Frontiers in Environmental Microbiology
2026, Vol. 12, No. 3, pp. 31–42
https://doi.org/10.11648/j.fem.20261203.11

Review Article

Biomarkers as Indicators of Environmental Stress in Animals

Prakarshi Kaushik[1], Bhawna Singh[1], Garima Supyal[1], Deepak Malkani[1], Pankaj Bhatt[1], Seeta Dewali[1], Netra Pal Sharma[1,*], Deepak Chandra Melkani[1], Satpal Singh Bisht[2]

[1]Dev Singh Bisht Campus, Kumaun University, Uttarakhand, India

[2]Soban Singh Jeena University, Almora, Uttarakhand, India

Abstract

Environmental stress arising from climate change, habitat loss, pollution, infectious agents, and human activities represents a growing threat to animal health in both terrestrial and aquatic ecosystems. Factors such as temperature fluctuations, ocean acidification, heavy metals, pesticides, and emerging pathogens can disturb physiological balance, weaken immune responses, and negatively affect growth and reproduction. Conventional health assessment methods often identify problems only after visible symptoms develop, highlighting the importance of early and sensitive detection tools. In this context, biomarkers serve as reliable early indicators by revealing subtle biological changes at molecular, cellular, biochemical, and physiological levels before clinical damage becomes apparent. This review compiles and evaluates major categories of biomarkers used in environmental health studies. Among these are signs of oxidative stress (like lipid peroxidation and antioxidant enzyme activity), stress and endocrine hormones, immune-related parameters, genotoxicity markers (including DNA damage assays), metabolic enzymes, and behavioral responses. Their relevance across diverse animal groups, including wildlife, livestock, and aquatic sentinel species, is discussed along with their advantages and limitations in ecological risk assessment. Furthermore, the review explores recent advances such as omics-based technologies, non-invasive sampling strategies, and integrated multi-biomarker approaches, which offer promising tools for improving environmental monitoring, conservation planning, and predictive assessments of animal health under changing environmental conditions.

Keywords

Environmental Stress, Biological Markers, Animal Health, Environmental Toxicology, Oxidative Damage, Biological Bioindicators

1. Introduction

Environmental stress encompasses any external factor—physical, chemical, or biological—that disrupts an animal's normal internal balance, or homeostasis. Common stressors include extreme temperatures, environmental pollutants, habitat degradation, infectious agents, radiation, and other anthropogenic activities. Prolonged or intense exposure to these

*Correspondence: Netra Pal Sharma (netrapal53@gmail.com)

Received: 22 April 2026; **Accepted:** 3 May 2026; **Published:** 17 August 2026

stressors can impair physiological processes, negatively affecting growth, reproduction, immune function, and overall survival, ultimately influencing population stability and ecosystem health [1-3].

Early detection of stress responses is essential to prevent long-term or irreversible damage. Subtle physiological, biochemical, and molecular changes often appear before overt symptoms or mortality, providing an opportunity for timely management, effective conservation measures, and accurate ecological risk assessment. This is particularly important in wildlife conservation, sustainable livestock management, and monitoring aquatic ecosystems [4, 5].

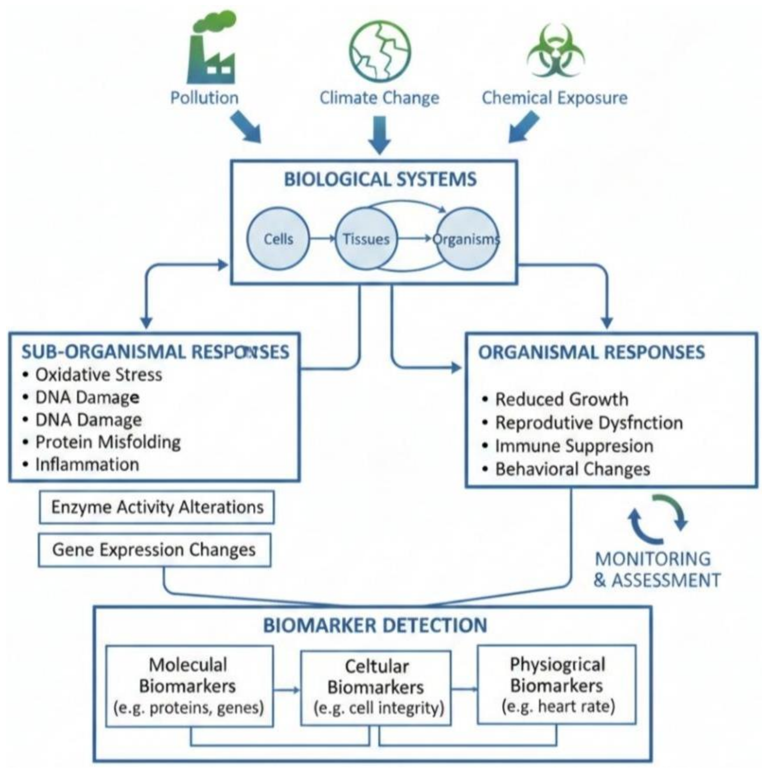

Figure 1. *Conceptual overview of environmental stressors and biomarker responses.*

Biomarkers offer significant advantages over traditional monitoring approaches, which typically rely on observable symptoms, population surveys, or habitat assessments. By measuring molecular, cellular, or biochemical responses, biomarkers can detect sub-lethal effects and provide sensitive, specific, and quantifiable information about environmental stress. Many biomarkers can be assessed using non-invasive methods, allowing repeated measurements in the same individuals, which is highly valuable for longitudinal studies and environmental monitoring programs [6-8].

This review aims to summarize the major categories of biomarkers used to assess environmental stress in animals, examine their applications and limitations, and explore recent advancements that improve early detection and ecological risk assessment under changing environmental conditions.

2. Environmental Stressors Affecting Animals

Environmental stressors are external factors that disturb the normal physiological balance, behavior, reproduction, and survival of animals. These stressors may be chemical, physical, biological, or ecological in origin and often interact with each other to intensify their impacts.

2.1. Chemical Stressors

Heavy metals- Lead, mercury, cadmium, and arsenic are examples of heavy metals that are persistent contaminants that stay in ecosystems for extended periods of time. These chemicals build up and become more concentrated in animal tissues throughout the food chain (bioaccumulation and biomagnification). When heavy metals are present, it can cause neurological damage, reproductive impairment, immune suppression, and oxidative stress in wildlife [9, 10]. Aquatic organisms and birds are particularly vulnerable to mercury contamination.

Pesticides- In agriculture, pesticides such as carbamates, organophosphates, and neonicotinoids are widely used to manage pests. However, they also negatively affect non-target species. These chemicals may disrupt endocrine function, impair nervous system activity, and reduce fertility and survival rates. Neonicotinoids have been strongly linked to pollinator declines and behavioral disturbances in insects [11]. Additionally, pesticide exposure has been shown to alter hormonal systems and immune responses in amphibians [12].

Industrial pollutants- Soil, water, and air are contaminated by industrial substances such dioxins, petroleum hydrocarbons, and polychlorinated biphenyls (PCBs). Because of their persistence, they accumulate in animal tissues and may cause cancer, developmental abnormalities, and immune dysfunction [13]. Major pollution events, such as oil spills, have demonstrated long-term ecological damage and population-level effects on marine species [14].

2.2. Physical Stressors

Temperature extremes- Rising global temperatures and increased climate variability expose animals to heat and cold stress beyond their tolerance limits. Extreme temperatures can disturb metabolism, decrease reproductive success, and shift species distributions [15]. Heatwaves, in particular, have resulted in mass mortality events among birds and marine organisms [16].

Noise- Human-generated noise from transportation systems, industrial activities, and urban expansion interferes with animal communication, orientation, and predator–prey interactions. Chronic noise exposure can elevate stress hormone levels and reduce breeding success [17]. Marine mammals are especially affected, as underwater noise disrupts their echolocation and migration behavior.

Radiation- Exposure to radiation from natural or anthropogenic sources can lead to genetic mutations, reproductive disorders, and increased mortality. Studies following the Chernobyl nuclear accident reported higher mutation rates and population declines in wildlife inhabiting contaminated areas [18]. Even prolonged low-dose radiation may weaken immune responses and impair development.

2.3. Biological and Ecological Stressors

Pathogens- Infectious agents such as bacteria, viruses, fungi, and parasites act as biological stressors in animal populations. Environmental changes, including climate change and habitat disturbance, can increase susceptibility to diseases and alter host–pathogen relationships. Global amphibian declines, for example, have been strongly associated with chytrid fungal infections [19]. Disease outbreaks may significantly reduce biodiversity and destabilize ecosystems.

Habitat degradation- Habitat destruction and fragmentation caused by deforestation, urbanization, and agricultural expansion limit access to food, shelter, and breeding sites. Such degradation often leads to population declines and increased vulnerability to additional stressors [20]. Fragmented habitats also restrict gene flow between populations, increasing the risk of inbreeding and long-term extinction.

Table 1. *Major environmental stressors and their sources.*

Stressor Type	Examples	Major Sources	References
Chemical Stressors	Heavy metals (Pb, Hg, Cd), pesticides (organophosphates, neonicotinoids), industrial pollutants (PCBs, dioxins, hydrocarbons)	Mining and smelting; agricultural runoff; industrial discharge; oil spills; fossil fuel combustion	[9-11, 13, 14]
Physical Stressors	Temperature extremes, anthropogenic noise, ionizing radiation	Climate change and heatwaves; transportation and urbanization; industrial machinery; nuclear accidents	[15-18]
Biological Stressors	Pathogens (fungi, bacteria, viruses), parasitic infections	Environmental disturbance; climate change; wildlife trade; increased host–pathogen interactions	[19]
Ecological Stressors	Habitat degradation, fragmentation, biodiversity loss	Deforestation; agricultural expansion; urban development; infrastructure projects	[20]

3. Concept and Classification of Biomarkers

Biomarkers are measurable indicators that reflect biological processes, the effects of environmental exposures, or an organism's susceptibility to stressors. They are frequently employed in ecological and environmental toxicology research to identify early indicators of animal stress or injury.

3.1. Definition of Biomarkers

Biomarkers are biological measurements that indicate exposure to environmental stressors, the effects of that exposure, or an organism's inherent sensitivity to stress. They can be detected at molecular, cellular, physiological, or behavioral levels and are used to identify early biological changes before clinical or population-level symptoms become apparent [6, 7, 21-24].

3.2. Characteristics of Ideal Biomarkers

An effective biomarker should:

1) Be specific to the stressor or biological effect of interest.
2) Demonstrate high sensitivity, detecting changes at low levels of exposure.
3) Be reliably measurable with consistent laboratory or field methods.
4) Allow non-lethal sampling, when possible.
5) Have ecological relevance, reflecting meaningful organismal or population responses [6, 8, 25-27].

3.3. Biomarkers' Classification

Based on what they show, biomarkers can be divided into three main categories:

3.3.1. Biomarkers of Exposure

These markers reveal that an organism has encountered a particular environmental stressor, such as a chemical pollutant, heavy metal, or endocrine disruptor. Examples include contaminant residues in tissues, induction of detoxification enzymes, and increased binding proteins like metallothioneins [6, 28].

3.3.2. Biomarkers of Effect

Biomarkers of effect reflect biological responses that result from exposure and often relate to impaired physiological function. These include oxidative stress markers, enzyme activity changes, DNA damage, endocrine disruption, and lowered immune competence [8, 29-31].

3.3.3. Biomarkers of Susceptibility

These indicators signal an organism's inherent potential to respond adversely to stressors. They include genetic polymorphisms in detoxification enzymes, differences in receptor sensitivity, and other physiological or developmental traits that make some individuals or species more vulnerable than others [22, 23, 27].

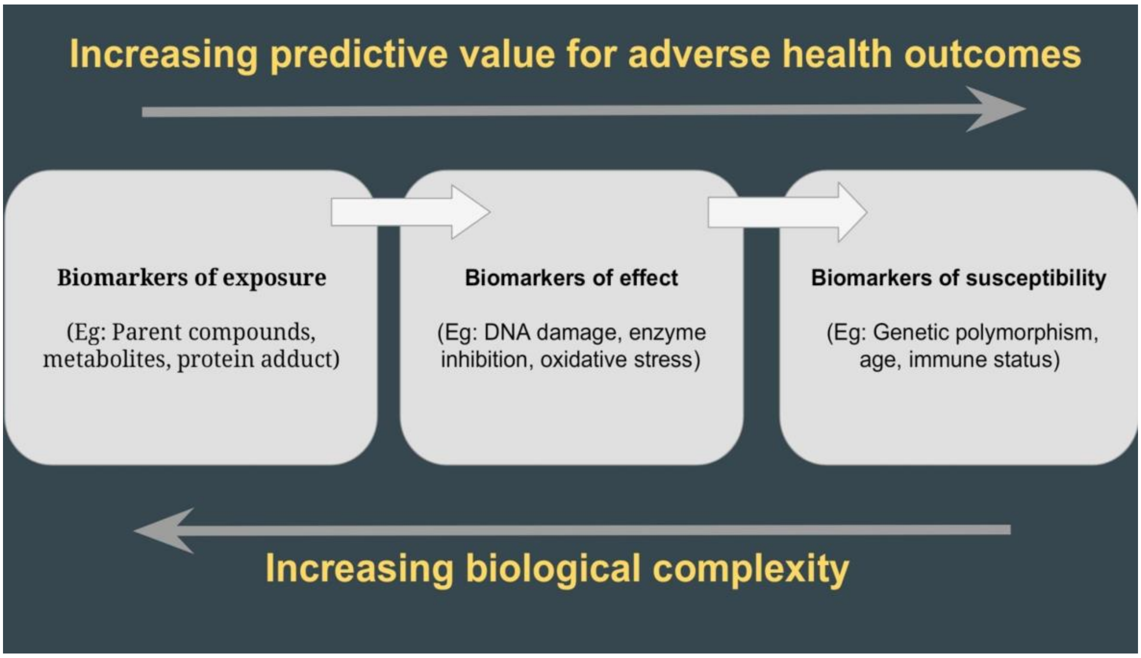

Figure 2. *Classification of biomarkers.*

4. Types of Biomarkers Used in Environmental Stress Assessment

Biomarkers serve as important indicators to monitor environmental stress in animals. They provide early evidence of physiological, biochemical, and behavioral changes before visible harm occurs. Based on the level of biological organization, biomarkers can be categorized into molecular, biochemical, physiological, and behavioral types.

4.1. Molecular Biomarkers

Molecular biomarkers reflect changes at the genetic or protein level caused by environmental stressors and are highly sensitive to early damage.

HSPs or heat shock proteins: These proteins are generated as a reaction to heat, chemical exposure, or oxidative stress, acting as protective agents and indicating cellular stress [32].

Metallothioneins (MTs): MTs are proteins that bind heavy metals, reducing their toxicity. They are commonly used to monitor exposure to metals like cadmium, zinc, and mercury [33].

DNA Damage Markers: Indicators such as DNA strand breaks, micronuclei formation, and comet assay results reveal genotoxic effects of pollutants or radiation [34].

4.2. Biochemical Biomarkers

Biochemical biomarkers measure metabolic or enzymatic changes induced by environmental stressors.

Enzyme Antioxidants: When pollutants produce oxidative stress, enzymes including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) rise [35].

Lipid Peroxidation: Malondialdehyde (MDA) levels indicate oxidative damage to cell membranes and are commonly elevated after exposure to metals or organic contaminants [36].

Stress Hormones: Cortisol or corticosterone levels rise under chemical, physical, or ecological stress, reflecting the physiological stress response in vertebrates [37].

4.3. Physiological Biomarkers

Physiological biomarkers assess changes in organ systems, immunity, or reproduction.

Immune Function: Alterations in leukocyte counts, antibody production, or other immune parameters show how environmental contaminants affect the organism's defense mechanisms [7].

Reproductive Parameters: Changes in gonad size, reproductive hormone levels, fertility, or hatchability show the effects of environmental pollutants regarding reproductive health [21].

4.4. Behavioral Biomarkers

Table 2. *Types of biomarkers and their biological significance.*

Biomarker Type	Example	Stress Response Indicated	References
Molecular Biomarkers	Heat shock proteins (HSPs)	Cellular stress due to heat, chemical, or oxidative exposure	[32]
	Metallothioneins (MTs)	Metal exposure and detoxification	[33]
	DNA damage markers (comet assay, micronuclei)	Genotoxicity and DNA strand breaks	[34]
Biochemical Biomarkers	Antioxidant enzymes (SOD, CAT, GPx)	Oxidative stress response	[35]
	Lipid peroxidation (LPO)	Membrane damage due to ROS	[36]
	Stress hormones (cortisol, corticosterone)	Physiological stress in vertebrates	[37]
Physiological Biomarkers	Immune function (leukocyte count, antibodies)	Impaired immunity due to pollutants	[7]
	Reproductive parameters (gonad size, fertility, hormone levels)	Endocrine disruption and reduced reproductive fitness	[21]
Behavioral Biomarkers	Feeding behavior	Reduced appetite or altered feeding patterns	[38]
	Locomotion	Altered movement or activity due to neurological/muscular impairment	[38]
	Avoidance behavior	Behavioral avoidance of contaminated areas	[39]

Behavioral biomarkers monitor modifications in normal animal behavior caused by environmental stressors and are useful for non-invasive assessment.

Feeding Behavior: Reduced food intake or altered feeding patterns may indicate toxic exposure or stress [38].

Locomotion: Changes in movement patterns, swimming speed, or activity levels can reflect neurological or muscular impairment.

Avoidance Behavior: Animals may actively avoid contaminated areas, which can signal environmental hazards [39].

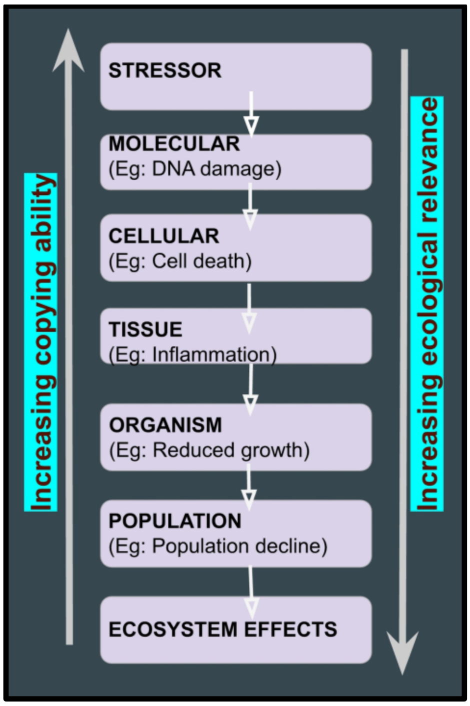

Figure 3. *Levels of biological organization affected by stress.*

5. Biomarkers in Different Animal Groups

Biomarkers are widely applied to detect environmental stress across different animal taxa. The type of biomarker used often depends on the species and habitat, as different animals exhibit unique physiological, biochemical, and behavioral responses to stressors.

5.1. Aquatic Animals

Fish- Fish are commonly used as bioindicators in freshwater and marine ecosystems due to their sensitivity to pollutants. Typical biomarkers include metallothioneins, antioxidant enzymes, heat shock proteins, DNA damage markers, and stress hormones such as cortisol. These biomarkers help assess exposure to heavy metals, pesticides, and other pollutants. Physiological and reproductive indicators, like gonad development and fertility changes, are also used to detect endocrine disruption [6, 21].

Mollusks- Bivalves and other mollusks are valuable for biomonitoring because they are sedentary and filter feeders. Commonly used biomarkers include metallothioneins, antioxidant enzyme activity, lipid peroxidation, and lysosomal stability. Behavioral responses, such as valve closure or decreased filtration rates, also provide rapid, non-invasive indicators of environmental stress [40, 41].

5.2. Terrestrial Animals

Mammals- Terrestrial mammals are studied to evaluate chemical contamination, habitat loss, and climate-related stress. Biomarkers include stress hormones (cortisol), antioxidant enzyme activity, metallothioneins for metal exposure, DNA damage markers, and immune function. Behavioral indicators, such as avoidance, feeding changes, and altered locomotion, also reflect environmental stress [42].

Birds- Birds are sensitive to pollutants in urban and agricultural areas. Frequently measured biomarkers include blood corticosterone, metallothioneins, antioxidant enzymes, DNA damage markers, reproductive hormones, and behavioral patterns such as nest attendance, foraging efficiency, and flight activity [43].

Amphibians- Amphibians are quite vulnerable to environmental stress because of their porous skin and dependence on both aquatic and terrestrial habitats. Biomarkers include stress hormones, antioxidant enzymes, heat shock proteins, metallothioneins, DNA damage, and reproductive hormone levels. Behavioral changes, such as reduced feeding, abnormal locomotion, or altered calling behavior, are also valuable indicators of environmental contamination [12].

Table 3. *Common biomarkers used in different animal groups.*

Animal Group	Biomarkers	Environmental Stress Indicated	References
Fish	Metallothioneins, antioxidant enzymes (SOD, CAT, GPx), heat shock proteins (HSPs), DNA damage markers, stress hormones (cortisol), reproductive parameters	Exposure to heavy metals, pesticides, organic pollutants, endocrine-disrupting chemicals	[6-21]
Mollusks	Metallothioneins, antioxidant enzymes, lipid peroxidation (MDA), lysosomal stability, valve closure or reduced filtration behavior	Contamination by heavy metals, hydrocarbons, and organic pollutants	[40, 41]
Mammals	Stress hormones (cortisol), antioxidant enzymes, metallothioneins, DNA damage markers, immune function, behavioral responses (feeding, movement, avoidance)	Chemical pollutants, habitat degradation, climatic stress	[42]
Birds	Blood corticosterone, metallothioneins, antioxidant enzymes, DNA damage markers, reproductive hormones, behavioral	Urban and agricultural contamination	[43]

Animal Group	Biomarkers	Environmental Stress Indicated	References
	changes (foraging, nest attendance, flight activity)		
Amphibians	Stress hormones, antioxidant enzymes, metallothioneins, heat shock proteins, DNA damage markers, reproductive hormones, behavioral changes (feeding, locomotion, calling activity)	Environmental pollution, habitat loss, chemical contaminants	[12]

6. Applications of Biomarkers in Environmental Monitoring

Biomarkers are extensively utilized in environmental monitoring to identify biological responses to pollutants and other environmental stressors. They are particularly valuable because they reveal early, sub-lethal changes in organisms before visible ecological damage becomes apparent. This makes them essential tools in environmental management and protection strategies.

6.1. Early Warning Systems

Biomarkers serve as sensitive early detection tools by identifying molecular, biochemical, or physiological alterations that occur before clear ecological consequences are observed. For instance, variations in antioxidant enzyme activity, metallothionein levels, and heat shock protein expression can indicate contaminant exposure at an early stage [6]. Such early responses enable prompt corrective actions to minimize long-term environmental harm [7].

6.2. Ecological Risk Evaluation

In Evaluation of ecological risk, biomarkers supply measurable evidence linking pollutant exposure to biological effects. Indicators such as oxidative stress markers, DNA damage, and endocrine disruption help determine the potential impact of contaminants on organisms and populations [25]. Incorporating biomarker responses into risk assessment frameworks enhances the reliability and scientific accuracy of environmental evaluations.

6.3. Conservation and Wildlife Health

Within conservation biology, biomarkers are employed to evaluate the health state of wildlife populations vulnerable to environmental pressures. Parameters comprising stress hormone concentrations, immune responses, reproductive indicators, and contaminant accumulation assist in understanding how pollution and habitat changes influence survival and reproductive success [44]. These assessments are particularly important for the management of threatened and endangered species.

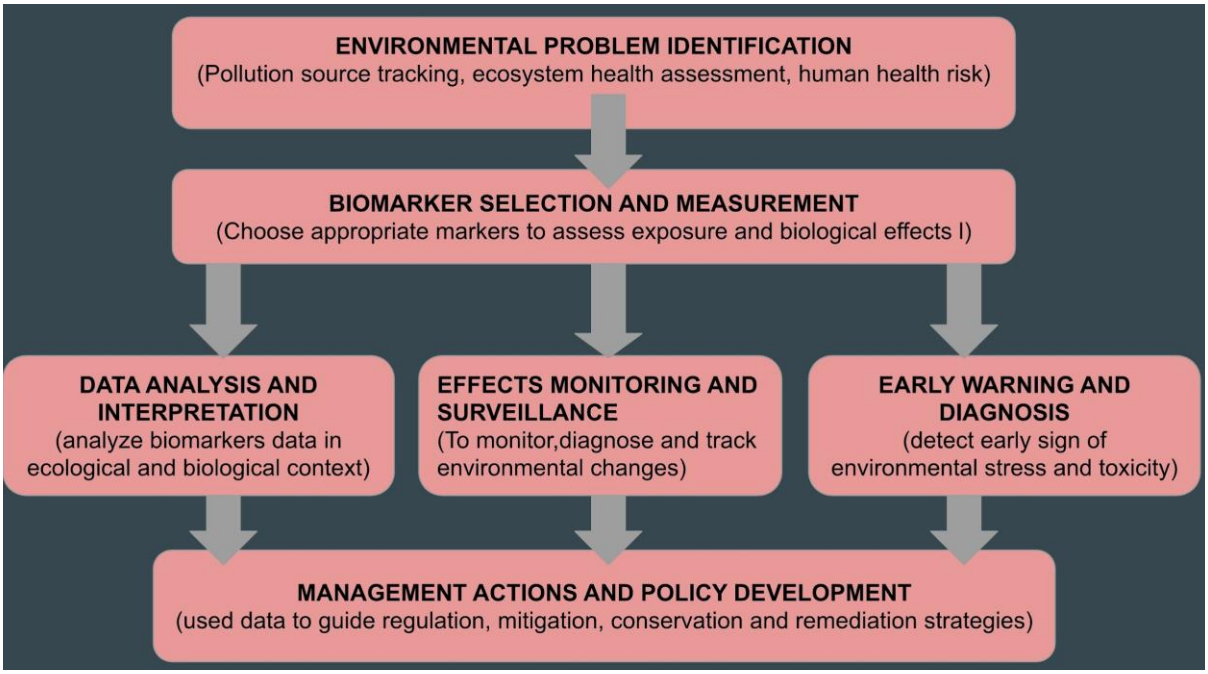

Figure 4. *Application framework of biomarkers in environmental monitoring.*

6.4. Livestock and Aquaculture Monitoring

Biomarkers are also applied in livestock production and aquaculture to monitor animal well-being and environmental quality. In aquaculture systems, measurements of antioxidant enzymes, stress hormones, and immune function help detect water quality issues and culture-related stress [45]. Similarly, in livestock, physiological and biochemical markers are used to assess exposure to environmental pollutants and heat stress, contributing to improved animal health and sustainable production practices.

7. Advantages and Limitations of Biomarker Approaches

Biomarkers are important tools in environmental toxicology because they provide measurable biological responses to environmental stressors. Although biomarker-based assessments offer several benefits, they also present certain challenges that must be carefully considered during interpretation.

7.1. Advantages

Sensitive and Early Detection- A key strength of biomarkers is their ability to identify subtle biological changes at the molecular, biochemical, or physiological level before visible symptoms or population declines occur [6]. This early responsiveness makes biomarkers valuable as preventive monitoring tools. Indicators such as antioxidant enzymes, metallothioneins, and DNA damage markers can signal contaminant exposure long before severe ecological consequences become evident [7].

Non-Lethal Sampling- Many biomarker analyses can be conducted using minimally invasive or non-destructive sampling techniques, including blood sampling or small tissue biopsies. This is particularly advantageous when monitoring rare or endangered species, as it allows repeated measurements without causing significant harm. Non-lethal methods also support long-term studies by enabling continuous observation of the same individuals [44].

7.2. Limitations

Species-Specific Variability- Biomarker responses may differ significantly among species because of variations in physiology, metabolism, and ecological adaptations. Consequently, a biomarker that performs well in one species may not yield comparable results in another, limiting the broader applicability of findings [25].

Environmental Confounding Factors- Natural environmental variables—like temperature, age, sex, reproductive condition, nutrition also seasonal changes—can affect the biomarker responses. These confounding factors may complicate the results' interpretation, which makes it challenging to distinguish between pollutant-induced effects and normal physiological variation [6]. Proper study design and baseline reference data are therefore essential for accurate evaluation.

Table 4. *Advantages and limitations of biomarker-based assessments.*

Category	Main Aspect	Description	References
Advantages	Sensitive and early detection	Biomarkers are capable of identifying subtle molecular, biochemical, or physiological alterations at an early stage, often before visible symptoms or population-level impacts become evident	[6, 7]
Advantages	Non-lethal or minimally invasive sampling	Many biomarker measurements can be performed using non-lethal techniques such as blood sampling or small tissue collection, allowing repeated monitoring of the same organisms	[44]
Limitations	Variation among species	Responses to biomarkers can differ between species due to differences in physiology and metabolism, which may restrict broader application of findings	[25]
Limitations	Influence of environmental variables	Natural factors such as temperature, age, sex, nutritional condition, and seasonal changes may affect biomarker responses, making interpretation more complex	[6]

8. Integration of Multiple Biomarkers

Relying on a single biomarker often provides an incomplete view of how organisms respond to environmental stressors. To obtain a more comprehensive assessment, multiple biomarkers are increasingly combined, which enhances the sensitivity, reliability, and ecological relevance of monitoring programs [6, 46].

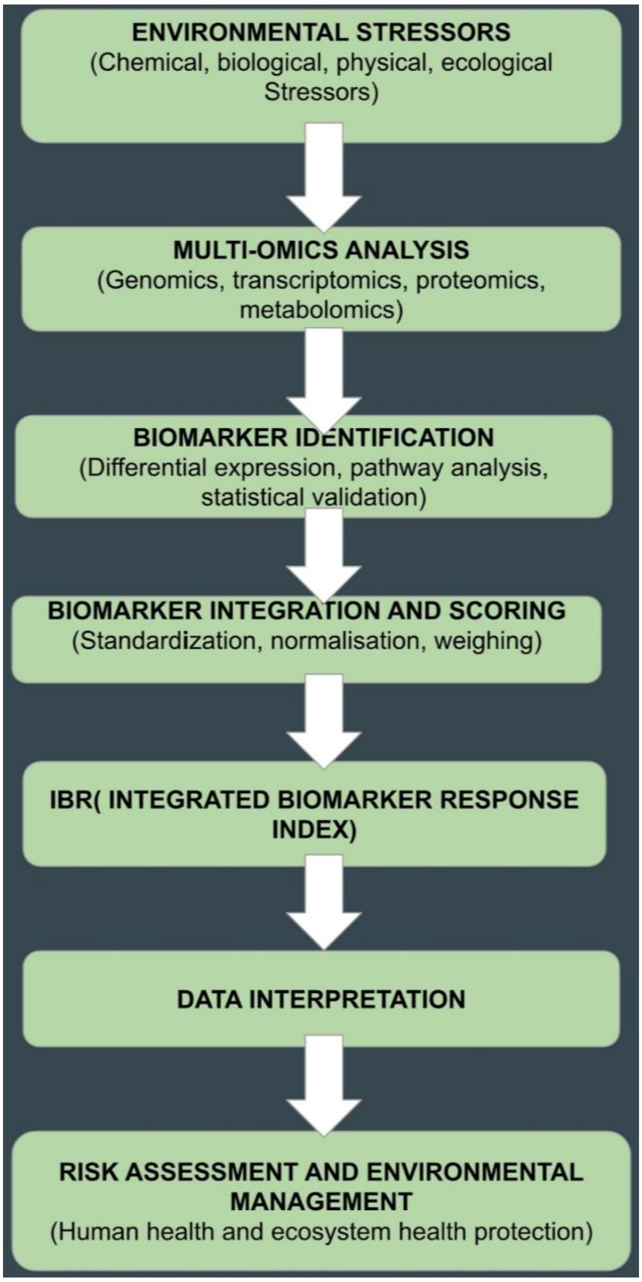

Figure 5. *Integrated biomarker approach (IBR/omics-based framework).*

8.1. Biomarker Indices

Biomarker indices combine data from several individual biomarkers into a single score, simplifying the interpretation of complex biological responses. A widely used approach is the Integrated Biomarker Response (IBR) index, which consolidates molecular, biochemical, and physiological measurements into one composite value. This method facilitates comparisons across sites, species, or pollution gradients and provides a clear indicator of overall stress levels [46, 47]. Field studies, including those on honeybees and aquatic organisms, have successfully applied IBR to assess the combined impacts of multiple environmental strains [48, 49].

8.2. Multi-Biomarker Approaches

Multi-biomarker strategies involve measuring several endpoints simultaneously, such as DNA damage, antioxidant enzyme activity, stress hormone levels, reproductive parameters, and immune responses. Evaluating multiple biomarkers together offers a holistic perspective of the organism's health and allows the detection of interactions among stressors that may not be evident from individual markers alone [25, 50]. Such integrated approaches improve ecological risk assessments by reflecting responses at different biological levels.

8.3. Omics Technologies

Advances in genomics, transcriptomics, proteomics, and metabolomics have enabled high-throughput omics approaches in biomarker research. These techniques allow large-scale monitoring of gene expression, protein profiles, and metabolic changes, providing insights into the mechanisms of stress responses. When combined with traditional biomarkers, omics technologies enhance sensitivity, uncover novel indicators of environmental stress, and improve predictive capabilities for ecological risk assessments [51-53]. This integration supports a more detailed and mechanistic understanding of how pollutants and other stressors affect organismal health and ecosystem stability.

9. Future Perspectives and Research Gaps

Despite significant progress in biomarker research for environmental monitoring, several limitations and unanswered questions still need attention. Addressing these gaps will improve the scientific robustness and ecological relevance of biomarker-based approaches.

9.1. Need for Standardization

A major concern in biomarker studies is the absence of uniform procedures for sampling, laboratory analysis, and data evaluation. Variations in methodologies, test species, and environmental conditions often make it challenging to compare findings between several studies. Developing standardized protocols and validated analytical techniques would enhance consistency and reliability in environmental assessments [6]. Such harmonization is particularly important when biomarkers are incorporated into regulatory and ecological risk assessment frameworks [25].

9.2. Long-Term Studies

Many biomarker studies focus on short-term or immediate biological responses to stressors. However, long-range investigations have essential to comprehend the effects of chronic exposure and delayed effects. Extended monitoring can provide insight into how persistent environmental stress influences growth, reproduction, survival, and overall fitness. Longitudinal research also helps differentiate between temporary adaptive mechanisms and permanent physiological damage [44].

9.3. Linking Biomarkers to Population-Level Effects

Another important research need is establishing stronger connections between individual biomarker responses and broader ecological outcomes. While biomarkers are effective in detecting early physiological disturbances, translating these findings into predictions about population declines or ecosystem disruption remains complex. Integrating biomarker data with ecological modeling tools and adverse outcome pathway (AOP) frameworks can improve the linkage between molecular-level changes and higher-level biological effects [53]. Advancing such integrative strategies will strengthen environmental risk assessment and conservation planning.

10. Conclusion

In summary, biomarkers provide an essential and versatile approach for evaluating environmental stress in both aquatic and terrestrial animals. They enable the detection of subtle molecular, biochemical, physiological, and behavioral changes that often occur long before visible effects appear at the organism or population level. This early warning capability makes biomarkers a powerful tool in environmental monitoring, as they can reveal sub-lethal stress impacts that may eventually influence survival, reproduction, and overall population dynamics.

The variety of biomarkers—ranging from molecular indicators such as DNA damage and heat shock proteins, to biochemical markers like antioxidant enzymes and metallothioneins, physiological measures including stress hormones and reproductive parameters, and behavioral responses—offers a multidimensional view of how organisms respond to environmental pressures. Integrating multiple biomarkers, using approaches such as biomarker indices, multi-biomarker strategies, and advanced omics technologies, further strengthens the sensitivity and reliability of assessments, allowing researchers to evaluate cumulative stress effects across multiple biological levels.

Beyond assessing individual health, biomarkers provide valuable insights into population and ecosystem-level consequences of environmental stress. They are instrumental for identifying contaminated sites, guiding conservation and wildlife management strategies, and monitoring the effectiveness of mitigation measures. In applied contexts such as livestock production and aquaculture, biomarkers also contribute to ensuring animal welfare and sustainable management by detecting environmental and husbandry-related stressors early. Despite their many advantages, biomarker-based assessments face challenges, including variability among species, influence of natural environmental factors, and the need for standardized protocols. Addressing these gaps through long-term studies, methodological harmonization, and integration with ecological and population-level models will enhance the ecological relevance and practical utility of biomarkers. Overall, biomarker approaches serve as a critical framework for understanding, predicting, and mitigating the effects of environmental stress on animals and ecosystems. Their ability to provide early, sensitive, and multidimensional insights into organismal responses makes them indispensable for preserving biodiversity, supporting ecosystem stability, and promoting sustainable interactions between humans and the environment amid increasing ecological pressures.

Abbreviations

CAT	Catalase
GPx	Glutathione Peroxidase
HSPs	Heat Shock Proteins
LPO	Lipid Peroxidation
MDA	Malondialdehyde
MTs	Metallothioneins
PCBs	Polychlorinated Biphenyls
SOD	Superoxide Dismutase

Acknowledgments

The authors wish to acknowledge the Dean, Faculty of Science and The Head, of the Zoology Department, Kumaun University, Nainital for providing the facilities utilized in the present study.

Author Contributions

Prakarshi Kaushik: Conceptualization, Writing – original draft
Bhawna Singh: Data curation
Garima Supyal: Formal Analysis
Deepak Malkani: Visualization
Pankaj Bhatt: Formal Analysis
Seeta Dewali: Investigation, Conceptualization
Netra Pal Sharma: Project administration, Writing – review & editing
Deepak Chandra Melkani: Validation
Satpal Singh Bisht: Supervision

Conflicts of Interest

Authors have no conflict of interest.

References

[1] McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. Hormones and Behavior, 43(1), 2–15. https://doi.org/10.1016/s0018-506x(02)00024-7

[2] Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G., & Sukhotin, A. A. (2012). Energy homeostasis as an integrative tool for assessing limits of environmental. https://doi.org/10.1016/j.marenvres.2012.04.003

[3] Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicology, 101(1), 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006

[4] Gorbi, S., Regoli, F., & Fattorini, D. (2011). Biomarkers in ecotoxicology: From molecular to ecosystem-level assessment. Marine Environmental Research, 72(5), 298–309.

[5] Bonier, F., Martin, P. R., Moore, I. T., & Wingfield, J. C. (2009). Do baseline glucocorticoids predict fitness? Trends in Ecology & Evolution, 24(11), 634–642.

[6] Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology, 13(2), 57–149.

[7] Depledge, M. H., & Fossi, M. C. (1994). The role of biomarkers in environmental assessment (2): Invertebrates. Ecotoxicology, 3(3), 161–172.

[8] Fazio, F., & Faggio, C. (2016). Biomarkers in fish ecotoxicology: A mechanistic approach. Environmental Science and Pollution Research, 23(2), 1331–1343.

[9] Scheuhammer, A. M., Meyer, M. W., Sandheinrich, M. B., & Murray, M. W. (2007). Effects of environmental methylmercury on the health of wild birds, mammals, and fish. AMBIO, 36(1), 12–19.

[10] Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. EXS, 101, 133–164.

[11] Goulson, D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology, 50(4), 977–987.

[12] Hayes, T. B., Khoury, V., Narayan, A., et al. (2010). Atrazine induces complete feminization and chemical castration in male African clawed frogs. Proceedings of the National Academy of Sciences, 107(10), 4612–4617.

[13] Walker, C. H., Sibly, R. M., Hopkin, S. P., & Peakall, D. B. (2012). Principles of ecotoxicology (4th ed.). CRC Press.

[14] Peterson, C. H., Rice, S. D., Short, J. W., et al. (2003). Long-term ecosystem response to the Exxon Valdez oil spill. Science, 302(5653), 2082–2086.

[15] Pörtner, H. O., & Farrell, A. P. (2008). Physiology and climate change. Science, 322(5902), 690–692.

[16] Stillman, J. H. (2019). Heat waves, the new normal: Summertime temperature extremes will impact animals. Journal of Experimental Biology, 222(Suppl 1), jeb214569.

[17] Shannon, G., McKenna, M. F., Angeloni, L. M., et al. (2016). A synthesis of two decades of research documenting the effects of noise on wildlife. Biological Reviews, 91(4), 982–1005.

[18] Møller, A. P., & Mousseau, T. A. (2006). Biological consequences of Chernobyl: 20 years on. Trends in Ecology & Evolution, 21(4), 200–207.

[19] Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica, 78(2), 103–116.

[20] Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics, 34, 487–515.

[21] Sumpter, J. P., & Johnson, A. C. (2005). Lessons from endocrine disruption in aquatic systems. Environmental Toxicology and Chemistry, 24(10), 2636–2646.

[22] Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D.,... & Villeneuve, D. L. (2010). Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 29(3), 730–741.

[23] Relyea, R. (2005). The impact of insecticides and herbicides on the stress physiology of amphibians. Ecology Letters, 8(3), 395–402.

[24] Hering, D., Borja, A., Carstensen, J., Carvalho, L., Elliott, M., Feld, C. K.,... & Pont, D. (2010). The European Water Framework Directive at the midpoint: Strategies to assess ecological quality. Science of the Total Environment, 408(19), 4007–4019.

[25] Forbes, V. E., Palmqvist, A., & Bach, L. (2006). The use and misuse of biomarkers in ecotoxicology. Environmental Toxicology and Chemistry, 25(1), 272–280.

[26] Soller, A., Ikonomou, M. G., & Smith, A. (2012). Advances in biomarker development: Integration with environmental monitoring. Journal of Environmental Monitoring, 14(6), 1440–1450.

[27] Scott, G. R., & Sloman, K. A. (2004). The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. Aquatic Toxicology, 68(4), 369–392.

[28] Hagger, J. A., Jones, O. A. H., & Scrimshaw, M. D. (2006). Biomarkers of exposure and effect in fish exposed to WWTP effluent. Ecotoxicology and Environmental Safety, 64(3), 329–342.

[29] Dara, M., Torregrossa, N., La Corte, C., Bisanti, L., Bertini, F., Parrinello, D., ... & Cammarata, M. (2024). Bioplastics and marine invertebrates: assessing immunological and environmental impacts in the transition from petrochemical to sustainable polymers. Invertebrate Survival Journal, 21(1), 127-142.

[30] Galloway, T. S., & Depledge, M. H. (2001). Immunotoxicity in invertebrates: Measurement and ecotoxicological relevance. Ecotoxicology, 10(1), 5–23.

[31] Moore, M. N., Wilson, J., & Blackhurst, D. (2004). Biomarkers in marine pollution monitoring. In J. G. Vos et al. (Eds.), Toxicology of Marine Pollution (pp. 1–27). Taylor & Francis.

[32] Sanders, B. M. (1993). Stress proteins in aquatic organisms: An environmental perspective. Critical Reviews in Toxicology, 23(1), 49–75.

[33] Roesijadi, G. (1992). Metallothioneins in metal regulation and toxicity in aquatic animals. Aquatic Toxicology, 22(2), 81–114.

[34] Olive, P. L., Banáth, J. P., & Durand, R. E. (1990). Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet" assay. Radiation Research, 122(1), 86–94.

[35] Livingstone, D. R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution Bulletin, 42(8), 656–666.

[36] Halliwell, B., & Gutteridge, J. M. C. (2007). Free radicals in biology and medicine (4th ed.). Oxford University Press.

[37] Boonstra, R. (2013). The ecology of stress: Effects of the social environment. Functional Ecology, 27(1), 66–80.

[38] Weis, J. S., Smith, G., Zhou, T., Santiago-Bass, C., & Weis, P. (2001). Effects of contaminants on behavior: Biochemical mechanisms and ecological consequences. BioScience, 51(3), 209–217.

[39] Dawson, R., Siebers, D., & Weis, J. S. (1991). Behavioral indicators of environmental contamination in fish. Environmental Toxicology and Chemistry, 10(9), 1309–1317.

[40] Rainbow, P. S. (2002). Trace metal concentrations in aquatic invertebrates: Why and so what? Environmental Pollution, 120(3), 497–507.

[41] Depledge, M. H., & Rainbow, P. S. (1990). Invertebrates as biomonitors. Marine Pollution Bulletin, 21(8), 321–324.

[42] McLaren, P. L., MacMillan, S., & Carey, P. D. (1998). Biomarkers of exposure and effect in terrestrial mammals. Environmental Toxicology and Chemistry, 17(5), 854–861.

[43] Furness, R. W., & Rainbow, P. S. (1990). Heavy metals in the marine environment. CRC Press.

[44] Wikelski, M., & Cooke, S. J. (2006). Conservation physiology. Trends in Ecology & Evolution, 21(1), 38–46.

[45] Ashley, P. J. (2007). Fish welfare: Current issues in aquaculture. Applied Animal Behaviour Science, 104(3–4), 199–235.

[46] Beliaeff, B., & Burgeot, T. (2002). Integrated biomarker response: A useful tool for ecological risk assessment. Environmental Toxicology and Chemistry, 21(6), 1316–1322.

[47] Mattos, J. J., Siebert, M. N., & Bainy, A. C. D. (2024). Integrated biomarker responses: Further improvement of IBR indexes to preserve data variability in statistical analyses. Environmental Science and Pollution Research International.

[48] Di Noi, A., Casini, S., & Others. (2021). First application of an Integrated Biological Response index to assess the ecotoxicological status of honeybees. Environmental Science and Pollution Research.

[49] Caliani, I., Campani, T., Conti, B., Cosci, F., Bedini, S., D'Agostino, A., … & Casini, S. (2021). Multi-biomarker approach and IBR index to evaluate the effects of different contaminants on the ecotoxicological status of Apis mellifera. Ecotoxicology and Environmental Safety, 208, 111486.

[50] Duarte, I. A., Reis-Santos, P., França, S., Cabral, H., & Fonseca, V. F. (2017). Biomarker responses to environmental contamination in estuaries: A comparative multi-taxa approach. Aquatic Toxicology, 189, 31–41.

[51] Smith, K. F., Pochon, X., Melvin, S. D., Wheeler, T. T., & Tremblay, L. A. (2025). Integration of "omics"-based approaches in environmental risk assessment to establish cause and effect relationships: A review. Toxics, 13(9), 714.

[52] Li, B., Zhang, Y., Du, J., Liu, C., Zhou, G., Li, M., & Yan, Z. (2025). Application of multi-omics techniques in aquatic ecotoxicology: A review. Toxics, 13(8), 653.

[53] Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., … & Villeneuve, D. L. (2006). Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 25(3), 589–598.