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Tracking environmental stress in aquatic species using biomarkers for conservation

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Abstract

Aquatic ecosystems are exposed to anthropogenic stressors and pollutants, including chemical pollutants, temperature, salinity, and habitat degradation, that threaten their biodiversity. Current conservation initiatives base their environmental assessment solely on the chemistry of water and sediments, neglecting the more telling indicators of ecological stress: organisms' physiological responses. This research investigates a set of biomarkers consisting of the oxidative-stress enzymes (catalase, superoxide dismutase, glutathione peroxidase), the neurotoxicity marker (acetylcholinesterase), and condition indices (hepatosomatic index, condition factor) on the freshwater carp Labeo rohita sampled from different reaches of the Hooghly River, India, under different environmental-stress situations. Findings indicate that condition and histological parameters respond to pollutant impacts over extended periods, whereas the oxidative-stress and neurotoxicity biomarkers react more rapidly. Supporting their diagnostic and predictive capabilities, correlation and multivariate analyses associated the biomarkers with critical physicochemical variables. The integrated biomarker response (IBR) index, with high precision, identifies a stress gradient, providing a quantitative method to support conservation. This biomarker system identifies stress to target specific sites and times for management action within aquatic conservation initiatives.

Keywords: Biomarkers, Oxidative stress, Fish conservation, Aquatic pollution, Early-warning, Environmental monitoring

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Introduction

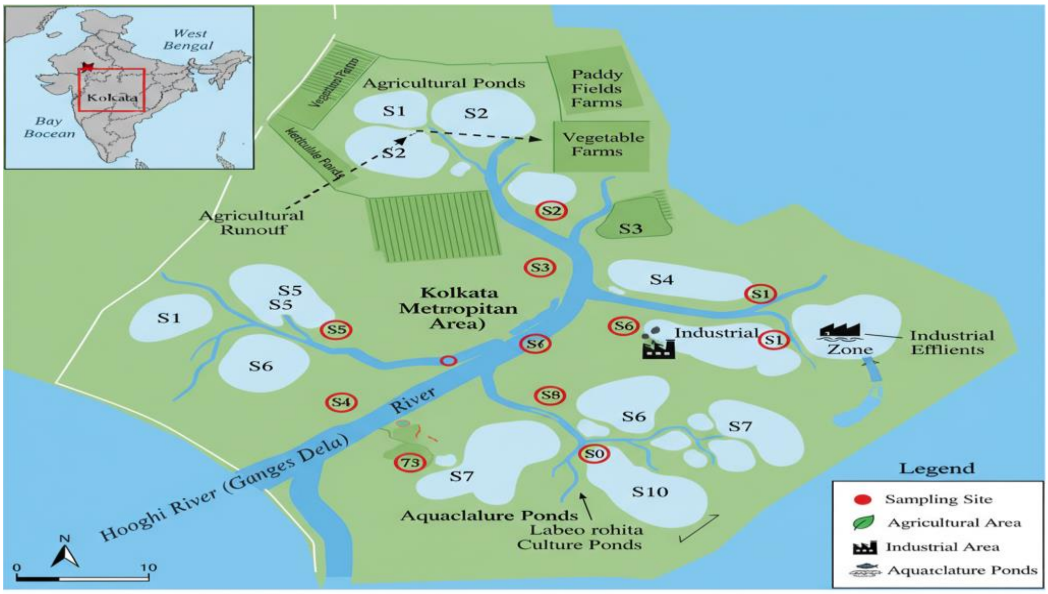
Aquatic ecosystems are complex and dynamic ecosystems that support ecological and biological diversity. Although environmental and biological diversity are under anthropogenic pressures of all sorts, including industrial effluents, agricultural runoff, urban waste discharge, and thermal changes, these stressors have led to the accumulation of multiple stressors within these systems. These changes affect the equilibrium of aquatic systems and may lead to oxidative stress, neurotoxicity, impaired growth, and reproductive failure in aquatic species (Sharma *et al.,* 2024). Disruption of sub-lethal physiological effects in organisms may impair the ecological integrity and resilience of freshwater systems. Previous environmental monitoring practices have focused on water and sediment, as well as the physicochemical components of the systems being assessed. These metrics may help define system quality, but they fail to reveal the biological impact of stress within organisms. Biomarkers, in contrast, reflect stress at the molecular, biochemical, and physiological levels and indicate exposure to stress well in advance of visible ecological impacts (Yamamoto *et al.*, 2023; Abdallah *et al.*, 2024). Antioxidant stress (catalase, superoxide dismutase, glutathione peroxidase) biomarker neurotoxicity (acetylcholinesterase), and condition indices (hepatosomatic index, condition factor) have become widely accepted in the appraisal of exposure to pollutants and the health of organisms.

The Indian major carp, or Labeo rohita, is a key freshwater bioindicator species of ecological and economic significance. Its wide distribution, bentho-pelagic feeding, and environmental tolerance make L. rohita an excellent candidate for assessing recipient systems and determining pollutant exposure and physiological response for a range of freshwater systems (Ashraf *et al.,* 2025; Kakavand and Chalechale, 2015). The responses of this species' biomarkers indicate the condition of polluted biomonitoring systems and working rivers, such as the Hooghly, which suffers dynamic contamination from industrial and agricultural sources (Nagarani *et al.*, 2023). Unlike biomarker studies, which are increasingly supported by evidence, the majority of conservation initiatives in India and other developing regions remain focused on chemical monitoring and abstracted, organism-removed assessments. This illustrates a gap in the biological interpretation of studies in environmental chemistry. The current study seeks to assess L. rohita using a multi-tier biomarker approach to examine the effects of ecological degradation and sub-lethal stress.

This study primarily characterizes the physicochemical stressors in the Hooghly River, focusing on the environmental aspects of ecosystem degradation (Chandravanshi and Neetish, 2023). Furthermore, this study sought to measure key biomarkers of oxidative stress, neurotoxicity, and general condition in Labeo rohita (L. rohita), which is of significant ecological and economic value (El-SiKaily and Shabaka, 2024). To integrate these biomarker data, the study used the Integrated Biomarker Response (IBR) index to quantify stress at each site. Additionally, the study offered a fresh perspective on freshwater regulation by investigating the application of biomarker-based indicators in conservation management. Improve the capacity to detect environmental stresses on aquatic ecosystems at an early stage by incorporating environmental chemistry and stress indicators into the "response" from this holistic viewpoint. Consequently, this improves overall environmental protection, which in turn optimizes aquatic conservation and management activities to achieve informed, proactive management of freshwater ecosystems.

Materials and Methods

Water quality varies with the seasons in the Kolkata region of India, where the research was conducted. Agricultural and industrial activity in the area impacts the water body by adding to environmental stresses such as nitrogen loading and pesticide residues. To capture annual variations in water quality and pollutant concentrations, sampling was conducted during three seasons: pre-monsoon (March–May), monsoon (June–September), and post-monsoon (October–February). The geographic distribution of the sites was highlighted in Figure 1, which maps the sampling locations. Labeo rohita was the intended focus of this research due to its high ecological and commercial importance in aquaculture and freshwater ecosystems (Oudah, 2024; Saglam *et al.*, 2014; Aadiwal *et al.*, 2025). The selection of this species was supported by its extensive usage in biomarker-based research evaluating neurotoxicity and oxidative stress. L. rohita is the focus species of this study for good reason, given its ecological significance and its history of successful application in environmental monitoring.



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Geographic Sistribution of Sampling Sites for for Labeo rohita Collection

Figure 1: Geographic distribution of sampling sites.

A mercury thermometer was used to measure temperature; a dissolved oxygen (DO) meter for dissolved oxygen; a pH meter for pH; an electrical conductivity (EC) meter for electrical conductivity; a refractometer for salinity; and a turbidity meter for turbidity at each sample location. Pesticide residues, heavy metals (such as lead, cadmium, and mercury), and nutrients (such as nitrates, phosphates, and ammonium) were among the stressor-specific variables examined, especially in regions affected by agricultural runoff (Zahran *et al.*, 2025).

Biomarker analyses were classified according to the rate of physiological response to environmental stress. Standard procedures were used to measure rapid biochemical indicators of oxidative stress, which include catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) (Formicki *et al.,* 2025). Protein damage was evaluated by measuring carbonyl content, and lipid peroxidation (MDA/LPO) was quantified using the thiobarbituric acid technique. Activity of the sensitive pollution marker acetylcholinesterase (AChE) in brain, gill, and muscle tissues was used to assess neurotoxicity. Glycogen and lactate levels were optionally evaluated, along with energy and condition indicators such as the hepatosomatic index (HSI) and condition factor (K). Score lesions, which relate tissue damage to environmental stresses, were identified by histopathological study of gill and liver tissues. To standardise the measures, biomarker data were normalised using Z-scores. With an emphasis on the effects of many stressors, the Integrated Biomarker Response (IBR) was calculated to combine the results of several biomarkers into a single composite health indicator. The associations between biomarker responses and environmental stressors were assessed using Pearson and Spearman correlation analysis. To examine how biomarker profiles and environmental factors classified sites, we used Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA). All statistical analyses were conducted using R version 4.0 and SPSS version 28, with a significance threshold of $\alpha = 0.05$.

Results

Each site's temperature, pH, dissolved oxygen, electrical conductivity, salinity, and turbidity readings were split down by season in Table 1. Particular metrics' noted constraints have been satisfied, including those about excessively concentrated nutrients, heavy metals, and pesticide residues (Oros *et al.,* 2025). The instances were more noticeable during the monsoon and post-monsoon seasons, suggesting periods of environmental stress (Sabullah *et al.,* 2015).

Table 1: Environmental Parameters and Stressor Exceedances Across Sampling Sites and Seasons.

Site	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (µS/cm)	Salinity (ppt)	Turbidity (NTU)	Nutrient Concentration (mg/L)	Heavy Metals (µg/L)	Pesticide Residues (ng/L)
Site A	28	7.2	6.5	500	1.2	10	1.2	0.8	5
Site B	30	6.8	5.2	800	2.5	25	3.5	1.5	20
Site C	29	7.4	7.1	550	1.1	8	0.8	0.5	3
Site D	32	7.1	6.8	600	1.8	5	2.0	2.0	30

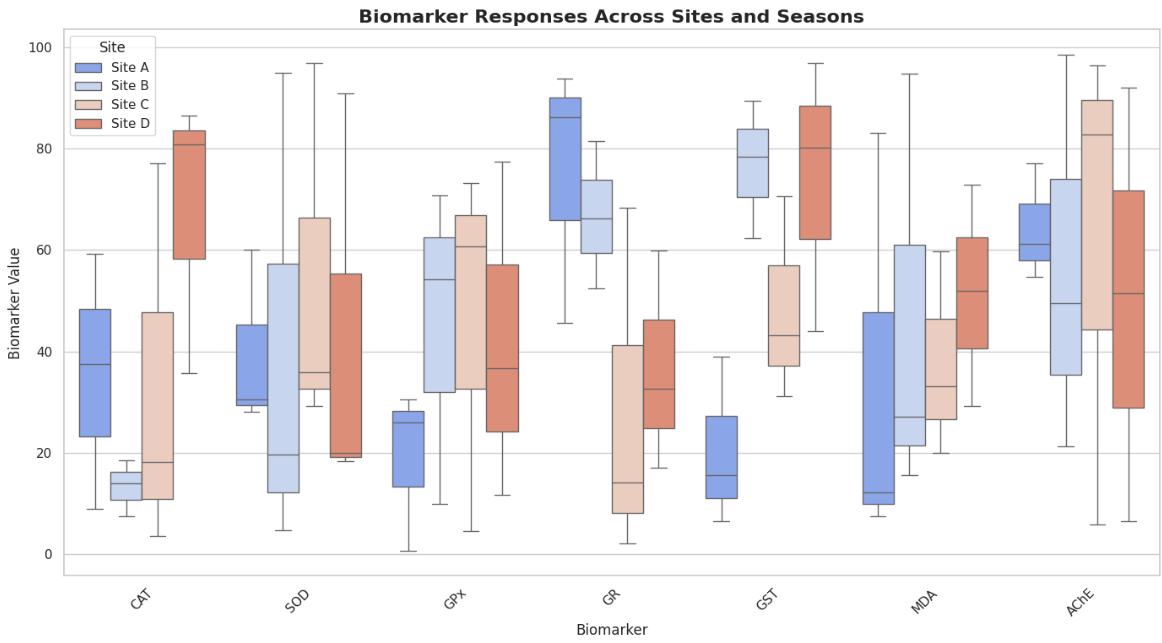

Figure 2: Seasonal variations in biomarker responses across sites.

The scores of the Integrated Biomarker Response (IBR) and the Conservation Biomarker Index (CBI) are shown in a radar plot for each site and season in Figure 2. The radar plot shows Site A had the highest IBR values in the post-monsoon season, when the highest oxidative stress and lipid peroxidation values were recorded (Menon *et al.*, 2023). In contrast, Site C recorded the lowest IBR scores throughout the study period, suggesting lower stress at this site. The CBI scores corroborate the considerable pollution stress documented at Site A, particularly during the monsoon and post-monsoon seasons.

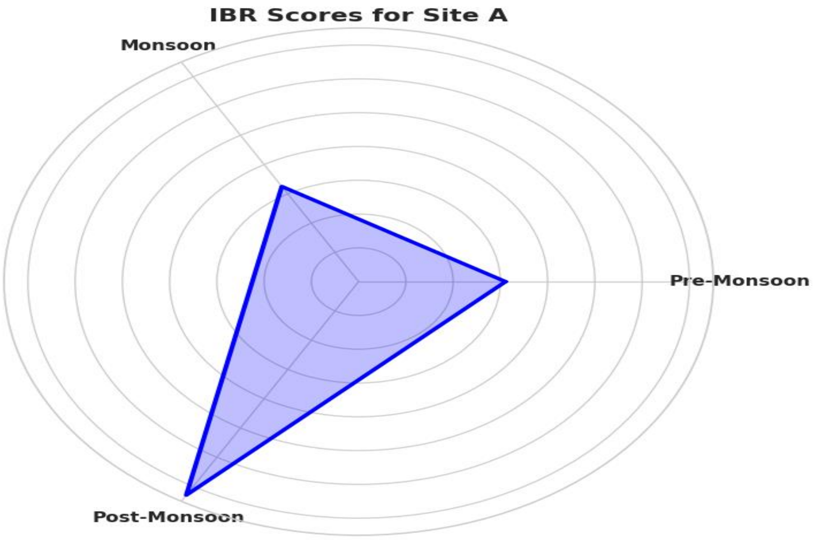

Figure 3: Integrated biomarker response scores for site a across seasons.

Results from Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA) of the relationships between environmental stressors and biomarker stress responses are shown in Figure 3. In the biplots, stressor sites (Sites A and B) anchor the cluster that exhibits high lipid peroxidation (LPO) and low acetylcholinesterase (AChE) enzyme activity. Sites A and B are situated mainly adjacent to high-nutrient, high-heavy-metal agricultural runoff. In contrast, the biomarker-stress sites (Sites C and D) are located away from the anthropogenic stressors.

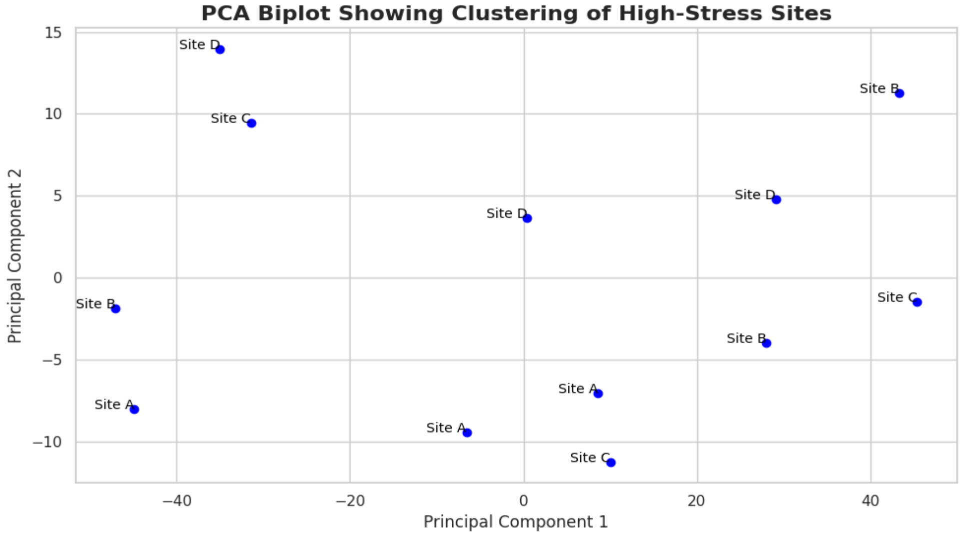

Figure 4: Clustering of high-stress sites based on pca analysis.

Figure 4 illustrates the changes due to polluted stress on fish gill and liver histopathological materials from different sampling locations. Site A showed gill hyperplasia and liver vacuolation and necrosis. The post-monsoon period showed the worst alterations, due to the highest levels of stressors, including heavy metals and pesticides. These alterations proved that the physiological stress pollution was causing the fish.

Discussion

Biomarkers exhibited evidence as early warning signs of stress from environmental factors and pollution (Yamamoto *et al.*, 2023). This research examined biomarker studies in which LPO, AChE, and various antioxidative enzymes (catalase, superoxide dismutase, and glutathione peroxidase) were shown to respond quickly to both mixed and pharmaceutical pollutants (see, for example, PMC). These quickly responding markers are critical for identifying stress in situations of episodic pollution and diffuse nonpoint-source pollution, such as agricultural runoff and aquaculture effluents. Proactive conservation treatments rely on our ability to identify early indicators of neurotoxicity and oxidative stress, long before the onset of actual symptoms of environmental damage (Menon *et al.*, 2023).

Find the same mixed response pattern when we examine genuine ecosystems that have been studied for numerous stresses; for example, research on Lake Qarun has shown this. Early stress exposure is indicated by biomarkers like LPO and AChE (Kocalar, Canli and Canli, 2023). Our findings corroborate previous research on the complex interactions among stressors, suggesting that environmental contaminants can trigger oxidative stress, which, in turn, can lead to other forms of physiological stress and malfunctions in neurotoxicity pathways, all of which worsen the damage. This shows how important it is to examine these systems periodically for biomarkers (Sarkar *et al.,* 2006). To better understand the state of ecosystems in these monitoring systems, surveillance stress biomarker systems can help pinpoint areas of high pollution and measure stress dynamics over time. Employing a multi-tiered biomarker strategy, as in this study, outweighs the advantages of individual principal biomarkers (Van der Oost, Beyer and Vermeulen, 2003). Although the sole principal biomarkers provide some insights, they tend to exhibit significant variances and are influenced by various external factors (Hook, Gallagher and Batley, 2014). The construction of IBR and the CBI provides a consolidated framework that addresses the variances in individual biomarkers and provides a solid estimate of the health of biological systems and the ecosystems in which they are embedded (Pramanik and Biswas, 2024). The conceptual shift of viewing biomarkers as ecological indices (Springer Open) is reminiscent of older literature in the ecological indices literature, which sought to improve the management of ecosystems. An ecological stress analysis can be best framed within an IBR system that combines rapid biochemical indices, physiological responses, and tissue necrosis to provide a comprehensive assessment.

The potential conservation uses of this multi-tiered biomarker approach are extensive. Conservationists can focus on the most polluted or ecologically compromised areas by identifying which reaches or ponds should be remediated based on elevated biomarker stress. Moreover, biomarkers can be used to schedule closed seasons during periods of high stress, enabling populations to recover during environmental hardships (Mahamood *et al.,* 2021). Aquaculture systems can be better protected from stressed and impaired organisms if biomarker levels in the hatchery and broodstock can be estimated. Last but not least, regulating practices may be ensured by providing scientific evidence for the necessity of pollution management, particularly in aquaculture clusters.

However, it is difficult to draw broad conclusions from this study due to its limited scope and small sample size, which focused on the Labeo rohita species. In addition, the snapshot sampling design offers only a limited temporal view of environmental stress, since biomarker fluctuations can reflect varying stress conditions. Longitudinal studies to monitor stress biomarkers and other indicators of the stress response would improve this line of research (Grădinariu *et al.,* 2025). Ultimately, the addition of genomics and other omics technologies would facilitate the description of more refined changes in pollutants and stressors at the molecular level, thereby deepening studies on pollutants in Labeo rohita and other similar aquatic species.

Conclusion

Using a multi-tiered biomarker approach is a good strategy for evaluating and quantifying sub-lethal stress in aquatic organisms. Most stressors are detected before passing classical thresholds for water-quality testing. In cases where water pollutants are not readily observable or are at variable concentrations, this is invaluable.

Specific stressors, mixed pollutants, and (eco)pharmaceuticals are rapidly detected using biomarkers such as lipid peroxidation and acetylcholinesterase activity. Through these sensitive biomarkers, pollution, ecosystem impacts, and impacts on aquatic biodiversity can serve as a routine standard for conservation monitoring in biomarker retrieval and assessment systems. The approach facilitates early detection and estimation of ecological pollution impacts. More generally, IBR analyses and the CBI approach integrate various biomarkers to assess cumulative stressor responses and simplify IBR systems for aquatic monitoring, especially for (local) environmental agencies. The effects of ecological stress and its mitigation can be better tracked through routine monitoring. The effectiveness of habitat restoration, pollution mitigation, and diversion efforts can only be measured using active IBR systems that incorporate periodic biomarker retrieval and evaluation. To make timely adjustments and improvements, it is essential to integrate treatments so their efficacy can be assessed in real time. Important insights may be gained by determining the degree of pollutant reduction and the responsiveness of the suggested actions to the ecosystem's recovery. The suggested biomarker panel focuses on ecosystem stress indicators that are both sensitive and easily measurable. Environmental quality frameworks and conservation initiatives at the national and regional scales may include this method, as it is fully compatible with the biomarker panel. These indicators, when used in conjunction with scientifically grounded frameworks, provide proactive evaluation and management of aquatic environment preservation.

References

Aadiwal, V., Upadhyay, S., Nagappan, B. and Wani, T.A., 2025. Investigating the Influence of Physiographic Factors on Habitat Selection by Cetacean Species in Marine Environments. *Natural and Engineering Sciences*, *10*(1), pp.301-311. https://doi.org/10.28978/nesciences.1646474

Abdallah, S.M., Muhammed, R.E., Mohamed, R.E., El Daous, H., Saleh, D.M., Ghorab, M.A., Chen, S. and El-Sayyad, G.S., 2024. Assessment of biochemical biomarkers and environmental stress indicators in some freshwater fish. *Environmental Geochemistry and Health*, *46*(11), p.464.

Ashraf, M.K., Ullah, S., Bilal, M., Multisanti, C.R. and Faggio, C., 2025. Multi-biomarker assessment of essential and toxic element accumulation in Labeo gonius from Khushab Lake (Pakistan). *Environmental Toxicology and Pharmacology*, p.104814. https://doi.org/10.1016/j.etap.2025.104814

Chandravanshi, N. and Neetish, K., 2023. Diurnal Variations in Greenhouse Gas Emissions from a Macrophyte-Covered River. *Aquatic Ecosystems and Environmental Frontiers*, *1*(1), pp.11-15.

El-SiKaily, A. and Shabaka, S., 2024. Biomarkers in aquatic systems: advancements, applications and future directions. *Egyptian Journal of*

Aquatic Research, *50*(2), pp.169-182. https://doi.org/10.1016/j.ejar.2024.05.002

Formicki, G., Goc, Z., Bojarski, B. and Witeska, M., 2025. Oxidative stress and neurotoxicity biomarkers in fish toxicology. *Antioxidants*, *14*(8), p.939. https://doi.org/10.3390/antiox14080939

Grădinariu, L., Crețu, M., Vizireanu, C. and Dediu, L., 2025. Oxidative Stress Biomarkers in Fish Exposed to Environmental Concentrations of Pharmaceutical Pollutants: A Review. *Biology*, *14*(5), p.472. https://doi.org/10.3390/biology14050472

Hook, S.E., Gallagher, E.P. and Batley, G.E., 2014. The role of biomarkers in the assessment of aquatic ecosystem health. *Integrated environmental assessment and management*, *10*(3), pp.327-341. https://doi.org/10.1002/ieam.1530

Kakavand, Z.M. and Chalechale, A., 2015. Comparison of Two Different Distance Functions of Image Retrieval for Detecting Species of Microscopic Fungi in Medical Mycology Laboratory. *International Academic Journal of Science and Engineering, 2 (4), 39, 44.*

Kocalar, K., Canli, E.G. and Canli, M., 2023. Responses of oxidative stress biomarkers of freshwater fish (Oreochromis niloticus) exposed to Cr6+, Hg2+, Ni2+ and Zn2+ in differing calcium levels. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *267*, p.109577. https://doi.org/10.1016/j.cbpc.2023.109577

Mahamood, M., Javed, M., Alhewairini, S.S., Zahir, F., Sah, A.K. and Ahmad, M.I., 2021. Labeo rohita, a bioindicator for water quality and associated biomarkers of heavy metal toxicity. *NPJ Clean Water*, *4*(1), p.17.

Menon, S.V., Kumar, A., Middha, S.K., Paital, B., Mathur, S., Johnson, R., Kademan, A., Usha, T., Hemavathi, K.N., Dayal, S. and Ramalingam, N., 2023. Water physicochemical factors and oxidative stress physiology in fish, a review. *Frontiers in Environmental Science*, *11*, p.1240813. https://doi.org/10.3389/fenvs.2023.1240813

Nagarani, N., Krishnaveni, G., Dharshini, V.D., Manohari, K.G., Jeyapandi, M.A., Mariappan, P.V. and Sangeetha, R., 2023. Biomarkers as ecological indices in monitoring the status of market fish. *The Journal of Basic and Applied Zoology*, *84*(1), p.2.

Oros, A., Coatu, V., Damir, N., Danilov, D., Ristea, E. and Lazar, L., 2025. Molecular Mechanisms and Biomarker-Based Early-Warning Indicators of Heavy Metal Toxicity in Marine Fish. *Fishes*, *10*(7), p.339. https://doi.org/10.3390/fishes10070339

Oudah, I.S., 2024. Common Biomarker for the Assessment of Water Pollution by Local Fish. *Applied Sciences*, *2*(4), pp.965-976.

Pramanik, S. and Biswas, J.K., 2024. Histopathological fingerprints and biochemical changes as multi-stress biomarkers in fish confronting concurrent pollution and parasitization. *IScience*, *27*(12).

Sabullah, M.K., Ahmad, S.A., Shukor, M.Y., Gansau, A.J., Syed, M.A., Sulaiman, M.R. and Shamaan, N.A., 2015. Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. *International Food Research Journal*, *22*(2).

Saglam, D., Atli, G., Dogan, Z., Baysoy, E., Gurler, C., Eroglu, A. and Canli, M., 2014. Response of the antioxidant system of freshwater fish (Oreochromis niloticus) exposed to metals (Cd, Cu) in differing hardness. *Turkish Journal of Fisheries and Aquatic Sciences*, *14*(1). https://doi.org/10.4194/1303-2712-v14_1_06

Sarkar, A., Ray, D., Shrivastava, A.N. and Sarker, S., 2006. Molecular biomarkers: their significance and application in marine pollution monitoring. *Ecotoxicology*, *15*(4), pp.333-340.

Sharma, A.K., Sharma, M., Sharma, S., Malik, D.S., Sharma, M. and Sharma, A.K., 2024. A systematic review on assessment of heavy metals toxicity in freshwater fish species: current scenario and remedial approaches. *Journal of Geochemical Exploration*, *262,* p.107472. https://doi.org/10.1016/j.gexplo.2024.107472

Van der Oost, R., Beyer, J. and Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental toxicology and pharmacology*, *13*(2), pp.57-149. https://doi.org/10.1016/S1382-6689(02)00126-6

Yamamoto, F.Y., Onishi, K., Ralha, T.R., Silva, L.F.O., Deda, B., Pessali, T.Y.C., Souza, C., Ribeiro, C.O. and Abessa, D.M.S., 2023. Earlier biomarkers in fish evidencing stress responses to metal and organic pollution along the Doce River Basin. *Environmental Pollution*, *329*, p.121720. https://doi.org/10.1016/j.envpol.2023.121720

Zahran, E., Mamdouh, A.Z., Elbahnaswy, S., El-Son, M.M., Risha, E., ElSayed, A., Barbary, M.I.E. and Sebaei, M.G.E., 2025. The impact of heavy metal pollution: bioaccumulation, oxidative stress, and histopathological alterations in fish across diverse habitats. *Aquaculture International*, *33*(5), p.371.