EXPLORING CURRENT FRONTIERS OF ENVIRONMENTAL CHALLENGES BY BIOINDICATORS AND BIOMARKERS

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Abstract

Anthropic pressure on the environment threaten its biodiversity and subsequently, its sustainability. Nowadays, the research focusses on the development of a new efficient screenings regarding contaminants toxic impact on aquatic and terrestrial environments based on specific biological models, as bioindicators. Cellular and molecular biomarkers are considered to appear very fast under a pollution or climate change stress, before any significant changes at the organism level, and to be specific and sensitive indicators of environmental quality and adaptation mechanisms. Changes at the molecular level could provide rapid information and prediction patterns regarding the occurrence of lethal, sub-lethal, or adaptive effects on biotopes under the influence of anthropogenic stressors. The combined use of bioindicators and biomarkers could provide a comprehensive picture of aquatic and terrestrial ecosystems health status and they could facilitate to identify the environmental issues. In this review, we aimed to establish a relationship between pollution and specific adaptation mechanism responses at the molecular level such as biomarkers. Moreover, we analyzed how biomarkers respond to exposure to different toxic substances and exposure levels, determining dose-response relationships, and biomarker response time.

Key words: *bioindicators, biomarkers, ecotoxicity, biodiversity, anthropogenic stress.*

INTRODUCTION

The environment is constantly exposed to various anthropogenic pollutants generated by industrial, domestic, and agricultural activities. A wide range of pollutants are susceptible to interact with physiological processes such as growth and reproduction from aquatic and terrestrial organisms. Unfortunately, pollutants could massively alter their life, leading to serious disruptions of populations number and diversity due to impairing reproductive functions and adaptation mechanisms. Environmental resources, resilience and sustainability are major concerns to governments and researchers which try to identify and turn polluted ecosystems into clean environments by new environmental policies and ecofriendly methodologies. In this respect, monitoring programs are used to evaluate the pollution status followed by mitigation strategies. A fast and reliable monitoring system of pollutants triggers an early warning long before environmental damage extension. The

ecotoxicological tests could predict the possible pollutant impact on the environment. The field of ecotoxicology is a complex scientific discipline based on principles of toxicology, biology, chemistry, and ecology, studying the effects of chemical compounds on living organisms other than humans. Ecotoxicology could evaluate pollutant dynamics based on mobility, migration, transformation, and degradation of different environmental areas as well as the effect of toxic compounds (acute, sub-acute or chronic effect) on sensitive biological models correlated with field data.

The studies in environmental field are innovative due to the diversity of test substances available on the national market (such as pesticides, surfactants, pharmaceutical compounds, biocides), complex laboratory experiments (acute, sub-acute or chronic exposure tests using different organisms and high-performance chemical detection methods), and data analyses, such as risk characterization, REACH (Registration, Evaluation, Authorization and Restriction of Chemicals)

classification, threshold approach system, or natural water classification according to Persoone et al. (2003). The ecotoxicological tests could use various biological models from bacteria to plants and vertebrates which could be used as bioindicators in analyzing the toxic effect of a wide range pollutants/chemical. Nowadays, monitoring and solving different environmental pollution issues relies on the use of bio-indicators / biomarkers as an ecotoxicological a top field for fundamental and applied research. The use of bioindicators could be further used to monitor the biodiversity and indirectly to give information about the environmental health and sustainability. Bioindicators include biological processes, species, or communities and are used to evaluate the environmental quality and how it changes over the time. Environmental changes are often linked to anthropogenic and natural stressors, although anthropogenic stressors are the main focus of bioindicator characterization. The development and widespread application of bioindicators have initially described in 1960s. Since then, introduction of biomarker tools continuously developed as a response of finding more sensitive indicators for sub-lethal effects. The scientific progress uncovered the biomarkers as tools for faster and reliable characterization of toxic effects. The 'biomarker' definition mentioned it as a 'biochemical, cellular, physiological, or behavioral variations that can be measured in tissue or body fluid samples, or at the level of whole organisms, to provide evidence of exposure and/or effects from one or more contaminants' (Depledge & Fossi, 1994). The combined use of bioindicators and biomarkers can provide a comprehensive picture of aquatic and terrestrial ecosystems health status and therefore they can identify environmental issues. Pollutants effect could be fast monitored at the molecular level by biomarkers long before perceiving the toxic effect at the organism level. Biomarkers at transcriptional level (DNA) or translational level (proteins) fasten the toxic effect detection and therefore increase the rapidity of ecological risk assessment (ERA) followed by mitigation measures to protect the environment. Recent studies indicated that biomarkers could contribute to ERA framework where gene expression (such as proteomics) showed a particular promise due to their cost-effectiveness and reliable results (Ali et al., 2017; Lill et al., 2021). Challenges in using biomarkers/ bioindicators for ERA were linked to difficulties in identifying a specific relationship stressor-biomarker/bioindicator, in quantifying a specific stressor dose-response functions, and in extrapolating from cellular or subcellular bioindicators to higher-order ecological effects (Bartell, 2006). The use of bioindicators and biomarkers in the context of environmental assessment should be included in a common European legislation regarding the environmental protection, public health, and biodiversity conservation. In the European Union, for example, the Water Framework Directive and the Habitat Directive, include provisions regarding the use of bioindicators and biomarkers in the assessment and monitoring of chemicals impact on the environment and human health. Additionally, international norms and standards, such as those established by the European Chemicals Agency (ECHA) and the World Health Organization (WHO), may provide further guidance on the use and interpretation of bioindicators and biomarkers. In the United States, agencies such as the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) may issue regulations and guidelines regarding the use of bioindicators and biomarkers in the context of assessing risks to the environment and human health. By analyzing and synthesizing previous research, this review study aims to identify current gaps and challenges in the field, as well as opportunities and future research directions. Through this study, the goal is to provide an overview of recent progress in identifying, validating, and using biomarkers and bioindicators in assessing and monitoring environmental health. Additionally, it aims to highlight new technologies and methodological approaches that could enhance our understanding of the anthropogenic impact on the environment and the sustainability of ecosystems.

MATERIALS AND METHODS

A literature search was conducted to access relevant material for this review. We comprehensively searched the Web of Science

and Google Scholar databases from 1990 to 2024 using the following terms and phrases: "*bioindicators*" or "*ecotoxicological tests*", "*biomarkers in ecotoxicology*" or "*Systems Biology in Ecotoxicology*" or "*Omics technologies in ecotoxicology*" or "*adaptive markers*" or "*DNA as a biomarker of pollution*". No restrictions were imposed regarding language, document type, or data category. Preliminary selection was based on information regarding ecotoxicological testing and the relevance of biomarkers. Secondary screening involved verifying details in the field of biomarkers at the cellular and molecular levels, with an emphasis on intensifying studies involving highly advanced technologies. A total of 78 publications were selected.

RESULTS AND DISCUSSIONS

Ecotoxicological tests, bioindicators and biomarkers develop an integrated and complementary framework for assessing the impact of anthropic activities on the environment. These tools provide detailed information about the environmental status and organisms' responses to various stressors, contributing to the development of more effective strategies for protecting and conserving ecosystems and human health. Stressors such as chemicals are a top public concern, especially regarding their bioaccumulation in organisms from the ambient environment and food.

Traditional ecotoxicological tests are standardized laboratory experiments used to assess the potential harmful effects of chemical substances on organisms and ecosystems by exposing, in controlled conditions, biological components (cells, individuals, populations, communities) to various concentrations of chemicals, mostly a single compound. A large number of bioassays have been developed to assess the toxicity of contaminants on plants, animals and microbes from terrestrial, marine and freshwater environments (Breitholtz et al., 2006). Biological effects are recording as survival, growth or reproduction endpoints (Connon et al., 2012; Schuijt et al., 2021). Overall, ecotoxicological tests cover acute and chronic toxicity tests, bioaccumulation studies, genotoxicity tests, reproductive toxicity tests, behavioral toxicity tests, sediment and soil toxicity tests and microbial toxicity tests (Vosylienė, 2007). Ecotoxicological dose-response bioassays are an important line of evidence for ERA, because they provide experimental evidence of cause and effect. Short-term tests quantified lethal concentrations (LC50 or LD50), while long-term tests assessed sublethal effects by providing values of EC50 or ED50 and determining NOEC and LOEC (Rand, 1991). These tests are regulated and standardized by organizations like the EPA and OECD.

An optimally designed ecotoxicological test should meet reliability, cost-effectiveness, sensitivity, and relevance criteria. Designing a test that meets all these criteria is challenging and often involves very high costs. However, tests at this level are often reproducible in terms of obtaining similar results upon test repetition, thereby making the results comparable between laboratories and easier to implement in regulatory hazard and risk assessments. Even though endpoints such as mortality and reproduction are ecologically relevant, they rarely provide information about effects at ecologically relevant concentrations because these endpoints are often assessed after acute or semi-acute exposures, using relatively high concentrations of xenobiotics (Furuhagen, 2015). Tests at the community and ecosystem levels were largely absent for organisms other than microorganisms and algae (Castaño-Sánchez et al., 2020). In addition to the shortage in biological models, the ecotoxicological tests cannot be accomplished within a reasonable timeframe, especially due to a large number of chemicals produced in Europe, more than 80,000 chemicals produced in quantities exceeding one ton, in spite of testing a "representative" class of chemicals on "standard" species. Defining "safe" concentrations of contaminants for ecosystems often relies on the use of an arbitrary "safety factor" (typically dividing a toxicity metric such as LCx, ECx, or NOEC by 100 or 1,000), based on data often available for only a small number of species (e.g., in aquatic risk assessment, often only Daphnia, fish, algae) or at most species sensitivity distributions (SSDs) constructed with only a few dozen species (Posthuma et al., 2019). The increasing number of chemicals to be

tested by standard ecotoxicological tests, relying on mortality or reproduction as endpoints, pushed the ecotoxicological field to extend the testing on biomarkers/bioindicators due to their superior efficiency, reduced costs and less workload of the testing procedure. Bioindicators and biomarkers are two interconnected concepts in ecotoxicology, providing information about the health of ecosystems and living organisms which are exposed to toxic chemicals. Moreover, both aquatic and terrestrial environments have particularities that must be considered for an accurate ecological assessment. For example, measuring pollutant concentrations in water has some disadvantages, such as low concentrations and random spatial and temporal variations. Sediment serves as a long-term integrator of pollution, where concentrations are higher than in seawater, but contaminants are not always available to organisms due to their physico-chemical forms. Furthermore, sediment heterogeneity (particle size and organic matter) could make comparisons between sites difficult. Therefore, the use of living organisms called bioindicators is preferable for quantifying pollutants. The health of an ecosystem or its surrounding environment is reflected on bioindicators as living organisms such as plants (lichens, algae, and vascular plants assessing air pollution or soil quality), invertebrates (earthworms, water scorpions or aquatic insects assessing water quality), fish and other aquatic organisms (assessing water quality and its contamination levels) or birds and mammals (assessing the health of terrestrial ecosystems and exposure to contaminants). Over time, bioindicators have been defined in various ways, one of them highlighted that ecotoxicology is ultimately analyzing the effects of pollutants on populations not individuals. In addition, it was mentioned that sub-lethal effects on the environment can have a greater impact on population size than acute toxicity (Moriarty, 1988). In 1993, Rainbow and Phillips defined bioindicators as animal(s) or plant(s) which accumulate contaminants in a direct ratio with the contaminant concentration from the surrounding environment. Another definition of bioindicators defined them as species or group of species that reflect the abiotic or biotic state of an impacted environment based on a subset of

taxa diversity or the whole diversity within an area (Gerhardt, 1999). The author considered that bioindicators are useful in three situations: (i) where the indicated environmental factor cannot be measured, (ii) where the indicated factor is difficult to measure, (iii) where the environmental factor is easy to measure but difficult to interpret based on its ecological significance. On the other hand, biomarkers are measurable biological indicators at the molecular, cellular, or physiological level that can indicate an exposure to toxic chemicals.

The use of bioindicators for pollution impact assessment is called biomonitoring, based on quantifying contaminants in aquatic organisms which is important from a human health perspective. However, they cannot provide information on the toxicological significance of accumulated pollutants or nor indicate the health status of organisms. Therefore, recent biomonitoring programs involved biomarkers, which are measurable parameters at different levels of biological organization (molecular, cellular, or physiological). Biomarkers reflect changes in regulatory metabolic processes resulting from the effect of anthropogenic stressors. Assessing published studies, it was found that tests at the whole-organismal level and biomarkers were most common for invertebrates and fish, whereas in vitro bioassays primarily relied on mammalian cell lines. Transitioning from bioindicators to biomarkers in ecotoxicology signifies an advancement in assessing the impact of chemicals on the environment and living organisms. While bioindicators provide insights into the overall condition of ecosystems, biomarkers offer detailed information at the molecular, cellular, and physiological levels regarding exposure to toxic substances and their effects on individual organisms. This shift allows for the detection of subtle changes resulting from exposure to toxic chemicals, including sublethal effects and molecular or genetic alterations (Rand, 1991). Furthermore, biomarkers are more likely to detect effects at lower and more ecologically relevant concentrations. Several chemicals interfere with organismal oxidative processes, causing an imbalance in oxidative status that can result in oxidative stress, a potentially harmful condition to organism health. Oxidative stress biomarkers

are therefore common in ecotoxicological studies to demonstrate exposure. While in theory, biomarkers could facilitate ecological risk assessments (ERAs), there are still considerable knowledge gaps regarding confounding factors and connections between biomarker response and effects at higher biological levels. These gaps limit the full potential of biomarker utilization in ecotoxicological studies and ERA (Furuhagen et al., 2014a).

The advantage of introducing biomarkers in environmental research
Biomarkers, through their distinct molecular structures, are essential tools for assessing environmental quality and biotechnological processes. Their importance lies in their ability to be identified and measured through various biochemical and molecular approaches. Many ecotoxicological biomarkers originated from the field of biomedical sciences and they were initially developed and validated in humans and model mammalian species before being applied in ecotoxicology. Biomarkers include enzymes (such as hepatic enzymes to evaluate the toxicity of chemicals on the liver), specific proteins (which can be expressed in a specific manner due an exposure to a particular contaminant), nucleic acids (changes at the DNA or RNA level to assess genetic damage caused by exposure to chemicals) or specific metabolites (specific chemical substances that are produced or metabolized in a specific manner following exposure to a contaminant). Molecular, biochemical, and cellular systems are often the most sensitive and can react quickly to environmental changes. These cellular processes are responsible for detoxification, adaptation, repair, and cellular integrity protection, ultimately contributing to organismal health in response to stress. Biomarkers linked to these processes could monitor very early and at lower levels of harmful exposure the environmental stress (Binelli et al., 2006; Furuhagen, 2014b). These early warning biomarkers can be used predictively, allowing the implementation of strategies before irreversible ecological damage occurs, acting as short-term indicators for long-term biological effects. Biomarker application range is very wide, they could be used in human

clinical and risk assessments studies to make predictions about an individual's future health and/or response to medical treatment. In addition, they could be used to reach ERA goals in predicting the integrity and functioning of ecosystems (population and community levels) under various stress factors (Hommen et al., 2010; Forbes et al., 2006). Some biomarkers are specific to a particular chemical or type of stressor, while others are nonspecific, responding to a wide range of stressors (Rossnerova et al., 2020). Susceptibility biomarkers indicate an organism's capacity to respond to a specific xenobiotic (Silvestre, 2020; Gonçalves et al., 2021). Exposure biomarkers are indicators of exposure to a particular stressor, while effect biomarkers are associated with the health and fitness effects on the organism (Depledge, 2020; Schuijt et al., 2021). Most biomarkers used in ecotoxicology would be classified as exposure biomarkers, as only a few of them have well-described connections to changes at higher biological levels (Furuhagen et al., 2014b).

Proteomics as translational-level biomarkers in the environment
Proteomics, as a branch of molecular biology, covers the entire set of proteins (proteome) expressed in a cell, tissue, or organism at a specific moment. In the context of the environment, proteomics becomes extremely useful in identifying and evaluating biomarkers, which are molecules or biological compounds that can indicate the presence or condition of a particular phenomenon, such as pollution. The use of protein biomarkers in environmental proteomics becomes a promising method for detecting and evaluating the impact of pollution on organisms and ecosystems. Organisms or cells exposed to new environmental conditions, such as pollution, modulates their protein expression pattern which could be correlated with specific protein biomarkers, indicating the level of stress or damage caused by pollutants. These proteins as biomarkers may be involved in various biological processes, such as detoxification, stress response, inflammation, or cellular damage, and can be detected and quantified using advanced techniques, such as mass spectrometry and two-dimensional gel electrophoresis. Furthermore, proteomics can

provide a deeper understanding of the molecular mechanisms involved in organisms' responses to pollution and can help identify biological pathways that are disrupted by environmental stressors. Proteins are more relevant than transcripts, because they are direct mediators of the resulting phenotype. Proteins direct all levels of the phenotype: structural proteins dictate physical form, enzymes catalyze biochemical reactions, and proteins act as signaling proteins, antibodies, transporters, ion pumps, and transcription factors to control gene expression. Molecular initiating events (MIEs) of adverse outcome pathways (AOPs) predominantly occur at the protein level (e.g., ligand-receptor binding), and proteomics can elucidate new MIEs and map key events in AOPs (Allen et al., 2014). Recent developments in environmental proteomics have shifted towards identifying and characterizing protein biomarkers in response to environmental stress. There is a growing interest in integrating proteomic methodologies with ecological applications, aiming to leverage their potential for translational-level biomarker monitoring in environmental management. Numerous studies have delved into employing proteomic analysis for monitoring environmental stress in aquatic organisms, examining its capability to identify protein biomarkers signaling exposure to pollutants and evaluating their suitability for environmental risk assessment and management (Brockmeier et al., 2017). Heat shock proteins (HSPs) are examples of general biomarkers. Their function is to prevent protein denaturation, a common effect of many environmental stressors; therefore, HSP induction is considered a general stress response. Other responses are induced only by a specific group of xenobiotics or stressors. Acetylcholinesterase (AChE) is considered a specific biomarker because it responds to organophosphate and carbamate pesticides and not to general environmental stressors or xenobiotics. General biomarkers often have multiple confounding factors that interfere with the toxic response (Aronson & Ferner, 2017). These biomarkers respond not only to the xenobiotics of interest but also to numerous other stressors and environmental factors, such as nutritional status, temperature, and UV irradiation (Moreira-de-Sousa et al., 2018; Demirci-Cekic et al., 2022). Commonly

used biomarkers, such as induction of cytochrome P450 1A enzyme, acetylcholinesterase activity, metallothioneins, and pigments, have been treated with great interest. Environmental markers for pollutants such as dioxins, furans, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons are necessary for pollution monitoring. Cytochrome P4501A (CYP1A) and ethoxyresorufin-O-dealkylase (EROD) are biomarkers used to detect the biotransformation of these pollutants in fish and marine bivalves (Kim et al., 2013, Cortés-Miranda et al., 2024). Metallothioneins (MTs) are biomarkers for oxidative stress, with the ability to chelate toxic metals from cells (Hemmadi, 2016). Pigments, such as chlorophylls and carotenoids, are biomarkers for the distribution and abundance of phytoplankton, also being used in cancer research (Mouzaki-Paxinou et al., 2016; Atta et al., 2018; Husayn & Guda, 2023). Other studies summarize recent advancements in the application of proteomics for environmental monitoring and assessment. Proteomic techniques can identify and characterize biomarkers in response to environmental stressors and pollutants, highlighting their potential as translational-level indicators of environmental health. This can facilitate the development of more efficient strategies for managing and reducing the impact of pollution on the environment and human health. Thus, proteomics as translational-level biomarkers in the environment represents an innovative and powerful approach for monitoring and evaluating pollution, offering significant opportunities for improving environmental management and protecting public health (Armengaud, 2016; Kumari & Kumar, 2021).

DNA Integrity /expression/mutation as a Pollution Biomarker (DNA biomarkers as transcriptional level)

Pollution and environmental changes are a stress factor for living organisms triggering changes at molecular level as a part of adaptation mechanisms. The adaptation mechanisms relied on genetic characteristics and physiological resilience which later modulates the phenotypic adaptive changes. Under the stress factors, the genetic markers are first to react, giving information about DNA changes and the

adaptive potential of certain populations to the environmental changes.

Genotoxic agents (as exogenous stress factors) can severely affected DNA integrity inducing DNA strand breaks, loss of methylation and DNA mutations. Exogenous agents can induce DNA strand breaks by a direct damage of nucleotide DNA sequence, impairing the DNA repair processes or by other physiological responses. Members of polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene (BaP) can form a complex with DNA and subsequently induced a direct chemical single-strand breaks due to ionizing radiation or oxidation-reduction chemical reactions (Paniagua-Michel & Subramanian, 2016). Studies on DNA integrity from marine snail *Planaxis sulcatus* have highlighted the impact of pollution in various harvesting locations contaminated with petroleum hydrocarbons from coastal waste (Sarker et al., 2018, D'Costa et al., 2017).

Environmental changes could also affect allele frequencies in populations with short reproductive cycles or they could be correlated to neutral and adaptive genetic markers. The neutral genetic markers give information about population decline under new environmental conditions (pollution or climate change). Neutral markers currently predominate in population genetics applications in the field of conservation and management, providing information about population demographic processes (Hohenlohe et al., 2021). However, neutral markers have limitations for monitoring the effects of environmental changes, but evaluating the adaptive genes directly involved in the response to environmental changes could provide more information about the nature of selection imposed by environmental changes and the potential of populations to respond through evolutionary adaptation (Chown et al., 2016). A useful set of adaptive markers should include loci that contribute significantly to the genetic variation of a trait within and between populations and undergo significant allele frequency changes with environmental changes (Stephan, 2016). Each locus must contribute significantly to genetic variation to be useful in monitoring adaptive changes; otherwise, the large number of required loci could be impractical, and the power to link genetic

changes to environmental effects would be reduced. Pleiotropic effects can influence adaptive evolution by hindering the increase in frequency of favored alleles under selection, such as in the evolution of pesticide resistance (Baucom, 2019). Studies showed that polymorphisms in unique markers genes can influence population growth rate, and adaptive changes can be observed in an increasing number of populations (Meyer et al., 2018). To identify sets of candidate genes, it is important to focus not only on plastic responses to stress but also on comparisons between populations adapted to that stress (Agrawal, 2020; Noble et al., 2019). Quantitative trait loci (QTL) mapping can identify genomic regions involved in adaptive changes, including modifications to protein structures and gene expression (Abraham & Croll, 2023). These studies can highlight genomic regions that control resistance to different stresses and provide information about genes involved in environmental adaptation, such as flowering time in *Arabidopsis thaliana*.

Various genetic tools are used to identify candidate genes involved in adaptive changes in response to environmental changes, including microarrays for measuring expression changes, selection experiments, QTL mapping, and strain comparisons (Franks et al., 2007; Alvarez et al., 2015, Bourdon-Lacombe et al., 2015). Most methods lead to the identification of sets of candidate loci rather than specific alleles, although the involved alleles can eventually be identified, including in non-coding and transcribed gene regions (Jeremias et al., 2020). Genes such as *Adh* in *Drosophila melanogaster* and *Gly* in *Pinus edulis* are considered candidates for monitoring adaptation to thermal and moisture changes, respectively. Knowledge of physiological pathways, such as abscisic acid synthesis in plants, may suggest genes involved in adapting to environmental changes (Hoffman & Willi, 2008). As knowledge of relevant pathways and gene functions in model species increases, the functional roles of candidate genes in relation to specific traits can be tested (Gómez et al., 2015). Recently invaded and established latitudinal climates, as well as newly introduced plants, are ideal experimental systems for identifying candidate genes (Chown et al., 2016), while spatial models of long-term

differential adaptation can provide less information, as evolution has occurred over long periods of time, with multiple genetic drift events (Hoban et al., 2016; Bock et al., 2016). Microarrays and other techniques are used to compare genetic expression patterns between environments and between strains of the same species (Alvarez et al., 2015). These techniques can be applied to non-model organisms, both from field-collected material and laboratory conditions. Genetic expression studies can help identify stress response genes by comparing the plastic responses of populations exposed to different conditions (DeBiasse & Kelly, 2016; Rivera et al., 2021; Kelly, 2019). Examples include identifying genes involved in water stress response in *A. thaliana* and salinity variation response in flounders (*Platichthys flesus*) (Knight et al., 2006). New techniques allow for the generation of dense genetic marker maps, which can be used in association studies to link markers to quantitative traits. The use of dense genetic maps can help identify causal genes in natural hybrid populations, but it requires the analysis of a large number of individuals and can be costly. Methods for identifying genes under selection fall into two categories: those that test divergence between populations at specific loci and those that test genetic variation within populations (Bernatchez, 2016). Tests for selective sweeps do not require crosses and can be performed on field-collected material but require a high-density map (Hoffmann & Willi, 2008). The approach relies on examining allele divergence and variation in allele size at markers located near expressed genes. This approach can identify loci with reduced diversity and/or variation, potentially linked to candidate genes under selection. Most genes are conserved in related organisms, suggesting that sets of candidate genes have the potential to be applied in related species (Wright et al., 2020). The predictability of adaptation can be improved by considering ecological context and the type of environmental stresses (Kristensen et al., 2020). There are universal mechanisms present at the organism level to cope with environmental stresses, and genetic changes in microorganisms seem to have some degree of predictability (Deans, 2021). Changes in allele frequencies can indicate the presence and impact of stress factors on populations (Ament-Velásquez et al., 2022; Brennan et al., 2022). Genetic variation influences traits under selection in natural populations, and changes at candidate markers are associated with adaptive changes. Alleles in structural or regulatory gene sequences are favored in new environments, accelerating adaptation (Lasky et al., 2023). Technological advances allow for rapid identification of genetic changes and candidate genes (Singh et al., 2016). Developing a high density of polymorphic markers allows for the isolation of candidate genes through association studies and marker scans (Hoffman & Willi, 2008; Pang et al, 2020; Tibbs Cortes et al., 2021).

Transition to Omics Technologies
The transition to omics technologies in ecotoxicology represents a significant shift towards a more comprehensive and high-throughput approaches for assessing the effects of contaminants on the environment and organisms. Omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics, enabled a simultaneous analysis of thousands of genes, transcripts, proteins, or metabolites within biological systems. This represents a holistic understanding of how organisms respond at molecular level to chemical exposures, providing insights into complex biological pathways and mechanisms of adapting to toxicity. Studies which integrated omics technologies into ecotoxicological studies could identify with greater accuracy and sensitivity biomarkers linked to stressors exposure and effects. These biomarkers can serve as early warning indicators of environmental stressors and predict ecological risks associated with chemical contaminants. Overall, the adoption of omics technologies in ecotoxicology enhances environmental health assessment, prediction of the potential ecological impacts, and development of more effective strategies for environmental management and conservation. The toxic effect of stressors could be assessed by Omics technologies combining toxicogenomics (Ankley et al., 2006) with transcriptomics and proteomics (De Wit et al., 2010). Biomonitoring of aquatic and terrestrial environments focuses on measurable biomarkers at different biological levels. Biomarkers can measure the effects of

pollutants or the host's response at biochemical, cellular, and molecular levels, and sometimes can also indicate effects at the whole organism level, such as behaviour, energy or metabolic processes. For example, marine mollusks are efficient indicators of xenobiotic impacts in the marine ecosystem. The term "exposure biomarkers" indicates that the organism has been exposed to pollutants, while "effect biomarkers" or "stress biomarkers" measure the organism's response to these pollutants (Paniagua-Michel et al., 2016). Technological advancements have led to the discovery and validation of new environmental biomarkers under the omics era (Garcia-Reyero & Perkins, 2010). Recent applications of omics technologies have redefined the roles of biomarkers in environmental biotechnology, with concurrent analyses of common biomarkers and new technologies for optimizing metabolic networks. Biomarkers, mainly molecular markers such as genes, proteins, and metabolites, are used for disease diagnosis and prognosis, as well as predicting response processes in living cells. Biomarkers include a variety of measures of the molecular, biochemical, cellular, and physiological responses of specimens of key species to exposure to contaminants or physical stressors (Kadim et al., 2022). Omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, are used to identify and quantify network components and interactions (Ebner, 2021). Recent developments in the analysis of mixed microbial communities, using culture-independent molecular tools, have brought new perspectives on catabolism in extreme and fragile environments. These approaches pave the way for identifying new biomarkers, increasing biodiversity expectations by about 99% compared to conventional classification. The use of functional genes through metagenomic matrices will enhance understanding of microbial interactions and metabolism, facilitating the development of appropriate strategies for environmental bioremediation. Genomic studies and metagenomic sequencing provide holistic approaches to microbial communities, enabling biotechnological exploitation. The use of 16S ribosomal RNA often precedes metagenomic analysis and can guide technological choices. Culture-independent technologies and next-generation sequencing have revealed a high microbial diversity, amplifying the knowledge about these communities (Sunagawa, 2015; Charles et al., 2017).

An ecotoxicological system is vast complex, consisting of numerous components and interactions, which could not be analyzed and understood separately. Omics technologies allow a deeper understanding of system biology and the complex interactions among different molecular components in living organisms. The fundamental principle of systems biology is that the emergence of disease or exposure to chemicals disrupts a network of biological pathways in the organism (Tantardini et al., 2019). They are used in a wide range of fields, including biology, medicine, ecology, and biotechnology, and are of great importance in studying ecological phenomena such as ecotoxicology, by providing detailed and comprehensive data on organism responses to stress factors and the environment (Sharma et al., 2022; Ebner, 2021). Once network components and interactions are clarified, quantitative predictions could be made about organism adaptive response and recovery from chemical stressors.

Future trends
There is a need in ecology, and not only there, to have a comprehensive view of how chemicals and other stressors impact organisms and ecosystems. Systems biology in ecotoxicology employ systemic approaches by integrating information about interactions between biological molecules, metabolic pathways, genes, and the surrounding environment to better understand organisms' responses to toxic substances. This combined approach provides valuable information for systems biology to identify effects undetectable by isolated technologies (Ankley et al., 2006; Garcia-Reyero & Perkins, 2010). Systems biology, already in work for human health, is finding a growing place in ecotoxicology by tackling future directions such as i) development and application of mathematical and computational models to simulate and predict the effects of chemical on organisms and ecosystems. These models can be used to assess ecotoxicological

risk and guide environmental management; ii) utilization of omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics, to identify biomarkers and molecular pathways involved in the response to ecotoxicological stress. These information can be used to develop diagnostic tests and pollution monitoring; iii) integration of data from different levels of biological organization, from the molecular level to the ecosystem level, to obtain a deeper understanding of the impact of chemicals on biodiversity and ecosystem functioning; iv) exploration of interactions between chemical stressors and environmental stressors, such as climate change and terrestrial pollution, to evaluate complex risks to the environment and human health. Ongoing research efforts aim to advance the development and application of bioindicators and biomarkers to enhance environmental monitoring, risk assessment, and biotechnological applications. Research directions in the development of bioindicators and biomarkers in ecotoxicology and biotechnology it should focus on identification of novel biomarkers and integration of omics technologies. Standardized protocols and assays for measuring biomarkers are also essential for ensuring reliability and comparability of results across different studies and laboratories. Research focuses on developing and validating robust methods for biomarker analysis. Future researches should aim to develop application of high-throughput screening and development of non-invasive biomonitoring techniques. Non-invasive biomonitoring techniques, such as remote sensing, bioimaging, and biosensors, are gaining attention for their ability to monitor environmental quality without harming organisms. The future research should to improve the sensitivity and specificity of these techniques for detecting biomarkers in situ. Having in view that bioinformatics and data analytics play a crucial role in processing and analyzing large omics datasets generated from biomarker studies the future research should focuses on developing computational tools and algorithms for data integration, interpretation, and visualization. Understanding the ecological relevance of biomarkers is essential for their application in ecotoxicology. Research explores the relationship between biomarker responses and ecological endpoints to assess the overall health and resilience of ecosystems. Last but not least field-based validation studies are necessary to assess the performance and applicability of biomarkers under real-world environmental conditions. Research involves conducting longitudinal studies in natural environments to evaluate the efficacy of biomarkers in predicting environmental health.

CONCLUSIONS

The levels of contaminants have increased in terrestrial and aquatic environments due to the raise of anthropogenic activities. Their complex harmful impact on the environment should be analyzed by a rapid assessment of the impact followed by an implementation of appropriate corrective measures. These continuous exposures to anthropogenic pollutants can also affect physiological processes which make more imperative to have a reliable bioscreening programs. The development and application of testing procedures at the experimental model level to highlight the impact of contaminants on both aquatic and terrestrial environments are essential for efficient screening of environmental matrix toxicity. Research and application strategies for biomarkers development in ecotoxicology are linked to specific environmental matrices, organisms and pollution. Thus, the development of non-invasive and non-destructive biomarkers will allow long-term monitoring without affecting the health or integrity of organisms. A series of steps must be taken, starting from the identification and validation of biomarkers, followed by the characterization of their responses for an efficient assessment of the anthropogenic stressors impact. Overall, long-term monitoring can be carried out to identify trends over time, and by combining biomarkers with other methods for assessing ecological risks, such as standardized toxicity tests and ecological modelling, a more comprehensive understanding of the impact of anthropogenic factors on the environment can be achieved. These studies contributed to the understanding of environmental adaptation and evolution to new anthropogenic challenges followed by new environmental policies.

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