; Reetz, 2002; Maurer, 2004; Cammarota and Freire, 2006; Hasan et al., 2006). Their high regioselectivity and stereospecificity are useful in the kinetic resolution of optical isomers and in the synthesis of optically pure compounds for pharmaceutical and chemical applications. Their ability to catalyse reactions without requiring additional cofactors is another important

advantage (Bornscheuer, 2002). Esterases also play important roles during insect metamorphosis (Quan-You Yu et al., 2009).

The present study was undertaken to examine the tissue-specific and species-specific distribution of esterases in six tissues of *Catla catla* and *Labeo rohita* using electrophoretic separation and inhibitor-sensitivity analysis.

II. MATERIALS AND METHODS

Fishes were collected from ponds and tanks located within a radius of approximately 60 km from the Kakatiya University campus using nets with the assistance of local fishermen. The fishes were transported immediately to the laboratory in water-filled plastic buckets and acclimatized to laboratory conditions for approximately one week in aquaria. They were fed with natural plankton collected from their respective habitats.

The fishes were immobilized by striking the head, and the required tissues were dissected. Six tissues—gill, liver, intestine, muscle, brain, and eye—were selected for analysis. Tissues obtained from approximately three large fish to six small fish were pooled, weighed to the nearest milligram, and homogenized in 0.01 M Tris-HCl buffer (pH 7.5) containing 0.9% NaCl.

The tissue homogenate concentrations were approximately 10% for gill, liver, intestine, brain, and eye, and 20% for muscle. After homogenization, the samples were placed in ice-jacketed centrifuge tubes and centrifuged at 2,000 rpm for 10 minutes in a clinical centrifuge at room temperature. The supernatants were mixed with an equal volume of 20% sucrose solution containing 0.05% bromophenol blue as the tracking dye. An aliquot of 0.1 mL of the resulting mixture was loaded onto the gel for electrophoretic separation of esterase zones.

Esterase zones were classified according to the procedures described by Holmes and Masters (1967), Hart and Cook (1976), Haritos and Salmastrakis (1982), and Lakshmipathi and Reddy (1989), based on their sensitivity to specific inhibitors. Physostigmine (eserine; a carbamate), pCMB (a thiol-reactive compound), and paraoxon (an organophosphorus compound) were used in the inhibitor-sensitivity analysis.

➤ Classification of Esterases Based on Inhibitor Sensitivity

Table 1 Classification of Esterases Based on Inhibitor Sensitivity

Classification	CHsp	ChE			CE	Esdp	ArE	ER	Ese
pCMB	-	+			+	-	-	+	+
Eserine	-	-			+	+	+	+	-
Paraoxon	-	-			-	-	+	+	+

III. RESULTS

➤ *Catla Catla*

The inhibitor-sensitivity patterns of individual esterase zones detected in *Catla catla* are presented in Table 2.

Table 2 Inhibitor Sensitivity of Individual Esterase Zones in *Catla catla*

Name of Tissue	Gill			Liver				Intestine				Muscle		Brain			Eye	
Rm values	.56	.45	.33	.76	.56	.45	.33	.96	.86	.56	.33	.56	.45	.76	.56	.45	.56	.33
Activity	+	++	+	+	++	++	++	+	+	++	++	+	++	++	++	++	++	++
pCMB	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+
Eserine	+	+	-	+	+	+	-	+	+	-	-	-	-	-	+	-	+	+
Paraoxon	+	+	-	+	-	+	-	+	-	-	-	-	-	-	+	-	-	-
Classification	ER	ER	CHsp	ER	CE	ER	ChE	ER	CE	ChE	ChE	CHsp	CHsp	CHsp	ArE	CHsp	CE	CE

- Gill: Three esterase zones were detected, with Rm values of 0.56, 0.45, and 0.33. The zones at Rm 0.56 and 0.45 were classified as ER esterases, whereas the zone at Rm 0.33 was classified as a CHsp esterase.
- Liver: Four esterase zones were detected at Rm values of 0.76, 0.56, 0.45, and 0.33. The zones at Rm 0.76 and 0.45 were classified as ER esterases. The zones at Rm 0.56 and 0.33 were identified as CE and ChE esterases, respectively.
- Intestine: Four zones were detected at Rm values of 0.96, 0.86, 0.56, and 0.33. The zones at Rm 0.56 and 0.33 showed higher activity and were classified as ChE esterases. The zones at Rm 0.96 and 0.86 showed lower activity and were classified as ER and CE esterases, respectively.
- Muscle: Two esterase zones were observed at Rm values of 0.56 and 0.45. Both were classified as CHsp esterases and showed low to moderate activity according to the activity symbols reported in the original table.
- Brain: Three esterase zones were detected at Rm values of 0.76, 0.56, and 0.45. The zones at Rm 0.76 and 0.45 were classified as CHsp esterases, while the zone at Rm 0.56 was identified as an ArE esterase and showed the highest activity among the brain zones.
- Eye: Two esterase zones were detected at Rm values of 0.56 and 0.33. Both were classified as CE esterases and showed moderate activity.

➤ *Labeo rohita*

The inhibitor-sensitivity patterns of individual esterase zones detected in *Labeo rohita* are presented in Table 3.

Table 3 Inhibitor sensitivity of Individual Esterase Zones in *Labeo rohita*

Name of Tissue	Gill			Liver			Intestine			Muscle		Brain			Eye	
Rm values	.50	.41	.25	.50	.33	.25	.58	.50	.33	.41	.33	.50	.41	.25	.41	.33
Activity	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++
pCMB	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+
Eserine	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+

Paraoxon	+	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-
Classification	ER	CE	ER	CE	CE	ER	Esdp	ER	ER	ChE	CHsp	ChE	ArE	ChE	CE	CE

- Gill: Three esterase zones were detected at Rm values of 0.50, 0.41, and 0.25. All showed low activity. The zones at Rm 0.50 and 0.25 were classified as ER esterases, while the zone at Rm 0.41 was classified as a CE esterase.
- Liver: Three zones were observed at Rm values of 0.50, 0.33, and 0.25. All showed moderate activity. The zones at Rm 0.50 and 0.33 were classified as CE esterases, whereas the zone at Rm 0.25 was classified as an ER esterase.
- Intestine: Three esterase zones were detected at Rm values of 0.58, 0.50, and 0.33. The zones at Rm 0.50 and 0.33 were classified as ER esterases and showed moderate activity. The zone at Rm 0.58 was classified as an Esdp esterase.
- Muscle: Two zones were detected at Rm values of 0.41 and 0.33. The zone at Rm 0.41 was classified as a ChE esterase, while the zone at Rm 0.33 was classified as a CHsp esterase.
- Brain: Three zones were detected at Rm values of 0.50, 0.41, and 0.25. The zones at Rm 0.50 and 0.25 were classified as ChE esterases, while the zone at Rm 0.41 was classified as an ArE esterase.
- Eye: Two active zones were detected at Rm values of 0.41 and 0.33. Both were classified as CE esterases and showed moderate activity.

IV. TISSUE-SPECIFIC DISTRIBUTION

Table 4 Tissue Specific Distribution of Esterases in *Catla catla*

Rm values	1	2	3	4	5	6
Tissues	.96	.86	.76	.56	.45	.33
1) Gill				+ER	++ER	+CHsp
2) Liver			+ER	+++CE	+++ER	++ChE
3) Intestine	+ER	+CE		++ChE		++ChE
4) Muscle				+CHsp	++CHsp	
5) Brain			++CHsp	+++ArE	++CHsp	
6) Eye				++CE		++CE

In *Catla catla*, six esterase zones were identified at Rm values of 0.96, 0.86, 0.76, 0.56, 0.45, and 0.33. The zone at Rm 0.56 occurred in all six tissues and displayed different esterase types depending on the tissue: CE in liver and eye, CHsp in muscle, ER in gill, ArE in brain, and ChE in intestine. The zone at Rm 0.45 occurred in gill, liver, muscle, and brain; it was classified as ER in gill and liver and as CHsp in muscle and brain.

The zone at Rm 0.33 occurred in gill, liver, intestine, and eye. It was classified as CHsp in gill, ChE in liver and intestine, and CE in eye. The zone at Rm 0.76 occurred in liver and brain and was classified as ER in liver and CHsp in brain. The zones at Rm 0.86 and 0.96 occurred only in intestine and were classified as CE and ER, respectively. Overall, the esterase pattern in *Catla catla* showed clear tissue-specific variation in electrophoretic mobility and inhibitor sensitivity. ER esterases were the most widely distributed, followed by CHsp and CE esterases.

➤ *Labeo rohita*

Table 5 Tissue Specific Distribution of Esterases in *Labeo rohita*

Rm values	1	2	3	4	5
Tissues	.58	.50	.41	.33	.25
1) Gill		+ER	+CE		+ER
2) Liver		++CE		++CE	++ER
3) Intestine	++Esdp	++ER		++ER	
4) Muscle			++ChE	++CHsp	
5) Brain		++ChE	++ArE		++ChE
6) Eye			++CE	++CE	

Labeo rohita exhibited five esterase zones, designated EST1–EST5, with Rm values of 0.58, 0.50, 0.41, 0.33, and 0.25, respectively. The zones at Rm 0.50, 0.41, and 0.33 occurred in four tissues each. The zone at Rm 0.50 was detected in gill, liver, intestine, and brain and was classified as ER in gill and intestine, CE in liver, and ChE in brain.

The zone at Rm 0.41 occurred in gill, muscle, brain, and eye. It was classified as CE in gill and eye, ChE in muscle, and ArE in brain. The zone at Rm 0.33 occurred in liver, intestine, muscle, and eye and was classified as CE in liver and eye, CHsp in muscle, and ER in intestine.

The zone at Rm 0.25 occurred in gill, liver, and brain; it was classified as ER in gill and liver and ChE in brain. The

zone at Rm 0.58 was detected only in intestine and was classified as Esdp. Overall, CE and ER esterases were

prominent in *Labeo rohita*, followed by the other esterase classes.

➤ Figures

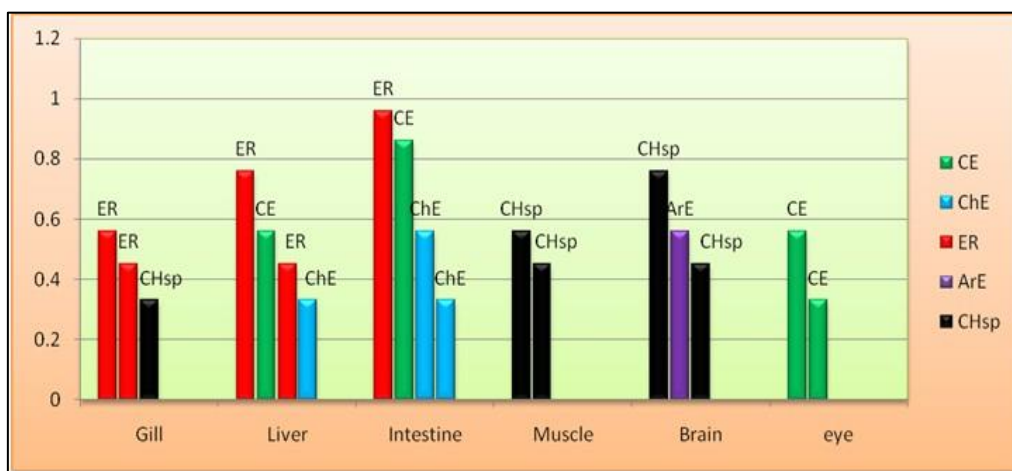


Fig 1 Tissue-Specific Distribution of Esterases in *Catla catla*.

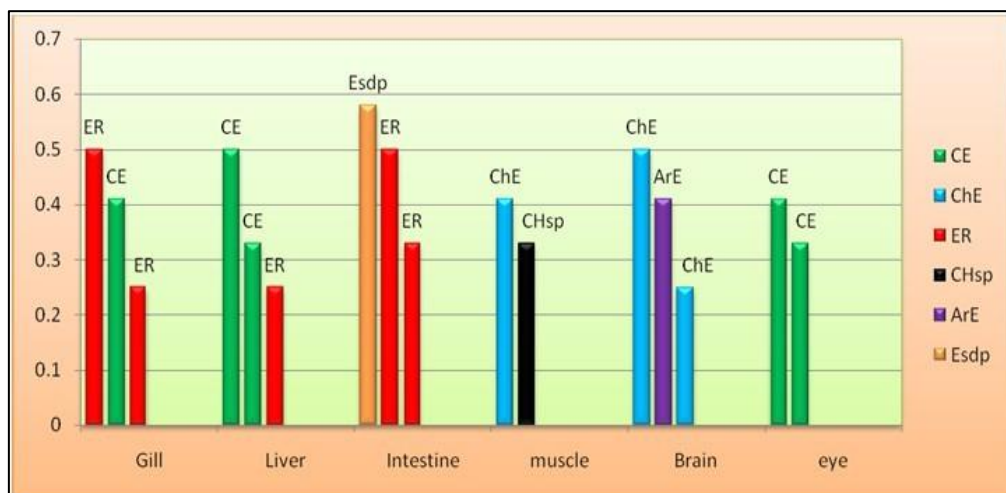


Fig 2 Tissue-Specific Distribution of Esterases in *Labeo rohita*.

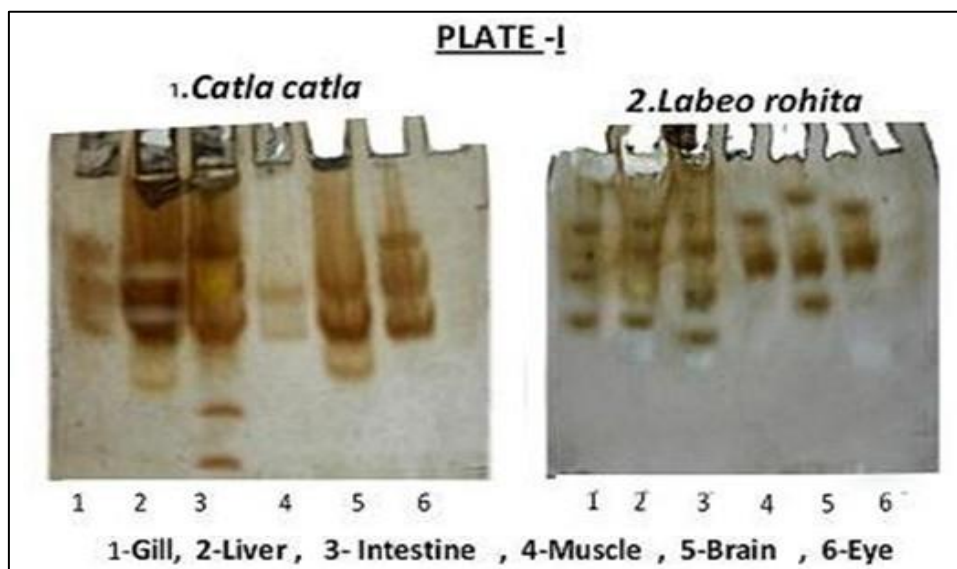


Plate 1 Electrophoretic Esterase Patterns in *Catla catla* and *Labeo rohita*. Lanes: 1—Gill; 2—Liver; 3—Intestine; 4—Muscle; 5—Brain; 6—Eye.

ABBREVIATIONS AND ACTIVITY SYMBOLS

Abbreviation	Meaning
Rm	Relative mobility, calculated as the fraction of the distance migrated by a zone from the origin of the tracking dye.
CE	Carboxylesterase
ChE	Cholinesterase
CHsp	Cholinesterase-like enzymes
ER	Esterases resistant to the inhibitors used in the study
ArE	Arylesterases
Esdp	Esterases sensitive to organophosphates and pCMB
Ese	Esterases sensitive to eserine alone
+++	High activity
++	Moderate activity
+	Low activity

REFERENCES

- [1.] Bornscheuer, U. T. (2002). Microbial carboxyl esterases: Classification, properties and applications in biocatalysis. *FEMS Microbiology Reviews*, 26, 73–81.
- [2.] Cammarota, M. C., & Freire, D. M. G. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology*, 97, 2195–2210.
- [3.] Haritos, A. A., & Salmastrakis, S. S. (1982). A comparison of muscle esterases in the fish genus *Trachurus* by vertical gel electrophoresis. *Comparative Biochemistry and Physiology Part B*, 72, 477–480.
- [4.] Hart, N. H., & Cook, M. (1976). Comparative analysis of tissue esterases in zebra danio (*Brachydanio rerio*) and the pearl danio (*B. albolineatus*) by disc gel electrophoresis. *Comparative Biochemistry and Physiology Part B*, 54, 357–364.
- [5.] Hasan, F., Shah, A. A., & Hameed, A. (2006). Industrial applications of microbial lipases. *Enzyme and Microbial Technology*, 39, 235–251.
- [6.] Holmes, R. S., & Masters, C. J. (1967). The developmental multiplicity and isoenzyme status of cavian esterases. *Biochimica et Biophysica Acta*, 132, 379–399.
- [7.] Jaeger, K. E., & Eggert, T. (2002). Lipases for biotechnology. *Current Opinion in Biotechnology*, 13(4), 390–397.
- [8.] Lakshmipathi, V., & Reddy, T. M. (1989). Esterase polymorphisms in muscle and brain of four freshwater fishes belonging to the family Cyprinidae. *Journal of Applied Ichthyology*, 5(2), 88–95.
- [9.] Loevenhart, A. S. (1906). *Journal of Biological Chemistry*, 2, 427. (Quoted by Pearse, 1972).
- [10.] Maurer, K. (2004). Detergent proteases. *Current Opinion in Biotechnology*, 15, 330–334.
- [11.] Yu, Q. Y., Lu, C., Li, W. L., Xiang, Z. H., & Zhang, Z. (2009). Annotation and expression of carboxylesterases in the silkworm, *Bombyx mori*. *BMC Genomics*, 10, 553.
- [12.] Rao, M. B., Tanksale, A. M., Ghatge, M. S., & Deshpande, V. V. (1998). Molecular and biotechnological aspects of microbial proteases. *Microbiology and Molecular Biology Reviews*, 62, 597–635.
- [13.] Reetz, M. T. (2002). Lipases as practical biocatalysts. *Current Opinion in Chemical Biology*, 6, 145–150.
- [14.] Seligman, A. M., & Nachlas, M. M. (1950). *Journal of Clinical Investigation*, 29, 31. (Quoted by Pearse, 1972).
- [15.] Sharma, R., Chisti, Y., & Banerjee, U. C. (2001). Production, purification, characterization and applications of lipases. *Biotechnology Advances*, 19, 627–662.
- [16.] Pereira, B. V. R., Silva-Zacarin, E. C. M., Costa, M. J., dos Santos, A. C. A., do Carmo, J. B., & Nunes, B. (2019). Cholinesterases characterization of three tropical fish species, and their sensitivity towards specific contaminants. *Ecotoxicology and Environmental Safety*, 173, 482–493. <https://doi.org/10.1016/j.ecoenv.2019.01.105>
- [17.] Martínez-Morcillo, S., Pérez-López, M., Míguez, M. P., Valcárcel, Y., & Soler, F. (2019). Comparative study of esterase activities in different tissues of marine fish species *Trachurus trachurus*, *Merluccius merluccius* and *Trisopterus luscus*. *Science of the Total Environment*, 679, 12–22. <https://doi.org/10.1016/j.scitotenv.2019.05.047>
- [18.] Lan, L., Ren, X., Yang, J., Liu, D., & Zhang, C. (2020). Detection techniques of carboxylesterase activity: An update review. *Bioorganic Chemistry*, 94, 103388. <https://doi.org/10.1016/j.bioorg.2019.103388>
- [19.] Park, J. M., Won, S. M., Kang, C. H., Park, S., & Yoon, J. H. (2020). Characterization of a novel carboxylesterase belonging to family VIII hydrolyzing β -lactam antibiotics from a compost metagenomic library. *International Journal of Biological Macromolecules*, 164, 4650–4661. <https://doi.org/10.1016/j.ijbiomac.2020.09.070>
- [20.] Santana, M. S., Sandrini-Neto, L., Di Domenico, M., & Prodócimo, M. M. (2021). Pesticide effects on fish cholinesterase variability and mean activity: A meta-analytic review. *Science of the Total Environment*, 757, 143829. <https://doi.org/10.1016/j.scitotenv.2020.143829>
- [21.] Johan, U. U. M., Raja Abd Rahman, R. N. Z., Kamarudin, N. H. A., & Ali, M. S. M. (2021). An

- integrated overview of bacterial carboxylesterase: Structure, function and biocatalytic applications. *Colloids and Surfaces B: Biointerfaces*, 205, 111882. <https://doi.org/10.1016/j.colsurfb.2021.111882>
- [22.] Olivares-Rubio, H. F., & Espinosa-Aguirre, J. J. (2021). Acetylcholinesterase activity in fish species exposed to crude oil hydrocarbons: A review and new perspectives. *Chemosphere*, 264, 128401. <https://doi.org/10.1016/j.chemosphere.2020.128401>
- [23.] Santana, M. S., Domingues de Melo, G., Sandrini-Neto, L., Di Domenico, M., & Prodocimo, M. M. (2022). A meta-analytic review of fish antioxidant defense and biotransformation systems following pesticide exposure. *Chemosphere*, 291, 132730. <https://doi.org/10.1016/j.chemosphere.2021.132730>
- [24.] Nanthanawat, P., Kiatprasert, P., Wattanakornsiri, A., Nanuam, J., Prasatkaew, W., Ruttanakorn, S., & Thanomsit, C. (2022). Development of an antibody technique for acetylcholinesterase expression detection in the gill of Nile tilapia (*Oreochromis niloticus*) as a glyphosate-based herbicide biomarker. *Toxicology Reports*, 9, 1548–1556. <https://doi.org/10.1016/j.toxrep.2022.07.009>
- [25.] Solé, M., Rivera-Ingraham, G., & Freitas, R. (2018). The use of carboxylesterases as biomarkers of pesticide exposure in bivalves: A methodological approach. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 212, 18–24. <https://doi.org/10.1016/j.cbpc.2018.06.002>
- [26.] Solé, M., & Sanchez-Hernandez, J. C. (2018). Elucidating the importance of mussel carboxylesterase activity as exposure biomarker of environmental contaminants of current concern: An in vitro study. *Ecological Indicators*, 85, 432–439. <https://doi.org/10.1016/j.ecolind.2017.10.046>
- [27.] Ghodke, P., et al. (2022). Environmental role of aromatic carboxylesterases. *Environmental Microbiology*. <https://doi.org/10.1111/1462-2920.15774>
- [28.] Ekeoma, B. C., Ekeoma, L. N., Yusuf, M., Haruna, A., Ikeogu, C. K., Merican, Z. M. A., Kamyab, H., Pham, C. Q., Vo, D.-V. N., & Chelliapan, S. (2023). Recent advances in the biocatalytic mitigation of emerging pollutants: A comprehensive review. *Journal of Biotechnology*, 369, 14–34. <https://doi.org/10.1016/j.jbiotec.2023.05.003>
- [29.] Gajula, S. K., Kanthala, S. K., Narra, M. R., & Vanamala, V. D. (2023). Individual and interactive biochemical profile damages in *Labeo rohita* (Cyprinidae) by imidacloprid and clothianidin. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 271, 109689. <https://doi.org/10.1016/j.cbpc.2023.109689>
- [30.] Sürmeli, Y., Vardar-Yel, N., & Tütüncü, H. E. (2025). Recent advances of structure, function, and engineering of carboxylesterases for the pharmaceutical industry: A minireview. *International Journal of Biological Macromolecules*, 307(Pt 3), 142206. <https://doi.org/10.1016/j.ijbiomac.2025.142206>