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Evaluation of Antioxidant Status and Oxidative Stress Biomarkers in Freshwater Fish Exposed to Pesticide Pollution

*Tanya and Neeru Singh
Department of Zoology, Keral Verma Subharti College of Science,
Swami Vivekanand Subharti University, Meerut (UP-250005), India
*Corresponding Author's email: tanyachaudhary30062002@gmail.com

The widespread use of pesticides in modern agriculture has led to the contamination of freshwater ecosystems, posing significant risks to aquatic life. Pesticides such as organophosphates, carbamates, pyrethroids, and neonicotinoids often enter rivers, lakes, and ponds through runoff, leaching, and atmospheric deposition. Freshwater fishes, due to their constant exposure to waterborne contaminants, serve as sensitive indicators of ecosystem health. Among the primary mechanisms by which pesticides exert toxicity is the induction of **oxidative stress**, which results from an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system. Evaluating antioxidant status and oxidative stress biomarkers in freshwater fish provides crucial insights into sub-lethal effects of pesticides, early warning signs of ecological disturbance, and the health of aquatic ecosystems.

Oxidative Stress and Its Mechanisms in Fish

Oxidative stress is a condition where the production of ROS exceeds the capacity of the organism's antioxidant defenses, resulting in cellular and tissue damage. Pesticides contribute to oxidative stress by interfering with normal metabolic processes. Organophosphates and carbamates, for example, disrupt acetylcholinesterase activity and cellular respiration, leading to the accumulation of ROS. Pyrethroids, widely used for pest control, can induce lipid peroxidation of cellular membranes, generating additional ROS, while neonicotinoids impair mitochondrial function, further contributing to oxidative stress. These ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are highly reactive and can attack lipids, proteins, and nucleic acids. The resulting damage manifests as lipid peroxidation, protein oxidation, and DNA strand breaks, ultimately affecting fish survival and reproduction. Fish possess inherent cellular adaptation mechanisms, including antioxidant enzymes and repair systems, but chronic exposure to pesticides can overwhelm these defenses, leading to persistent oxidative damage.

Antioxidant Defense Systems in Freshwater Fish

Fish have evolved complex antioxidant defense systems to neutralize ROS and maintain cellular homeostasis. These defenses include both enzymatic and non-enzymatic components. Enzymatic antioxidants such as superoxide dismutase (SOD) play a crucial role in converting highly reactive superoxide radicals into hydrogen peroxide, which is less harmful. Catalase (CAT) further detoxifies hydrogen peroxide into water and oxygen, preventing the formation of even more reactive hydroxyl radicals. Glutathione peroxidase (GPx) reduces hydrogen peroxide and organic peroxides using reduced glutathione as a cofactor, protecting critical cellular components. Glutathione-S-transferase (GST) provides an additional detoxification mechanism by conjugating electrophilic pesticide metabolites with glutathione, facilitating their excretion.

Non-enzymatic antioxidants complement these defenses by directly scavenging free radicals. Reduced glutathione (GSH) maintains the redox balance within cells and acts as a substrate for GPx activity. Vitamins such as C and E prevent oxidative damage to proteins and lipids, while carotenoids and flavonoids stabilize cell membranes and enhance resistance to ROS. The efficiency of these antioxidant systems is influenced by species, age, nutritional status, and environmental conditions. Assessment of antioxidant enzyme activities and non-enzymatic components provides critical information on the physiological status of fish exposed to pesticide stress.

Oxidative Stress Biomarkers in Pesticide Exposure

Oxidative stress biomarkers serve as sensitive indicators of sub-lethal pesticide toxicity in fish. Lipid peroxidation, measured as malondialdehyde (MDA) content, is one of the most widely used biomarkers and reflects damage to cell membranes. Elevated MDA levels in gills, liver, and kidney tissues indicate that ROS generated by pesticides have disrupted membrane integrity, impairing essential physiological functions such as gas exchange and metabolism. Protein oxidation is another key biomarker, with increased protein carbonyl content indicating damage to structural and enzymatic proteins. Such modifications can reduce enzyme activity, destabilize structural proteins, and interfere with cellular signaling.

DNA damage markers, including strand breaks and chromosomal aberrations detected through comet assays and micronucleus tests, provide insight into the genotoxic effects of pesticide-induced oxidative stress. Changes in antioxidant enzyme activity, such as altered SOD, CAT, GPx, and GST levels, reveal the organism's compensatory response to ROS production and indicate whether the antioxidant defense system is overwhelmed. Tissue-specific responses are particularly informative, as liver, gills, and kidney are major sites of pesticide metabolism and ROS production. By examining these biomarkers collectively, researchers can gain a comprehensive understanding of the physiological and molecular stress experienced by freshwater fish under pesticide exposure.

Integrative Assessment Approaches

To fully understand the impact of pesticide pollution, oxidative stress biomarkers are increasingly evaluated in conjunction with other biochemical, molecular, and ecological indicators. Simultaneous assessment of ROS levels, antioxidant enzyme activity, gene expression of detoxification enzymes, and heat shock protein induction allows for a multi-layered understanding of sub-lethal toxicity. Correlating these biomarkers with histopathological observations, such as gill lamellar fusion, liver vacuolation, and kidney tubular degeneration, provides a mechanistic link between molecular changes and tissue-level damage.

Developing multi-biomarker indices by integrating various oxidative stress parameters allows for quantitative comparisons between different sites, species, or pesticide concentrations. These indices help identify trends in environmental stress and facilitate risk prioritization. Furthermore, linking biomarker responses to measured pesticide concentrations in water and sediment enables researchers to evaluate ecological relevance and determine safe exposure thresholds. This integrative approach bridges the gap between molecular biomarkers and ecosystem-level consequences, making it a powerful tool for environmental monitoring and management.

Applications and Case Studies

The application of oxidative stress biomarkers in freshwater fish has provided valuable insights into pesticide toxicity across diverse aquatic ecosystems. In agricultural landscapes, studies on common carp (*Cyprinus carpio*) exposed to organophosphate-contaminated runoff revealed elevated malondialdehyde levels, decreased SOD and CAT activity, and histopathological liver damage. These findings highlight the early sub-lethal effects of pesticide exposure that precede observable mortality. In industrial and urban water bodies, tilapia (*Oreochromis niloticus*) exposed to mixed pesticide contamination exhibited altered

GPx and GST activity, increased protein carbonylation, and DNA damage in gill and liver tissues, indicating both oxidative stress and genotoxicity.

From a regulatory perspective, oxidative stress biomarkers are increasingly used in environmental monitoring programs to evaluate water quality, assess sub-lethal pesticide toxicity, and guide mitigation strategies. These biomarkers provide early-warning signals that can prevent long-term ecological damage and support sustainable water management practices. The global applicability of this approach is evident from studies conducted in Asia, Europe, and North America, which demonstrate that oxidative stress biomarkers are a sensitive, reliable, and reproducible tool for assessing pesticide pollution.

Challenges and Future Directions

Despite their utility, oxidative stress biomarkers face several challenges. Species-specific variability in antioxidant responses and life-stage differences can complicate interpretation and standardization. In real-world scenarios, fish are exposed to complex mixtures of pesticides, which may produce additive, synergistic, or antagonistic effects, further complicating the assessment of oxidative stress.

Future research should focus on omics-based approaches, including transcriptomics, proteomics, and metabolomics, to identify novel biomarkers and elucidate molecular mechanisms underlying pesticide-induced oxidative stress. Predictive modeling that links biomarker changes to population and ecosystem-level effects can enhance ecological risk assessment. Finally, sustainable monitoring programs integrating oxidative stress biomarkers with water quality assessment, fish community surveys, and GIS-based mapping will improve environmental management and policymaking, ensuring the protection of freshwater biodiversity

Conclusion

Oxidative stress biomarkers and antioxidant status provide a sensitive and early-warning system for evaluating pesticide toxicity in freshwater fish. By monitoring enzymatic and non-enzymatic antioxidant defenses, lipid peroxidation, protein oxidation, and DNA damage, researchers can detect sub-lethal effects before population-level impacts occur. Integrating these biomarkers with histopathological observations, gene expression studies, and environmental measurements offers a comprehensive understanding of pesticide toxicity and its ecological implications. Adoption of such multi-tiered monitoring strategies is essential for safeguarding freshwater biodiversity, promoting sustainable pesticide use, and maintaining the health of aquatic ecosystems worldwide.

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