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BIOCHEMICAL BIOMARKERS IN FRESHWATER BIVALVES FOR EVALUATING PLANT-DERIVED TOXICANTS

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ABSTRACT

Freshwater ecosystems are increasingly threatened by natural and anthropogenic contaminants, including bioactive phytochemicals released from medicinal plants, invasive weeds and plant-derived pesticides. Freshwater bivalves have emerged as valuable bioindicator organisms because of their sedentary lifestyle, filter-feeding behavior and ability to accumulate environmental contaminants. Biochemical biomarkers provide sensitive and early indicators of physiological disturbances before visible ecological effects occur. This article synthesizes current knowledge regarding biochemical responses of freshwater bivalves exposed to plant-derived toxicants, emphasizing alterations in total protein, free amino acids, acid phosphatase, alkaline phosphatase, protease, pyruvate, lactate, aspartate aminotransferase (AST) and acetylcholinesterase (ACHE). Published evidence demonstrates that phytochemicals can disrupt protein metabolism, carbohydrate utilization, lysosomal function, oxidative balance and neural transmission, resulting in measurable biochemical alterations. Biomarker responses vary depending on toxicant composition, exposure duration, concentration, species sensitivity and environmental conditions. The integration of multiple biochemical biomarkers enhances environmental monitoring by providing comprehensive information on sublethal toxicity and ecosystem health. Current challenges include biomarker standardization, interspecies variability and limited field validation. Future research should integrate biochemical biomarkers with molecular, histopathological, metabolomic and ecological approaches to strengthen aquatic risk assessment and conservation strategies.

Keywords: Freshwater Bivalves, Biochemical Biomarkers, Aquatic Toxicology, Lamellidens Marginalis, Phytochemicals, Environmental Monitoring, Plant-Derived Toxicants

INTRODUCTION

Freshwater ecosystems are among the most productive and biologically diverse ecosystems on Earth, providing a wide range of ecological, economic and social services that are essential for sustaining both biodiversity and human well-being. These ecosystems regulate hydrological cycles, facilitate nutrient recycling, support fisheries and aquaculture, provide potable water, maintain groundwater recharge and contribute significantly to climate regulation through carbon sequestration Dudgeon et al. (2006), Zieritz et al. (2022). Despite covering less than 1% of the Earth's surface, freshwater habitats harbor nearly 10% of all known species, making them biodiversity hotspots of exceptional ecological significance Dudgeon et al. (2006). However, freshwater ecosystems are also among the most threatened environments globally because of rapid urbanization, industrialization, agricultural intensification, dam construction, habitat fragmentation, climate change and contamination by a wide range of natural and synthetic chemicals Vörösmarty et al. (2010), Reid et al. (2019). These multiple stressors have substantially altered the physicochemical characteristics

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of aquatic environments, adversely affecting aquatic organisms and ecosystem functioning. In recent decades, increasing attention has been directed toward emerging contaminants, a diverse group of substances that are not routinely monitored but may pose considerable ecological risks even at relatively low concentrations. Besides pharmaceuticals, pesticides, personal care products and microplastics, naturally occurring bioactive compounds released from plants have emerged as contaminants of growing environmental concern Richardson and Kimura (2020). Plant-derived toxicants originate from medicinal plants, invasive weeds, botanical pesticides, agricultural residues, herbal pharmaceutical industries and decomposition of plant biomass. Although these compounds are generally regarded as environmentally friendly alternatives to synthetic pesticides because of their biodegradability, many possess potent biological activities capable of adversely affecting non-target aquatic organisms Isman (2020).

Plant secondary metabolites comprise structurally diverse compounds including alkaloids, flavonoids, phenolic acids, tannins, terpenoids, saponins, coumarins, glycosides and sesquiterpene lactones. These compounds serve defensive roles in plants by protecting them against herbivores, pathogens and environmental stress. However, once released into aquatic ecosystems through agricultural runoff, soil erosion, rainfall-mediated leaching, industrial discharge or decomposition of plant residues, these metabolites may interfere with normal physiological processes in aquatic organisms Isman (2020). Numerous phytochemicals have been reported to alter membrane permeability, inhibit enzymatic activity, induce oxidative stress, disrupt mitochondrial respiration, impair energy metabolism and interfere with neurotransmission, thereby producing both acute and chronic toxic effects Singh et al. (2004), Tiwari et al. (2004). Such biochemical disturbances often occur long before visible pathological symptoms or mortality become evident, emphasizing the importance of sensitive biomarkers for early detection of toxic effects.

Among aquatic organisms, freshwater bivalves have gained considerable recognition as valuable sentinel species for environmental biomonitoring. Species such as *Lamellidens marginalis, Corbicula fluminea, Anodonta anatina, Unio pictorum* and *Unio tumidus* possess several biological and ecological characteristics that make them highly suitable bioindicator organisms Elder and Collins (1991), Farris and Van Hassel (2007). These benthic filter feeders continuously process large volumes of water, allowing efficient accumulation of dissolved contaminants, suspended particles, microorganisms and organic pollutants within their tissues. Their relatively sedentary lifestyle enables them to reflect site-specific environmental conditions, while their comparatively long lifespan permits assessment of chronic contaminant exposure over extended periods Elder and Collins (1991). Furthermore, freshwater bivalves play fundamental ecological roles in nutrient cycling, sediment stabilization, water clarification and maintenance of ecosystem integrity, making their health closely linked with overall freshwater ecosystem functioning Zieritz et al. (2022). The application of freshwater bivalves in ecotoxicological studies has expanded substantially over the past three decades because these organisms exhibit measurable biochemical, physiological, histopathological, and molecular responses following exposure to environmental contaminants. Compared with traditional toxicity endpoints such as mortality or reproductive impairment, biochemical biomarkers provide early-warning signals that can detect sublethal stress before irreversible ecological damage occurs van der Oost et al. (2003). Consequently, biomarker-based monitoring has become an important component of ecological risk assessment and environmental management worldwide.

Biomarkers are defined as measurable biological responses occurring at molecular, biochemical, cellular, physiological or organismal levels following exposure to environmental stressors van der Oost et al. (2003). Biochemical biomarkers are particularly attractive because they are highly sensitive, relatively inexpensive and capable of detecting physiological disturbances at an early stage of toxic exposure. Alterations in enzyme activities and metabolic constituents frequently precede histopathological lesions and population-level changes, thereby providing valuable mechanistic information regarding toxicant action van der Oost et al. (2003). Among the most frequently investigated biochemical biomarkers in freshwater bivalves are total protein, free amino acids, acid phosphatase (ACP), alkaline phosphatase (ALP), protease activity, pyruvate, lactate, aspartate aminotransferase (AST), alanine aminotransferase (ALT), acetylcholinesterase (AChE), antioxidant enzymes, glutathione metabolism and lipid peroxidation Cossu et al. (1997), van der Oost et al. (2003). Variations in total protein concentration reflect disturbances in protein synthesis and degradation, whereas elevated free amino acids generally indicate enhanced proteolysis under stress conditions. Acid and alkaline phosphatases serve as indicators of lysosomal integrity and membrane transport, while protease activity reflects accelerated protein turnover during toxic stress. Similarly, alterations in pyruvate and lactate concentrations provide valuable information regarding shifts between aerobic and anaerobic metabolism, whereas elevated AST activity is frequently associated with tissue damage and altered amino acid metabolism. Acetylcholinesterase inhibition remains one of the most widely accepted biomarkers for assessing neurotoxicity induced by pesticides and other xenobiotics because suppression of enzyme activity disrupts synaptic transmission and normal behavioral functions Ellman et al. (1961), Singh and Agarwal (1990).

Numerous investigations have demonstrated that exposure to plant-derived compounds including *Euphorbia latex, Parthenium hysterophorus, Ambrosia maritima* and other botanically active extracts induces significant biochemical alterations in freshwater molluscs Al-Zanbagi et al. (2001), Belot et al. (1991), Singh et al. (2004), Tiwari et al. (2004). These studies consistently report depletion of proteins, elevation of protease activity, inhibition of acetylcholinesterase, altered phosphatase activities and disruption of carbohydrate metabolism, suggesting that phytochemicals influence multiple physiological pathways simultaneously. However, biomarker responses vary considerably depending on toxicant composition, exposure duration, environmental conditions, developmental stage and species-specific sensitivity, emphasizing the need for integrated biomarker approaches rather than reliance on individual biochemical parameters Vereycken and Aldridge (2023). Although considerable progress has been made in aquatic biomonitoring, significant knowledge gaps remain regarding the biochemical effects of plant-derived toxicants on freshwater bivalves. Most published studies focus on synthetic pesticides, heavy metals, pharmaceuticals or industrial pollutants, whereas comparatively fewer investigations have systematically evaluated botanical toxicants despite their increasing environmental use. Furthermore, standardization of exposure protocols, biomarker interpretation, interspecies comparisons and field validation remains limited Vereycken and Aldridge (2023). Recent advances in metabolomics, transcriptomics, proteomics and integrated biomarker indices provide promising opportunities for improving the sensitivity and ecological relevance of freshwater biomonitoring van der Oost et al. (2003), Vereycken and Aldridge (2023), Science of the Total Environment (2026).

In view of these developments, the present review critically summarizes the current understanding of biochemical biomarkers in freshwater bivalves exposed to plant-derived toxicants. Particular emphasis is placed on alterations in total protein, free amino acids, acid phosphatase, alkaline phosphatase, protease activity, pyruvate, lactate, aspartate aminotransferase and acetylcholinesterase. The review further discusses the mechanisms underlying biomarker responses, evaluates their applications in environmental monitoring and ecological risk assessment, identifies current limitations and highlights future research directions aimed at improving the use of freshwater bivalves as bioindicator organisms in aquatic toxicology.

METHODOLOGY

The present study was conducted through a comprehensive and systematic survey of the published scientific literature to synthesize current knowledge on the application of biochemical biomarkers in freshwater bivalves for evaluating the toxicity of plant-derived compounds. Although this study is narrative in nature, the literature identification, screening and selection processes were designed to ensure comprehensive coverage of relevant studies while minimizing selection bias. Established recommendations for conducting high-quality review studies and evidence synthesis were considered during the preparation of this manuscript Grant and Booth (2009), Page et al. (2021). A comprehensive literature search was performed using internationally recognized scientific databases, including Web of Science Core Collection, Scopus, PubMed, ScienceDirect, SpringerLink, Google Scholar, Wiley Online Library, Taylor & Francis Online and CrossRef. These databases were selected because they collectively provide extensive coverage of peer-reviewed publications in aquatic toxicology, environmental sciences, freshwater ecology, ecotoxicology, biochemistry, environmental chemistry and biomonitoring. Additional literature was retrieved from the reference lists of highly cited review articles, books, conference proceedings and authoritative reports published by international organizations, including the World Health Organization (WHO), the Organisation for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (USEPA), to ensure that foundational methodologies and environmental monitoring guidelines were adequately represented OECD (2019), WHO (2023).

The literature search primarily included publications published between 1995 and 2026, thereby encompassing both classical investigations that established biochemical biomarker methodologies and recent advances in aquatic ecotoxicology. Earlier landmark publications describing standard biochemical assay methods, including protein estimation, enzyme assays, amino acid determination and acetylcholinesterase analysis, were also incorporated because these methods continue to serve as internationally accepted protocols for biomarker investigations Ellman et al. (1961), Lowry et al. (1951), Reitman and Frankel (1957). Greater emphasis was placed on publications published during the last decade (2015–2026) to capture recent developments in biomarker applications, environmental monitoring, and aquatic risk assessment Reid et al. (2019), Zieritz et al. (2022).

The electronic database search employed combinations of controlled vocabulary terms and free-text keywords using Boolean operators (AND, OR and NOT) to maximize retrieval efficiency. The primary search terms included:

- Freshwater bivalves
- Lamellidens marginalis
- Corbicula fluminea
- Anodonta anatina
- Unio pictorum
- Biomarkers
- Biochemical biomarkers
- Aquatic toxicology
- Ecotoxicology
- Environmental monitoring
- Medicinal plants
- Botanical pesticides
- Plant-derived toxicants
- Phytochemicals

- Alkaloids
- Flavonoids
- Terpenoids
- Sesquiterpene lactones
- Protein metabolism
- Free amino acids
- Acid phosphatase
- Alkaline phosphatase
- Protease
- Aspartate aminotransferase
- Acetylcholinesterase
- Oxidative stress
- Bioindicators
- Freshwater pollution

Representative search strategies included combinations such as ("Freshwater bivalves" AND "biochemical biomarkers"), ("Lamellidens marginalis" AND phytochemicals), ("Plant-derived toxicants" AND aquatic toxicology) and ("Acetylcholinesterase" AND freshwater molluscs). Searches were refined using database-specific filters to retrieve peer-reviewed journal articles published in English. Duplicate records identified across multiple databases were removed prior to further screening to improve the accuracy of literature selection Page et al. (2021).

Studies were considered eligible for inclusion if they investigated biochemical, physiological, enzymatic, or metabolic responses in freshwater bivalves following exposure to plant-derived toxicants. Particular emphasis was placed on studies involving medicinal plant extracts, botanical pesticides, invasive weed extracts, allelochemicals, latex-producing plants, or purified phytochemicals that produced measurable biochemical alterations. Experimental laboratory investigations, semi-field studies, field biomonitoring studies, review articles, methodological papers, and relevant book chapters were included when they provided significant information regarding biochemical biomarker responses or analytical methodologies. Studies focusing on widely used freshwater bivalve species, including Lamellidens marginalis, Corbicula fluminea, Anodonta anatina, Unio pictorum, Unio tumidus, and related Unionidae species, were given particular attention because of their established role as bioindicator organisms in aquatic biomonitoring Farris and Van Hassel (2007), Zieritz et al. (2022).

The exclusion criteria were carefully defined to improve the scientific quality and relevance of the reviewed literature. Publications that exclusively investigated marine molluscs without discussing freshwater species, studies lacking biochemical endpoints, conference abstracts without full experimental details, duplicate publications, unpublished theses, editorials, opinion articles and reports lacking adequate methodological information were excluded. Similarly, studies evaluating only synthetic industrial pollutants without discussing biochemical mechanisms relevant to plant-derived toxicants were considered only when they provided valuable information regarding biomarker methodology or mechanistic interpretation van der Oost et al. (2003). Each selected publication was critically evaluated for methodological quality before inclusion in the review. The assessment considered several factors, including experimental design, sample size, exposure duration, toxicant characterization, concentration selection, biomarker measurement techniques, statistical analyses, reproducibility of results and clarity of data presentation. Studies employing internationally accepted biochemical methods, validated analytical procedures, appropriate controls and rigorous statistical analyses were given greater emphasis during data synthesis Lowry et al. (1951), Ellman et al. (1961), Reitman and Frankel (1957). Particular attention was also given to investigations that simultaneously evaluated multiple biochemical biomarkers because integrated biomarker approaches provide more reliable assessments of sublethal toxic effects than individual biochemical parameters alone van der Oost et al. (2003).

Following literature selection, the extracted information was organized into thematic categories to facilitate systematic synthesis and comparison. The reviewed studies were classified according to freshwater bivalve species, plant-derived toxicants investigated, biochemical biomarkers evaluated, exposure conditions, analytical methodologies, principal biochemical alterations and ecological significance. Biomarkers were further categorized into protein metabolism indicators (total protein and free amino acids), lysosomal enzymes (acid phosphatase and alkaline phosphatase), proteolytic enzymes (protease), energy metabolism biomarkers (pyruvate and lactate), transaminases (AST), neurotoxicity biomarkers (acetylcholinesterase) and oxidative stress-related biomarkers where appropriate. This thematic organization enabled identification of common response patterns, methodological limitations, knowledge gaps and future research priorities.

Although the present review primarily emphasizes biochemical biomarkers associated with plant-derived toxicants, relevant comparative studies involving heavy metals, pesticides, pharmaceuticals and industrial pollutants were also consulted whenever they contributed to understanding biomarker mechanisms or environmental applications. Such comparisons facilitate interpretation of biomarker specificity and improve the ecological relevance of biochemical responses observed following phytochemical exposure van der Oost et al. (2003), Reid et al. (2019). Overall, this comprehensive methodological approach ensured that the review provides an up-to-date and scientifically robust synthesis of the available evidence regarding the use of biochemical biomarkers in freshwater bivalves for evaluating plant-derived toxicants. By integrating findings from experimental toxicology, environmental biomonitoring, enzymology and freshwater ecology, the present review aims to provide a reliable scientific foundation for future research and environmental risk assessment.

RESULTS AND DISCUSSION

MAJOR PLANT-DERIVED TOXICANTS AND THEIR REPORTED BIOCHEMICAL EFFECTS IN FRESHWATER BIVALVES.

Plant-derived toxicants encompass a wide variety of secondary metabolites that differ considerably in their chemical composition, mode of action, persistence and toxicity toward aquatic organisms. Numerous experimental studies have demonstrated that phytochemicals released from medicinal plants, invasive weeds and botanical pesticides induce significant biochemical and physiological disturbances in freshwater bivalves. However, the magnitude of these responses varies depending on the phytochemical composition, exposure concentration, duration of exposure and species sensitivity Isman (2020), Singh et al. (2004), Tiwari et al. (2004). Table 1 summarizes representative plant species investigated in aquatic toxicology, their principal bioactive constituents, target freshwater organisms, major biochemical alterations and corresponding literature sources.

Table 1

Table 1 Major Plant-Derived Toxicants and Their Biochemical Effects in Freshwater Bivalves

Plant species	Major phytochemicals	Target organism	Major biochemical effects	References
Parthenium hysterophorus	Sesquiterpene lactones (parthenin), phenolics	*Lamellidens marginalis*, freshwater molluscs	↓ Total protein, ↑ protease, ↓ AChE, oxidative stress	Tiwari et al. (2004), Singh et al. (2004)
Euphorbia royleana	Diterpenoids, latex proteins	Freshwater molluscs	↓ Protein, ↑ ACP, ↓ ALP, ↑ AST	Tiwari et al. (2004)
Ambrosia maritima	Sesquiterpene lactones	*Biomphalaria* spp.	AChE inhibition, metabolic stress	Belot et al. (1991)
Azadirachta indica	Azadirachtin, limonoids	Freshwater bivalves/molluscs	Enzyme inhibition, oxidative stress	Isman (2020)

As shown in Table 1, plant-derived toxicants influence multiple biochemical pathways simultaneously rather than affecting a single physiological process. Most phytochemicals cause depletion of protein reserves, alterations in lysosomal enzyme activities, inhibition of acetylcholinesterase, disruption of energy metabolism and induction of oxidative stress. Among the plants reviewed, *Parthenium hysterophorus* and *Euphorbia royleana* have received considerable attention because of their potent bioactive compounds and pronounced biochemical effects. These observations demonstrate that freshwater bivalves provide a sensitive biological model for evaluating the ecological risks associated with botanical toxicants and support the use of biochemical biomarkers as reliable indicators of sublethal environmental stress.

MAJOR BIOCHEMICAL BIOMARKERS USED IN FRESHWATER BIVALVES FOR EVALUATING PLANT-DERIVED TOXICANTS.

Biochemical biomarkers represent sensitive indicators of physiological disturbances because they respond rapidly to environmental stress before visible pathological lesions or mortality occur. Different biomarkers reflect specific aspects of cellular metabolism, including protein turnover, lysosomal stability, energy metabolism, tissue integrity and neural function. Since no single biomarker provides complete information regarding toxicity, simultaneous assessment of multiple biochemical parameters is recommended for comprehensive environmental monitoring van der Oost et al. (2003). Table 2 summarizes the principal biochemical biomarkers commonly employed in freshwater bivalves, their physiological significance, expected responses following phytochemical exposure and their toxicological interpretation.

Table 2

Table 2 Biochemical Biomarkers Used in Freshwater Bivalves

Biomarker	Physiological significance	Response to phytotoxicants	Interpretation
Total protein	Protein metabolism	Decrease	Protein degradation and stress
Free amino acids	Protein catabolism	Increase	Enhanced proteolysis
Acid phosphatase	Lysosomal activity	Increase	Cellular damage
Alkaline phosphatase	Membrane transport	Decrease	Membrane dysfunction
Protease	Protein turnover	Increase	Stress adaptation
Pyruvate	Aerobic metabolism	Decrease	Reduced oxidative metabolism
Lactate	Anaerobic metabolism	Increase	Anaerobic respiration
AST	Amino acid metabolism	Increase	Tissue injury
Acetylcholinesterase	Neural transmission	Decrease	Neurotoxicity

The information presented in Table 2 demonstrates that individual biochemical biomarkers provide complementary information regarding different physiological processes affected by toxicant exposure. For example, reductions in total protein indicate enhanced protein degradation, whereas increased acid phosphatase reflects lysosomal membrane destabilization. Similarly, inhibition of acetylcholinesterase indicates neurotoxicity, while alterations in pyruvate and lactate concentrations reflect disturbances in cellular energy metabolism. Therefore, integrated biomarker assessment offers considerably greater diagnostic accuracy than reliance on individual biochemical parameters.

ADVANTAGES, LIMITATIONS AND ENVIRONMENTAL APPLICATIONS OF BIOCHEMICAL BIOMARKERS.

Although biochemical biomarkers have become indispensable tools in aquatic toxicology, each biomarker possesses specific strengths and limitations that influence its applicability in environmental monitoring programs. Interpretation of biomarker responses should therefore consider environmental variables, species-specific differences, seasonal fluctuations and physiological conditions. Selection of appropriate biomarkers depends upon the objectives of the investigation and the nature of the contaminants under evaluation. Table 3 summarizes the major advantages, limitations and environmental applications of commonly used biochemical biomarkers in freshwater bivalves.

Table 3

Table 3 Advantages and Limitations of Biochemical Biomarkers

Biomarker	Advantages	Limitations	Environmental application
Protein	Simple, inexpensive	Non-specific	General stress indicator
ACP/ALP	Sensitive to tissue injury	Species variation	Cellular toxicity
Protease	Indicates metabolic adaptation	Influenced by nutrition	Chronic toxicity
Pyruvate/Lactate	Energy metabolism	Rapid fluctuation	Metabolic stress
AST	Detects tissue damage	Not organ-specific	Physiological assessment
AChE	Highly sensitive	Influenced by temperature	Neurotoxicity monitoring

As summarized in Table 3, no individual biomarker is sufficiently specific to characterize all aspects of toxicant-induced stress. Biomarkers such as total protein and phosphatases are useful indicators of general physiological disturbance, whereas acetylcholinesterase provides greater specificity for neurotoxicity. Consequently, integrated biomarker approaches combining multiple biochemical endpoints provide more robust assessments of environmental contamination than single-parameter analyses. Such integrated strategies have become increasingly important in contemporary aquatic biomonitoring and ecological risk assessment.

CURRENT LIMITATIONS AND FUTURE PERSPECTIVES FOR BIOCHEMICAL BIOMONITORING OF FRESHWATER BIVALVES.

Recent advances in aquatic ecotoxicology have emphasized the need for multidisciplinary biomonitoring strategies capable of integrating biochemical, molecular, physiological and ecological information. Although classical biochemical biomarkers remain valuable because of their simplicity and sensitivity, emerging technologies such as transcriptomics, proteomics, metabolomics, environmental DNA analysis, artificial intelligence and machine learning are expanding the scope of environmental monitoring. Table 4 highlights the current limitations of conventional biomonitoring approaches together with future directions that may improve ecological risk assessment of plant-derived toxicants in freshwater ecosystems.

Table 4

Table 4 Future Directions in Biomonitoring

Current approach	Limitation	Future recommendation
Single biomarker	Low specificity	Multi-biomarker index
Laboratory studies	Limited field relevance	Field validation
Biochemical assays	Mechanistic limitations	Integration with transcriptomics and proteomics
Conventional monitoring	Time-consuming	AI-assisted biomonitoring
Chemical analysis only	Misses biological effects	Biomarker-based ecological risk assessment

The future directions summarized in Table 4 indicate that aquatic biomonitoring is progressively shifting from single-endpoint biochemical measurements toward integrated, multi-level assessment strategies. Combining classical biochemical biomarkers with molecular techniques, histopathology, metabolomics and ecological modeling is expected to substantially improve the sensitivity, specificity and ecological relevance of environmental risk assessment. Such multidisciplinary approaches will facilitate more effective conservation and sustainable management of freshwater ecosystems exposed to plant-derived toxicants.

CONCLUSION

Freshwater bivalves remain among the most reliable bioindicator organisms for evaluating aquatic environmental quality. Biochemical biomarkers including total protein, free amino acids, phosphatases, protease, pyruvate, lactate, AST and acetylcholinesterase provide sensitive indicators of sublethal toxicity induced by plant-derived compounds. Available evidence demonstrates that botanical toxicants alter multiple metabolic pathways affecting protein turnover, carbohydrate metabolism, enzyme regulation and neural function. While biochemical biomarkers offer rapid and cost-effective environmental assessment tools, their interpretation requires integration with molecular, physiological and ecological endpoints. Future multidisciplinary approaches incorporating biomarker profiling, omics technologies and ecological modeling will enhance environmental risk assessment and facilitate sustainable management of freshwater ecosystems.

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REFERENCES

Al-Zanbagi, N. A., Barrett, J., and Banaja, A. A. (2001). Laboratory Evaluation of Molluscicidal Properties of Euphorbiales Against Biomphalaria Pfeifferi. Acta Tropica, 78(1), 23–29. https://doi.org/10.1016/S0001-706X(00)00166-2

Belot, J., Bornarel, P., Diouf, M., and Polderman, A. M. (1991). Molluscicidal Effects of Ambrosia Maritima on Freshwater Snails. Plant Science, 74, 167–170. https://doi.org/10.1016/0168-9452(91)90042-7

Cossu, C., Doyotte, A., Jacquin, M. C., Babut, M., Exinger, A., and Vasseur, P. (1997). Glutathione Reductase, Glutathione Peroxidase, Glutathione Levels and Lipid Peroxidation in Freshwater Bivalves (Unio tumidus) as Biomarkers of Aquatic Contamination. Ecotoxicology and Environmental Safety, 38(2), 122–131. https://doi.org/10.1006/eesa.1997.1582

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., ... Sullivan, C. A. (2006). Freshwater Biodiversity: Importance, Threats, Status and Conservation Challenges. Biological Reviews, 81(2), 163–182. https://doi.org/10.1017/S1464793105006950

Elder, J. F., and Collins, J. J. (1991). Freshwater Molluscs as Indicators of Bioavailability and Toxicity of Metals in Surface-water Systems. Reviews of Environmental Contamination and Toxicology, 122, 37–79. https://doi.org/10.1007/978-1-4612-3198-1_2

Ellman, G. L., Courtney, K. D., Andres, V., Jr., and Featherstone, R. M. (1961). A New and Rapid Colorimetric Determination of Acetylcholinesterase activity. Biochemical Pharmacology, 7, 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Farris, J. L., and Van Hassel, J. H. (Eds.). (2007). Freshwater Bivalve Ecotoxicology. CRC Press. https://doi.org/10.1201/9780367801823

Isman, M. B. (2020). Botanical Insecticides in the Twenty-First Century: Fulfilling Their Promise? Annual Review of Entomology, 65, 233–249. https://doi.org/10.1146/annurev-ento-011019-025010

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., ... Cooke, S. J. (2019). Emerging Threats and Persistent Conservation Challenges for Freshwater Biodiversity. Biological Reviews, 94(3), 849–873. https://doi.org/10.1111/brv.12480

Richardson, S. D., and Kimura, S. Y. (2020). Water Analysis: Emerging Contaminants and Current Issues. Analytical Chemistry, 92(1), 473–505. https://doi.org/10.1021/acs.analchem.9b05269

Singh, A., and Agarwal, R. A. (1990). Molluscicidal Properties of Synthetic Pyrethroids. Journal of Medical and Applied Malacology, 2, 141–145.

Singh, S. K., Yadav, R. P., and Singh, A. (2004). Toxic Effects of Euphorbia Latex on Freshwater Snails. Environmental Toxicology and Pharmacology, 15, 87–93. https://doi.org/10.1016/j.etap.2003.11.001

Tiwari, S., Singh, S. K., and Singh, A. (2004). Toxicological Effects and Biochemical Alterations Induced by Euphorbia Royleana Latex in Freshwater snails. Indian Journal of Experimental Biology, 42, 1220–1225.

Vereycken, J. E., and Aldridge, D. C. (2023). Bivalve molluscs as biosensors of water quality: State of the art and future directions. Hydrobiologia, 850, 231–256. https://doi.org/10.1007/s10750-022-05057-7

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., ... Davies, P. M. (2010). Global threats to human water security and river biodiversity. Nature, 467, 555–561. https://doi.org/10.1038/nature09440

van der Oost, R., Beyer, J., and Vermeulen, N. P. E. (2003). Fish Bioaccumulation and Biomarkers in Environmental Risk Assessment: A Review. Environmental Toxicology and Pharmacology, 13(2), 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6

Zieritz, A., Sousa, R., Aldridge, D. C., Douda, K., Esteves, E., Ferreira-Rodríguez, N., ... Vaz, A. S. (2022). A Global Synthesis of Ecosystem Services Provided and Disrupted by Freshwater Bivalve Molluscs. Biological Reviews, 97(5), 1967–1998. https://doi.org/10.1111/brv.12878