BIOTECHNOLOGICAL ADVANCEMENTS IN ECO-TOXICOLOGY PERTAINING TO MARINE-BIOME

Abstract

Modern biotechnology advancements are transforming marine ecotoxicology in dramatic ways. Environmental DNA (eDNA), DNA barcoding, and Next-Generation Sequencing (NGS), which are all based on DNA, are at the forefront of methods that allow for accurate species identification, biodiversity monitoring, and in-depth research of marine microbial communities. In addition, satellite remote sensing, a key component of imaging methods, provides unmatched capabilities for monitoring water quality and identifying variations in it. Another notable advancement is the development of biosensors, particularly enzymatic versions that can detect environmental toxins like pesticides and heavy metals. Additionally, there is a greater emphasis on comprehending nanomaterial interactions with aquatic creatures because of the widespread use of nanotechnology.

But these technologies immense potential also comes with several problems. They produce vast amounts of data that require sophisticated computational abilities to analyse. The landscape is further complicated by addressing the complex interactions of emerging contaminants in marine environments and ensuring uniform techniques across research venues. However, the future seems bright. A comprehensive understanding of marine ecosystems is possible thanks to the convergence of multi-omics data, sophisticated bioinformatics, high-throughput screenings, and non-invasive monitoring approaches. The implications of nanoparticles for eco-toxicology also call for additional investigation given the growing interest in nanotoxicology. These technology

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advancements have the potential to fundamentally alter how we perceive, manage, and conserve aquatic habitats.

I. INTRODUCTION

Marine ecosystems play a crucial role in supporting biodiversity, providing essential resources, and regulating global climate patterns. Unfortunately, these critical environments are facing increasing threats due to human activities, resulting in the release of toxic pollutants into marine waters. This has led to growing concerns about the consequences of marine pollution on marine organisms and ecosystems, leading to the emergence of a multidisciplinary field known as marine eco-toxicology. Marine eco-toxicology encompasses a diverse range of scientific disciplines, including ecology, toxicology, environmental chemistry, and molecular biology, aimed at understanding the interactions between pollutants and marine life. This field endeavors to assess the ecological implications of toxicant exposures and their potential impacts on marine ecosystems and human health. The sources of marine pollution are extensive, originating from industrial discharges, agricultural runoff, coastal development, shipping activities, and improper waste disposal (USEPA, 2009; GESAMP, 2020). These pollutants include chemical contaminants, heavy metals, plastics, pesticides, pharmaceuticals, and hydrocarbons (Galloway et al., 2017; Lebreton et al., 2018; Lusher et al., 2016). The accumulation of these toxic substances in marine environments can lead to adverse effects on marine organisms, including developmental abnormalities, reproductive impairments, immune system disruptions, and even mortality (Oost et al., 2002; Rakib et al., 2023).Marine eco-toxicology plays a vital role in identifying potential risks to marine ecosystems and species and guiding the development of effective environmental management and conservation strategies. Researchers utilize molecular biomarkers, bioassays, and other advanced biotechnological tools to decipher the mechanisms of toxicity, detect subtle effects of pollutants, and monitor marine pollution levels.

II. BIOTECHNOLOGICAL TOOLS FOR MARINE ECO-TOXICOLOGY

Molecular biomarkers for marine eco-toxicology include bioaccumulation markers, biomarkers of exposure (CYP1A, EROD, SOD, LPOX, HSP, MT, DNA strand breaks, micronuclei, apoptosis), and biomarkers of effect. As per Kroon, F (et al., 2017) water bodies have accumulated the metals aluminum (Al), Cd, Cu, gallium (Ga), Pb, selenium (Se), Zn, As, cobalt (Co), Cr, iron (Fe), mercury (Hg), Mn, and Ni (Gladstone Harbour Report Card 2015, Angel et al., 2010, Angel et al., 2012, Apte et al., 2006, Arango et al., 2013, Mortimer et al., 2013, Port Curtis Ecosystem Health Report 2011, Anastasi et al., 2010,Update on the quality of sediment from Port Curtis and Tributaries. 2012)from anthropogenic activity and natural sources. From marine water heavy metals accumulate in different parts of fish like gills, liver, and muscle. It was analysed through the ICP and AAS methods (Waltham et al., 2011, Chen et al., 2002, De Mora et al., 2004, Hariharan et al., 2016, Soto et al., 2008, Jara et al., 2009, Padmini et al., 2009, Kalantzi et al., 2014, Nel et al., 2015, Peters et al., 1999). Omics technologies, including genomics, proteomics, and metabolomics, have revolutionized the field of marine eco-toxicology by offering comprehensive insights into the molecular responses of marine organisms to pollutants. These high-throughput approaches allow researchers to analyze thousands of genes, proteins, or metabolites simultaneously, providing a detailed understanding of the pathways and processes affected by toxicant exposures.

1. **Genomics: Unraveling Genetic Responses:** Genomics explores the entire genome of marine organisms to identify genetic variations associated with pollutant tolerance or sensitivity. By analyzing the gene expression profiles of marine organisms exposed to

pollutants, researchers can identify specific genes involved in stress responses, detoxification mechanisms, and adaptation processes (Ralph et al., 2010). For instance, the upregulation of certain genes, such as cytochrome P450, Glutathione-S-transferase, and metallothionein, indicates the activation of detoxification pathways in response to pollutant exposure Additionally, the identification of single nucleotide polymorphisms (SNPs) associated with pollutant tolerance provides valuable information for understanding the adaptive capacity of marine species in polluted environments. From a metabolic perspective, pollution impacts the health of eels because key genes involved in energy synthesis, respiration, and RNA expression are down regulated in Tiber fish, possibly contributing to the individuals' low levels of energy (Pujolar et al., 2012).

2. **Proteomics: Understanding Protein Responses:** Proteomics investigates how contaminants affect the expression of proteins and post-translational modifications in marine species. Researchers can identify specific proteins essential for pollutant detoxification, oxidative stress responses, and cellular repair mechanisms by analyzing the proteome of marine creatures exposed to toxicants (Bassey et al., 2019). For instance, an increase in stress-related proteins, such as heat shock proteins and metallothioneins, shows that cellular defense systems are being activated in response to toxicant exposure. Additionally, the discovery of protein biomarkers can be a useful tool for tracking pollution exposure levels and assessing the general well-being of marine animals.

3. **Metabolomics: Profiling Metabolic Responses:** Metabolomics investigates changes in metabolic pathways and small molecule metabolites as a response to toxic substances. Through the analysis of metabolic profiles in marine organisms exposed to pollutants, researchers can detect disturbances in particular metabolic pathways and specific metabolic signatures that indicate pollutant exposure. For example, compared to conventional parameters like morphology and physiological responses, metabolomics approaches provide higher sensitivity, as observed in various parameters such as carcass weight, standard length, and CF, with most showing minimal or no differences between sites (Long et al., 2020).

4. **Acute Toxicity Tests:** Acute toxicity tests are short-term experiments designed to determine the lethal effects of pollutants on marine organisms within a relatively brief exposure period. These tests are conducted with a wide range of marine species, including marine crustaceans, fish, and mollusks. Commonly used marine organisms in acute toxicity tests include the Brine shrimp Artemia salina, mysid shrimp, zebrafish Danio rerio, and mussels. Acute tests involve exposing the organisms to various concentrations of pollutants for a specified period, and the observed mortality or adverse effects are used to establish lethal concentration values, such as LC50 (the concentration at which 50% of the exposed organisms exhibit mortality).

5. **Chronic Toxicity Tests:** Chronic toxicity tests are long-term experiments that assess the sublethal effects of pollutants on marine organisms over extended exposure periods. Unlike acute tests that focus on immediate lethal effects, chronic tests provide insights into the cumulative impacts of chronic exposure to low concentrations of pollutants. These tests are particularly relevant for evaluating the effects of pollutants on the growth, reproduction, and survival of marine organisms. Chronic bioassays are conducted using

various marine species, such as fish, crustaceans, and bivalves, to understand the long-term effects of pollutants on their overall health and well-being.

6. **Biochemical Biomarker Assays:** Biochemical biomarker assays measure specific molecular or enzymatic responses of marine organisms exposed to pollutants. These biomarkers serve as early indicators of exposure to toxicants and can provide valuable information about the cellular responses to pollutants. For example, oxidative stress biomarkers, such as superoxide dismutase and catalase, indicate the activation of antioxidant defense systems in marine organisms exposed to pollutants. Phase I and II detoxification enzymes, such as cytochrome P450 and glutathione-S-transferase, are also commonly measured as biomarkers of exposure to specific classes of pollutants. Biochemical biomarker assays offer insights into the cellular mechanisms involved in pollutant detoxification and can help identify the presence and potential impacts of pollutants in marine environments.

7. **Behavioral Bioassays:** Behavioral bioassays assess changes in the behavior of marine organisms in response to pollutants. These tests are based on the premise that certain pollutants can alter the normal behavioral patterns of marine species. For example, exposure to pollutants may lead to altered swimming behavior, feeding activity, or shoaling behavior in fish, which can serve as indicators of toxicity. Behavioral bioassays are particularly relevant for understanding the sublethal effects of pollutants on marine organisms' ecological interactions and survival strategies. By observing behavioral changes, researchers can gain insights into the potential impacts of pollutants on marine species' overall health and fitness.

8. **Microbiological Assays:** Microbiological assays use specific microorganisms as indicators of toxicity in marine environments. Bacteria and algae are commonly employed in these tests to assess the effects of pollutants on microbial communities. Changes in microbial growth, photosynthetic efficiency, or respiration rates can indicate the presence and toxicity of pollutants in marine waters. Microbiological assays provide valuable information about the potential impacts of pollutants on the base of the marine food web and the overall health of marine ecosystems.

9. **Whole Organism Bioassays:** Whole organism bioassays involve using intact marine organisms, such as fish, crustaceans, or algae, to evaluate the overall toxic effects of pollutants. These assays consider the integrated responses of the entire organism, providing a more holistic assessment of pollutant toxicity. Whole organism bioassays are valuable for understanding the ecological implications of pollutants on marine populations and communities. These tests allow researchers to assess multiple endpoints, such as growth, reproduction, behavior, and survival, simultaneously, offering a comprehensive understanding of the effects of pollutants on marine organisms' fitness and ecological roles.

III. DNA-BASED TECHNIQUES IN MARINE ECO-TOXICOLOGY

1. **DNA Barcoding:** DNA barcoding is a molecular technique that utilizes a short standardized DNA fragment to identify and assess biodiversity in marine ecosystems. By analyzing specific gene regions, such as the mitochondrial cytochrome c oxidase I (COI)

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gene in animals, researchers can accurately identify various marine species. DNA barcoding provides rapid and precise species identification, making it invaluable for monitoring changes in marine biodiversity and assessing the impacts of pollutants on different species. Rapid species identification by DNA barcodes has been applied in forensic research, food supply chain management, and disease understanding, among other domains (Antil et al 2023).

2. **Environmental DNA (eDNA):** Environmental DNA (eDNA) is a non-invasive DNA-based technique that involves collecting and analyzing DNA fragments shed by organisms into their surrounding environment. In marine eco-toxicology, eDNA is used to monitor aquatic biodiversity and detect invasive species without direct contact with the organisms. By analyzing DNA samples from water or sediment, researchers can identify the presence of various marine species and assess changes in community compositions caused by pollutants or invasive species introductions. The use of eDNA for biodiversity monitoring may offer a quick, low-cost, and standardized method to gather vital data on the distribution and population size of subterranean and aquatic invasive species, enabling more efficient use of limited conservation resources and taxonomic knowledge. For studies that seek to pinpoint changes in species diversity, hotspots for invasive species, and other relevant information, as well as those that concentrate on conservation initiatives or reveal processes at the ecosystem level, the study of eDNA will be a significant source of data. As a supplement to traditional evaluation paradigms, which have been steadily enhanced over a sizable period, eDNA-based biomonitoring is currently most effectively used (Sakib et al 2023).

3. **Next-Generation Sequencing (NGS):** Next-generation sequencing (NGS) technologies have revolutionized the field of genomics, allowing for high-throughput sequencing of DNA and RNA. In marine eco-toxicology, NGS is applied to assess microbial communities and their responses to pollutants and environmental changes. Metagenomic and metatranscriptomic analyses using NGS provide comprehensive insights into the functional and taxonomic diversity of marine microbial communities, helping researchers understand how pollutants influence these vital components of marine ecosystems.The genomes and transcriptomes of fish and other aquatic creatures can now be partially or completely read using NGS methods. This data has helped create linkage maps, look for QTLs, reveal and/or reconsider population structures and phylogenetic relationships, identify the functions of different genes, identify SNPs (mutations) and their effects on the physiology of organisms, identify species and the origin of populations, etc (Kumar et al., 2017).

IV. REMOTE SENSING AND IMAGING TECHNIQUES

1. **Satellite Remote Sensing:** Remote sensing techniques have become vital tools in monitoring water quality, particularly in compliance with the Water Framework Directive (WFD). Satellite remote sensing provides extensive coverage of water bodies, offering frequent temporal updates and cost-effectiveness. The strength of remote sensing lies in its ability to provide both temporal and spatial views of surface water quality parameters, which are challenging to obtain through in situ measurements (Sheela et al., 2011; Sheela et al., 2013).Remote sensing enables the identification of significant water quality issues in water bodies and facilitates efficient landscape monitoring for the development of

management strategies (Sheela et al., 2013). Regression models using landscape TM images aid in assessing salinity, depth, and organic matter. Modern satellites can detect various parameters, including chlorophyll a and phycocyanin for green algae, total suspended matter, dissolved organic matter (yellow substance), water transparency, and surface temperature (Odermatt et al., 2012).Additionally, remote sensing provides valuable information on catchment features, such as changes in land cover and distribution of aquatic vegetation over spatial and temporal scales. Traditional methods for catchment monitoring are time-consuming and suitable only for smaller areas. In contrast, remote sensing offers repeatability and synoptic spatial coverage, making it a practical and feasible solution for larger-scale processes (Glasgow et al., 2004; Odermatt et al., 2012, Tavakoly et al., 2014).

V. BIOSENSORS AND NANOTECHNOLOGY

1. **Biochemical Sensors:** Naresh et al. 2021 describe the workings of enzymatic biosensors. There are two different types of mechanisms of action: either the enzyme metabolizes the target analyte or the analyte activates, inhibits, or modifies the enzyme. the use of terrestrial and aquatic biota as microbial biosensors to identify environmental pollutants, such as pesticides, heavy metals (As, Cu, Hg, Pb, or Cd), phenols, and other hazardous chemicals (Bilal et al., 2019, Chung et al.,2021, Gupta et al., 2019, Do M.H et al., 2016, Gavrilaș et al., 2022). Over the past few years, various other microbial biosensors have also been produced and proposed, with outstanding environmental monitoring potential.

2. **Nanotechnology Applications:** Griffitt et al. 2008 investigated the impact of particle composition on the toxicity of metallic nanomaterials in aquatic organisms. They utilized zebrafish, daphnids, and algal species as representative models for various trophic levels and feeding strategies. The study exposed different organisms to silver, copper, aluminum, nickel, and cobalt in the form of nanoparticles and soluble salts, along with titanium dioxide nanoparticles. Their findings revealed that nanosilver and nanocopper exhibited toxicity in all tested organisms, with 48-hour median lethal concentrations as low as 40 and 60 µg/L, respectively, in Daphnia pulex adults. In contrast, titanium dioxide did not demonstrate any toxicity in the conducted tests (Ray et al., 2009). Asharani et al. 2008 also reported on the toxicity of silver nanoparticles in zebrafish models. To explore their deleterious effects and distribution patterns in zebrafish embryos, silver nanoparticles were synthesized using starch and bovine serum albumin (BSA) as capping agents. (Ray et al., 2009)

VI. CHALLENGES AND FUTURE DIRECTIONS

1. Challenges in Applying Biotechnological Approaches:

- **Handling Data Complexity:** The sheer volume of data generated by biotechnological techniques can be overwhelming to analyze and interpret. Effectively integrating omics data and utilizing bioinformatics analyses to comprehend the intricate interactions between pollutants and marine organisms require advanced computational tools and multidisciplinary expertise.

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- **Ensuring Consistency and Replicability:** Maintaining uniformity and reproducibility of biotechnological assays across different laboratories is crucial to obtaining reliable and comparable results. Inconsistencies in experimental conditions and protocols can lead to discrepancies in findings, impeding the advancement of marine eco-toxicology.

- **Addressing Novel Pollutants and Mixtures:** The discovery of new pollutants and the presence of pollutant mixtures in marine environments present challenges in assessing their eco-toxicological impacts. Traditional biotechnological approaches may not fully capture the complex interactions and cumulative effects of these pollutants on marine ecosystems.

VII. FUTURE RESEARCH DIRECTIONS AND EMERGING BIOTECHNOLOGIES

1. **Integration of Multi-Omics:** By combining diverse omics data, including genomics, proteomics, and metabolomics, researchers can gain a holistic understanding of how marine organisms respond to pollutants. Multi-omics approaches will uncover novel biomarkers and shed light on complex toxicological pathways.

2. **Harnessing Advanced Bioinformatics:** The progress of bioinformatics tools and machine learning algorithms will revolutionize data analysis and enhance the predictive power of eco-toxicological assessments. Standardized bioinformatics pipelines will promote collaboration and data sharing among researchers.

3. **Embracing High-Throughput Screening:** Cutting-edge screening technologies, like automated robotic systems and microfluidic platforms, enable efficient evaluation of the toxicity of numerous chemicals simultaneously. This empowers researchers to prioritize chemicals for further eco-toxicological investigations.

4. **Pioneering Non-Invasive Monitoring:** Innovative non-invasive techniques, such as environmental DNA (eDNA) sampling and remote sensing, allow real-time monitoring of marine ecosystems without disturbing marine life. These methods yield crucial information on environmental changes and pollutant levels.

5. **Unraveling Nanotoxicology:** With the growing utilization of nanotechnology in various industries, exploring the toxicity of nanomaterials in marine ecosystems is vital. Nanotoxicology studies will provide insights into the potential risks nanoparticles pose to marine organisms and the environment.

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