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Bioindicators: A Promising Tool for Detecting and Evaluating Water Pollution

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ABSTRACT

The aquatic environment has various living resources, making it unique among other environments and economically significant. The lack of guidance, environmental awareness, and health control has decreased water quality, impacting living organisms and humans along the food chain. This is due to the increasing number of pollutants, including physical, chemical, radioactive, and infectious microscopic biological substances, that infiltrate water resources, raising concerns about the aquatic environment. Traditional chemical and physical assays for pollution detection have some limitations, such as providing information only during sampling and requiring expensive analysis for sensitive detection of contaminants when present in very low concentrations. In contrast, biological communities (bioindicators) offer reliable and cost-effective tools for assessing toxic pollutants. Various bioindicators, such as plants, plankton, animals, and microorganisms, are considered vital indicators for detecting water pollution compared to their benefits and drawbacks. Using indicator species as ecological indicators is a reliable and cost-effective way to assess environmental changes and reflect the overall water quality by integrating the effects of various stressors over time. Therefore, bioindicators are considered a valuable model for predicting the presence and extent of pollution (acting as an early warning system) before the onset of pollutant effects and characterizing ecosystem health.

INTRODUCTION

The increasing population and industrialization have led to the generation of significant amounts of wastewater from various human activities, including residential, industrial, and municipal operations, as well as those occurring by the aquatic ecosystems. This effluent frequently constitutes a substantial contaminant of water sources since it typically flows into rivers and streams without undergoing any treatment **(Bhatia *et al*., 2020; Taha *et al*., 2023b)**.

Water, an invaluable natural resource, is essential for the life of human beings **(Ahmed *et al*., 2011)**. Life support systems are complex and ever-changing, comprising both living and non-living elements, as well as soluble and insoluble organic and inorganic substances. Alterations in water quality have the potential to disturb the equilibrium of aquatic ecosystems, rendering them unfit for their intended functions. Securing freshwater (surface and groundwater) resources from contamination is crucial due to the fact that just 1% of freshwater is suitable for drinking, agricultural, and residential use **(Karthika & Dheenadayalan, 2015)**.

Human-induced disruptions pose a significant threat to global freshwater ecosystems **(Taha *et al*., 2023b)**. Physical habitat disturbances caused by recreational activities **(Barletta *et al*., 2010)**, dam building, agricultural chemicals, and urbanization **(Kripa *et al*., 2013)** have a significant impact on aquatic ecosystems. The primary source of water contamination on a global scale has been attributed to agricultural pollutants **(Jayawardana *et al*., 2017)**. The excessive use of fertilizers, hormones, and pesticides in agriculture, together with the presence of heavy metals, often leads to the contamination of water bodies **(Kripa *et al*., 2013; Taha *et al*., 2023b)**. The pollution can have hazardous impacts on aquatic organisms, either promptly or over an extended period, possibly leading to changes in the species composition of these ecosystems **(Brühl & Zaller, 2019)**. In addition, there is a tendency for persistent organic pollutants and heavy metals to build up in aquatic food chains, which can have an adverse effect on species that were not the primary focus of concern **(Cui *et al*., 2015; Taha *et al*., 2023b)**.

The current water quality evaluation methods mostly consist of laboratory-based analyses, focusing on chemical and physical testing. Chemical parameters encompass measurements of redox potential, salinity, as well as biological and chemical oxygen demand. The physical characteristics of the environment, such as the surrounding temperature, nutritional levels, presence of pollutants, amount of accessible light, and gas concentrations, are directly quantified by measurement **(Holt & Miller, 2010)**. These processes need a significant amount of time, specialized staff, and new chemical reagents. The increasing want for straightforward, dependable, and instantaneous techniques to identify pollutants and impurities has stimulated the advancement of remote detection and monitoring systems that use biological indicators such as microbes, plants, and animals **(Korostynska *et al*., 2013)**. Monitoring pollution levels is crucial not only for developing effective pollutant control management methods but also for implementing restorative bioremediation techniques **(Garg *et al*., 2022)**.

Generally, biomonitoring and bioindication have more precise definitions. Bioindicators assess biological reactions to ecological stress in a qualitative manner, whereas biomonitors measure and quantify these responses. The "bioindicator" is a specific term used to describe any terms associated with the identification of biological responses to ecological constraints **(Gökçe, 2016)**. Biological indicators (bioindicators) have been utilized in bioassays to analyze specific chemicals in water, sediment, and soil samples. These bioassays help evaluate the ecological toxicity of these substances in various matrices **(Viegas, 2021)**. Due to their high sensitivity and ability to be replicated, bioindicators provide a substantial deviation from standard ways of measuring environmental quality and provide several benefits over traditional physical or chemical pollution detection techniques. Assessing the effects of indirect pollution through bioaccumulation poses significant difficulties when using chemical or physical assays. As toxins and other micropollutants build up in living creatures, the concentration of metals in food chains also increases. According to **Holt and Miller (2010)**, this buildup can lead to assessments that underestimate the actual amounts of contamination in higher trophic levels. Bioindicators are essential tools for identifying environmental contamination. Therefore, our study emphasized the significance of employing diverse bioindicators as possible instruments for recognizing and detecting water pollution.

1. Bioindicators

Naturally occurring bioindicators are employed to evaluate environmental well-being and function as a crucial instrument for detecting environmental alterations, whether beneficial or detrimental, and their subsequent effects on human civilization. It is a common mistake to refer to all sources of biotic and abiotic reactions linked to alterations in particular ecosystems as "bioindicators" **(Zaghloul *et al*., 2020)**. Bioindicators are defined as organisms that employ living things, such as microorganisms, plankton, plants, and animals, to detect pollution in a specific area. They are reproducible, objective, helpful, and relevant at different scales **(Parmar *et al.*, 2016)**. Taxa are not only utilized as indicators of natural changes but also to illustrate the consequences of environmental or ecosystem changes. They are frequently employed in natural ecosystems to indicate either positive or negative consequences **(Holt & Miller, 2010; Zaghloul *et al*., 2020)**.

The abundance of bioindicators in the environment is affected by several elements, including temperature, light, water, and suspended particles. Bioindicators may be utilized to forecast the ecological state or the degree of pollution in a certain region **(Khatri & Tyagi, 2014; Parmar *et al*., 2016)**. When considering an ecosystem, it is crucial to carefully choose biological indicators, such as species or groups, that are well-known and specific to the area's disturbances. Ecologists have recently developed a complete set of criteria that must be met for organisms to be used as good indicators (Fig. 1).

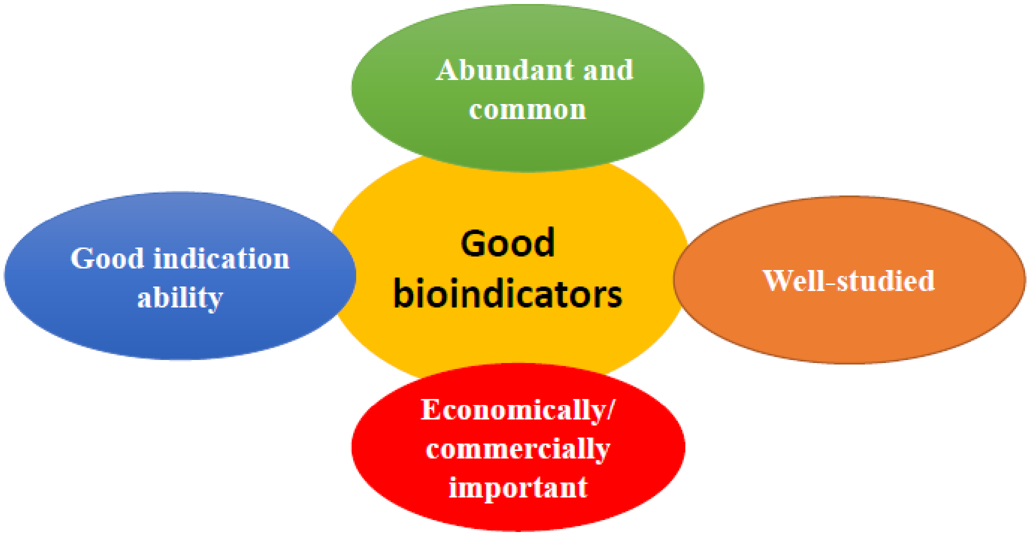

Fig. 1. Briefly key characteristics of good bioindicators

2. The positive and negative aspects of bioindicators

Like every instrument used for management, it is crucial to understand its limits. Nevertheless, the benefits of bioindicators outweigh their drawbacks. Bioindicators may be employed across many levels of measurement, ranging from cellular to ecological, in order to evaluate the overall well-being of the ecosystem. They combine information from the physical, biological, and chemical elements of our surroundings, which appear as changes in the number of organisms, ecological activities, individual health, and composition of the community. Bioindicators assist in determining the biological feasibility of management actions.

Every organism within a biological system has the potential to serve as a bioindicator of its environment. The intensity of a bioindicator serves as a warning flag for contamination before it becomes too late. Bioindicators have many advantages that have led to their usage and inclusion in several international agreements by legislative authorities. They are widely utilized to identify the expected detrimental effects of contaminants on biota as well as the synergistic and antagonistic effects of several pollutants **(Zaghloul *et al*., 2020)**. Bioindicators offer the advantage of complementing traditional chemical assays and direct physical measurements of water, such as temperature, salinity, nutrients, pollutants, light, and gas levels. They provide a comprehensive assessment of water quality by integrating the cumulative effects of different stressors over time. Furthermore, there is a possibility of the presence of pollutants in very small amounts, which necessitates the use of costly and intricate studies employing very sensitive technology for detection. Conversely, regular surveillance of biological populations is a dependable and economical approach when compared to evaluating harmful contaminants **(Jindal & Sharma, 2011)**. In addition, bioindicators can reveal the secondary biological impacts of contaminants that may go undetected by physical or chemical assessments. Phosphorus enrichment in a lake can cause certain species to develop and reproduce more, as postulated in the study of **Khan and Ansari (2005)**.

There are some drawbacks to using bioindicators. First and foremost, it might be difficult to differentiate between changes caused by humans and those that occur naturally and also limits their ability as scale-dependent which restricts the application of bioindicators in various environments **(Holt & Miller, 2010; Zaghloul *et al*., 2020)**. Moreover, an imbalanced number of responses from different species might obscure a robust biological indication signal, as some species may have a rise while others experience a fall **(Zaghloul *et al*., 2020)**. In addition, the bioindication signal might be complex due to the diverse reactions observed across different species (some may flourish while others decline). The benefits of this method over its drawbacks are due to its biological significance and cost-effectiveness for monitoring.

3. Classification of bioindicators

As shown in Fig. (2), bioindicators can be categorized into various groups according to the following criteria: the purpose of using bioindicators (usage) and their aims and application **(Muhar *et al*., 2000; Butterworth *et al*., 2001)**.

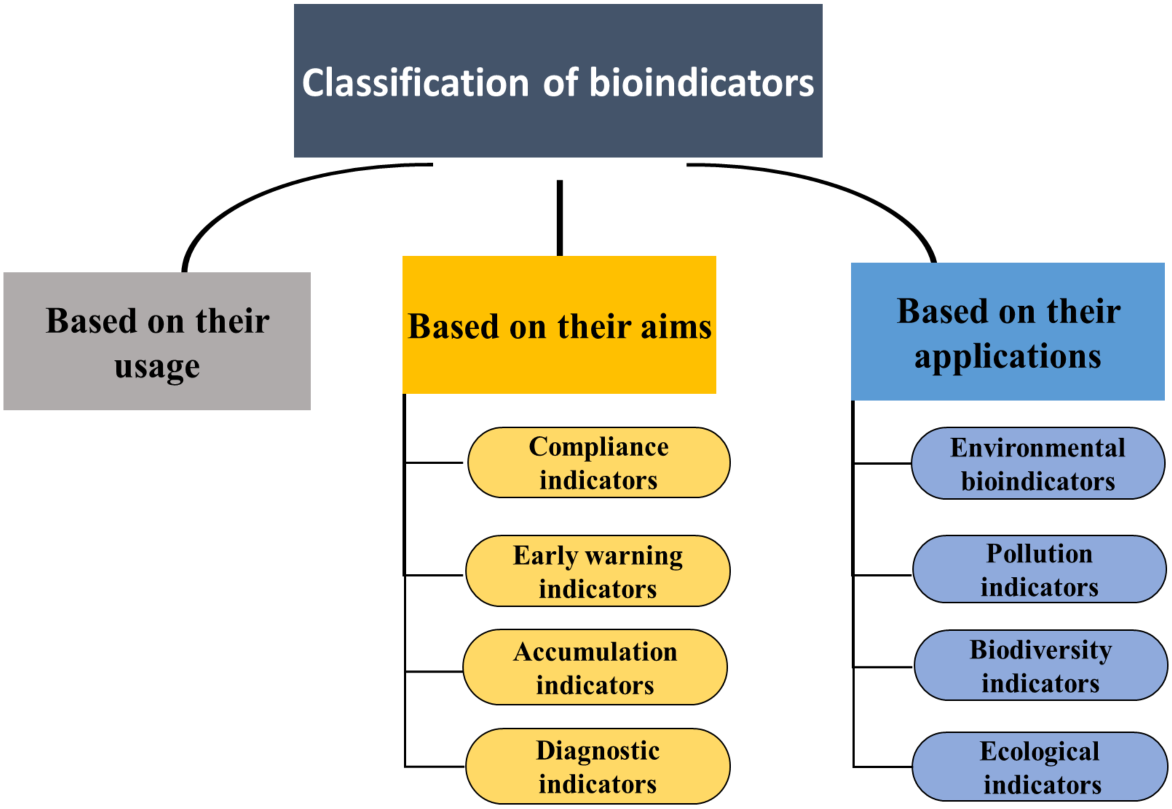

Fig. 2. Classification of bioindicators

3.1. Based on their usage

There are three situations when bioindicators are helpful:

1. In cases where the intended environmental component is not measurable, such as when historical environmental factors, such as climatic change, are rebuilt and investigated in paleo biomonitoring.
2. When the indicated factor is challenging to quantify, such as in the case of complex toxic effluents with multiple interacting chemicals or pesticides and their residues.
3. When the environmental factor is simple to quantify but challenging to interpret, such as in the case of whether the observed changes have ecological significance.

3.2. Based on their aims (objectives)

- i. **Compliance indicators:** Indicators that help verify the achievement of maintenance or restoration objectives. For example, measuring fish characteristics can serve as a bioindicator for the overall sustainability of a population or community.
- ii. **Diagnostic indicators:** Indicators that assist in examining the detected environmental changes or disturbances. These indicators are generally measured on the individual or sub-organismal level.
- iii. **Early warning indicators:** Indicators that can show the first signs of disturbance in the environment. These indicators reveal signs before most other species are affected since they have very quick and sensitive responses to any environmental change.
- iv. **Accumulation indicators:** Indicators that assist in studying the effects on different biological organization levels.

3.3. Based on their applications

- i. **Environmental bioindicators:** These species respond to environmental changes, providing insights into alterations or disruptions in the ecosystem. They are crucial for diagnosing the environmental state when formulating environmental policies. Such as sentinel species, animals, and macroinvertebrates.
- ii. **Ecological bioindicators:** These are species sensitive to environmental stressors, pollution, habitat fragmentation, and other ecological disturbances, serving as ecological indicators. Examples: plant indicators and lichens.
- iii. **Biodiversity bioindicators:** These indicators reflect the species diversity within a community and are used to measure biodiversity aspects, such as genetic and landscape parameters. Examples: microbial, plant, and animal indicators.
- iv. **Pollution bioindicators:** These are organisms that produce signals as responses to the presence of pollutants in an environment, such as various plant and animal indicators.

4. Criteria for choosing bioindicators

The process of identifying dependable bioindicators is a challenging endeavor. No single species can encompass all types of environmental stress or disruption in every habitat. When choosing bioindicator species or groups, it is important to consider the particular ecosystem, the species that are found there, and any local disruptions.

Ecologists have defined specific requirements that species must fulfill to be considered good bioindicators. These criteria are briefly depicted in Fig. (1), and further elaborated in Table (1) **(Holt & Miller, 2010)**.

Table 1. Characteristics of good bioindicators

Criteria	**Definition**
Specificity	The biological reaction is unique to the specific stressor and is not influenced by other environmental stressors.
Monotonicity	The size of the biological response should reflect the strength and duration of the relevant stressor.
Variability	Consistency in the biological response should be observed across a range of spatial and temporal scales. A low background level would be the ideal variability, even if it involves a shift in variance that can indicate an impact.
Practicality	Biological response measurements should be affordable inexpensive, easy to conduct, nondestructive, and independent of observer.
Relevance	The biological reactions should be significant and ecologically relevant in the eyes of the general population to support communication.

5. Types of bioindicators

Biotas are often used as indirect indicators to determine the levels of pollutants in their ecosystems. They can also help monitor changes in population density over time that may result from ecosystem modifications. Due to the high susceptibility of many organisms to environmental pollutants, they may change morphology, physiology, or behavior. Numerous species of biota have been identified in various environments on Earth, as bioindicators shown in Fig. (3).

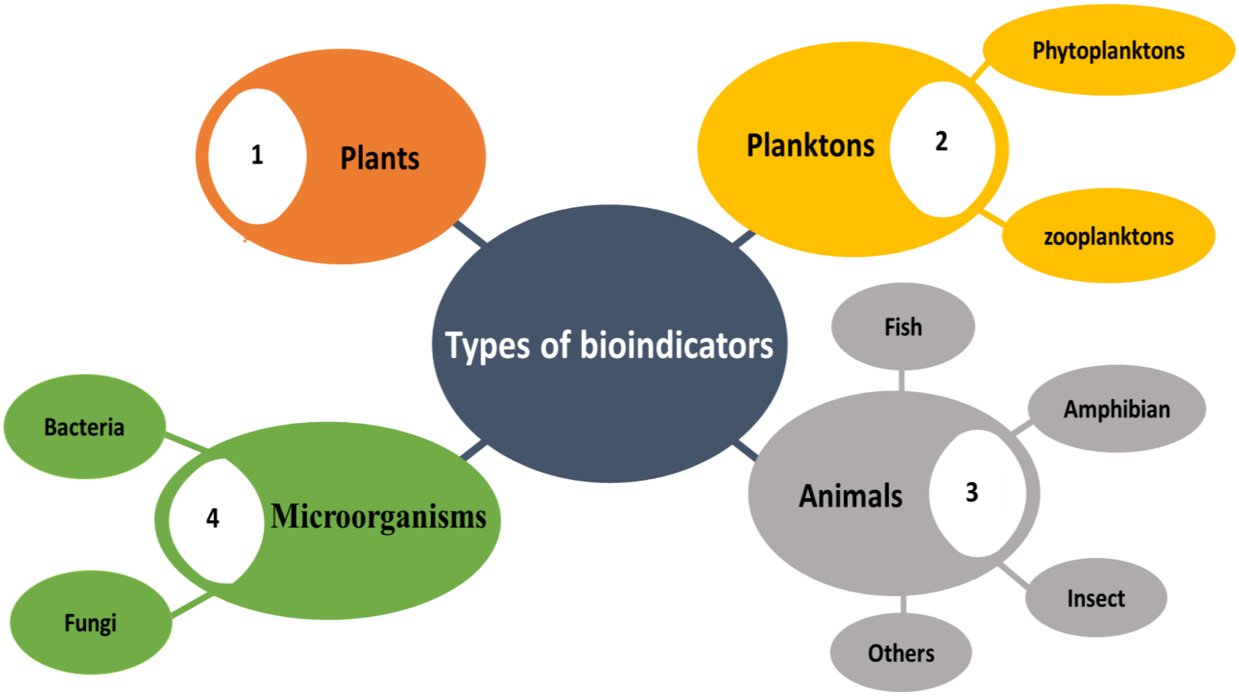

Fig. 3. Types of bioindicators

5.1. Plant indicators

Plants serve as highly responsive indicators for forecasting and identifying ecological disturbances. The pollution of land and water ecosystems has increased in recent times as a result of industrialization and urbanization. The majority of plants are sessile and possess a high capacity to acclimatize to their native surroundings, rendering them highly useful for assessing the condition of contaminated ecosystems. Plant species that are plentiful, such as higher plants and lichens, typically offer valuable information on the health of a certain ecosystem **(Zaghloul *et al*., 2020)**. The presence or absence of particular plants or vegetation provides valuable insights into the overall condition of the ecosystem **(Jain *et al*., 2010)**.

Marine plants have a vital role in predicting the condition of the marine environment since they are immobile and quickly adapt to their environments **(Plafkin *et al*., 1989)**. However, the equilibrium can be disrupted by variables such as increasing levels of sulphur dioxide (SO_2), sulphur, and nitrogen pollution (N_2), which can have a substantial influence on marine ecosystems **(Gerhardt, 2002; Holt & Miller 2010; Khatri & Tyagi, 2014)**. *Wolffia globosa* is an important bioindicator of sensitivity to cadmium and is used to indicate the presence of cadmium pollution.

5.2. Planktons

Planktons are organisms that often reside in different aquatic settings and have the ability to form dense populations that can actively move against water currents. Microscopic plankton encompasses bigger species such as certain crabs and jellyfish.

Lee and Stokes (2006) propose a fundamental taxonomy of plankton that categorizes them into two main groups: phytoplankton, which is photosynthetic and resembles plants, and zooplankton, which are animal-like. The final classification (zooplankton) is further subdivided into holoplankton and meroplankton, depending on their respective durations of existence in the aquatic environment. Holoplankton are planktonic organisms that remain in the planktonic stage throughout their entire lifespan, while meroplankton are temporary members of the zooplankton group that only spend a part of their life cycle as plankton.

Plankton exhibits rapid responses to environmental changes and are regarded as exceptional indicators of water quality and trophic status owing to their short lifespans and high rates of reproduction. The occurrence of planktonic organisms in their native habitats is associated with a variety of abilities to withstand abiotic environmental conditions (such as temperature, oxygen concentration, and pH) and interactions among different species. Plankton community variations are used to evaluate the trophic condition of aquatic bodies **(Parmar *et al*., 2016)**. Cyanophyta, a well-recognized plankton indicator, is a clear indication of fast eutrophication in aquatic habitats due to the production of blooms **(Thakur *et al*., 2013)**. Plankton, particularly those that have chlorophyll, exhibit substantial biological activity in aquatic habitats such as rivers, lakes, streams, and wetlands. They absorb and circulate significant quantities of energy, which is then transported to higher levels of the food chain **(Zaghloul *et al*., 2020)**.

5.2.1. Phytoplankton

These organisms are little aquatic creatures that hover on the surface of water and act as indicators of changes in water quality and production **(Siregar *et al.*, 2014)**. **Bazhenova and Krentz (2018)** assert that phytoplankton are well-suited for monitoring water quality since they exhibit a fast reaction to external influences. These organisms have a high level of adaptability to changes in their environment and are commonly employed to evaluate ecological modifications, which indicate important interactions within the system **(Ali & Shehawy, 2017; Wu *et al*., 2019)**. Phytoplankton, as a rule, serve as dependable bioindicators of water quality. Fluctuations in the composition of phytoplankton can provide insights into the state of water conditions, which can help evaluate its appropriateness for tourism **(Lathifah *et al*., 2020)**.

Algal species (phytoplankton) and quantities serve as reliable indicators for assessing water quality. For instance, the presence of contaminants such as heavy metals can lead to cellular mutations, suppression of photosynthesis, depletion of cytochrome, disruption of normal biological processes and metabolism, and even the death of algae.

An accurate assessment of water pollution may be made by analyzing the quantities of algae present, as well as their physiological and biological reactions, and the residues they carry **(Parmar *et al*., 2016)**. **Hosmani (2013)** suggests that using a composite rating of many types of algae, such as *Euglena* sp., *Chlamydomonas* sp., *Scenedesmus* sp., and *Chlorella* sp., might be a useful method for detecting pollution in aquatic environments.

5.2.2. Zooplankton

These organisms are small aquatic animals that cannot move or are very poor swimmers. They float in the water of oceans, seas, or freshwater bodies to travel long distances. Typically, they inhabit the sunny zone, which has the highest concentration of food resources and may also be found in the depths of the ocean. Zooplankton are organisms that rely on obtaining nutrients from other sources and can sometimes feed on decaying organic matter. They are recognized as the primary food source for marine fish and the early stages of other marine animals due to their abundant protein, mineral, and lipid content **(Al-Ghanim, 2012)**. In addition, zooplankton serves a crucial function in the food chain by connecting the primary producers (by ingesting phytoplankton, primarily different types of bacterioplankton, and occasionally zooplankton) with higher trophic levels.

Zooplankton is widely recognized as the primary indication of trophic status **(Kovalev *et al*., 1999)**. Due to their dependence on environmental conditions, these occurrences and reactions might be used as "bioindicators" in studies about water pollution. Zooplankton possesses the capacity to facilitate the production or absorption of CO_2 and other greenhouse gases in marine ecosystems. Zooplankton combines minuscule sedimented particles with bigger ones (faecal pellets) during feeding, which have the ability to sink to the bottom before undergoing recycling. This mechanism facilitates the storage of biogenic carbon in the sediment and postpones the emission of CO_2 **(Alcaraz & Calbet, 2009)**.

Several species of zooplankton can collect and degrade pollutants, which makes them potentially valuable for monitoring the quality of water. Certain molecular biological techniques can be used to specifically identify aquatic metals. One method that may be used to detect mercury pollution in water is slot-blot hybridization. This technique targets the Hg reductase gene of microbial communities and allows for monitoring of the presence of mercury **(Chakrabortty & Paratkar, 2006)**. Multiple studies have demonstrated that zooplankton species, including *Alona guttata*, *Mesocyclops edax*, Cyclops, and *Aheyella*, may be used as indicators of pollution by observing their distribution in various zones **(Ferdous & Muktadir, 2009; Jain *et al*., 2010; Hosmani, 2014)**. Zooplankton, including protozoa, crustaceans, amphipods, copepods, bivalve mollusks, and other creatures, play a vital role in aquatic ecology.

5.2.2.1. Protozoa

Protozoa have comparable traits with entire aquatic ecosystems and play a vital part in their food chains. Collecting samples of protozoa necessitates uncomplicated apparatus and fundamental experimental arrangements. Due to their unique dispersion characteristics, specialized arrangements, and wide range of species, they are well-suited as bioindicators. Unicellular protozoa are very susceptible to water contamination due to their tiny size, huge surface-to-volume ratio, simple structure, and lack of defense systems. Protozoa and other microbes make up the majority of biomass in several aquatic ecosystems, as determined by the number of species and the weight per unit area or volume. Their high rate of reproduction allows for fast evaluation of the effects of hazardous substances on the growth, reproduction, metabolism, and biochemical activities of numerous generations of protozoa. Conversely, experiments conducted on higher animals may require a significantly longer period, ranging from days to months or even years, to get similar outcomes **(Zhou *et al*., 2008)**.

Moreover, protozoa serve as excellent indicators for evaluating the toxicity and pollution levels in water. They function as biological indicators of pollution when their presence or absence corresponds to certain environmental circumstances, and as test organisms for assessing the toxicity of relevant hazardous chemicals **(Nicolau *et al*., 2001)**. Protozoa play a crucial role in evaluating pollution levels during the biological treatment of wastewater and in managing pollution by consuming scattered itself **(Mostafa *et al*., 2023)**. This helps maintain a well-balanced food chain in man-made environments. The protozoan population present in the aeration tanks of activated sludge plants remains a cutting-edge and effective method for monitoring biological wastewater treatment. There is a strong anticipation for future studies that will gather data and analyze the effects of harmful substances on this group.

5.2.2.2. Crustacean

Multiple studies have verified that the grass shrimps are dependable bioindicators of pollution in aquatic ecosystems. These crustaceans have a high sensitivity to a range of pesticides, such as fenvalerate, endrin, DDT, azinphosmethyl, parathion, endosulfan, and malathion **(Scott *et al*. 1987; AL-Khazraji *et al*., 2020)**.

Hatakeyama and Sugaya (1989) found that after assessing the susceptibility of freshwater prawns and *Paratya improvisa* to five types of pesticides and five herbicides, they compared the results to those of two species of Cladocera, namely *Daphnia magna* and *Moina macrocopa*. The study revealed that shrimp, particularly the species *Paratya australiensis*, exhibited greater sensitivity to pesticides, specifically fenitrothion, and fenthion, compared to the Cladocera species. Additionally, the shrimp demonstrated higher sensitivity to herbicides, with LC_{50} values being two to eight times lower than those of the Cladocera species. These findings were based on a 96-hour exposure period **(Kumar *et al*., 2010)**.

Copepods and amphipods are types of small crustaceans. It is crucial to measure the biological traits and life-history parameters of aquatic invertebrates to determine the quantities of trace metals they acquire in natural environments. This information is necessary for using them as biomonitors. A study conducted in the Greenland Sea investigated the amphipods *Themisto libellula* and *T. abyssorum*, together with the copepod *Calanus hyperboreus*. The study revealed exponential correlations between the concentrations of Cd, Pb, Cu, and Ni and the body length of these organisms. Nevertheless, the element Zn did not display any variation in relation to the length of *T. libellula*, as reported by **Zhou *et al.* (2008)**.

5.2.2.3. Bivalve mollusks

Filter-feeding bivalve mollusks can collect metals, which can have detrimental effects on other species. Professor Goldberg, from the Scripps Institution of Oceanography, created a programme known as "Mussel Watch" to monitor alterations in chemical pollution in coastal and estuary habitats across different locations and periods. A global-scale monitoring system has been suggested to detect patterns in the levels of several marine pollutants using the "sentinel organism concept." Mussels and other species are commonly used to monitor metal pollution in aquatic environments due to their superior qualities compared to other organisms **(Tanabe & Subramanian, 2003)**.

Gastropods are mollusks that have a resemblance to oysters and bivalve clams. Most gastropods are benthic organisms that feed on a variety of food sources. By increasing their buoyancy, they are able to rise to the surface in order to find food, which consists of fish and other creatures that they consume **(Guo & Lin, 1997)**. It has been widely recognized that gastropods have a natural tendency to collect significant amounts of metals. The mud snail, scientifically known as *Cipangopaludina cahayensis*, has been recognized as a valuable bioindicator for assessing the toxicity and bioavailability of heavy metals in a cumulative exposure test. Hence, the levels of the mud snails can be used to make inferences regarding the bioavailability of certain heavy metals **(Guo & Lin, 1997)**. Nevertheless, various gastropod species may exhibit differing abilities to collect diverse metal compounds, offering a variety of possible bioindicators for monitoring metal contamination in aquatic environments **(Liang *et al*., 2004a, b)**.

5.3. Animal indicators

Fluctuations in animal populations may indicate harmful alterations caused by ecological pollution. Fluctuations in population density may suggest adverse impacts on the environment. The relationship between populations and food supplies can impact population dynamics. When food resources become limited and are unable to sustain the population's requirements, a decrease in population size can ensue **(Jain *et al*., 2010)**. Animal indicators can be used to identify the presence of poisons in animal tissues **(Joanna 2006; Khatri & Tyagi, 2014)**. Animal indicators encompass a variety of organisms such as fish, amphibians, insects, and others.

5.3.1. Fish

Fish have a high degree of sensitivity toward alterations in their surroundings, particularly the escalating levels of water contamination. Conducting health evaluations on fish can therefore provide insights into alterations occurring in aquatic environments.

Trace metals in marine settings have been reported to disturb the intricate equilibrium of these ecosystems. Preliminary signs of pollution's harmful effects can be observed at the cellular or tissue level in fish before any visible changes in behavior or appearance occur **(Galadima & Garba, 2012; Gyampo *et al*., 2013)**.

The distinct biological features of fish, including their substantial size, long life cycles, and the convenience of growing them, have generated considerable interest in using fish for monitoring water pollution. The use of fish for biomonitoring is of great importance due to their position at the apex of the aquatic food chain and their potential to directly affect human health. The primary method of biomonitoring employed since the early 1990s was the fish lethal test, specifically designed to evaluate marine pollution. One might also examine the impact of pollutants on fish behavior, as suggested by **Cairns (1981)**. Aquatic pollution biomonitoring can make use of fish growth, reproduction, metabolism, fecundity, and acute lethal rate. Several fish species, such as medaka, loaches, zebrafish, and the Chinese unusual minnow, have been seen and recorded in relation to this matter **(Zhou *et al*., 2008)**.

5.3.2. Amphibians

Frogs serve as significant bioindicators for assessing environmental changes and determining environmental quality. Changes in both aquatic and terrestrial environments have a major influence on them, making them useful markers of ecological health. Because of its distinctive biological characteristics, such as its capacity to respire via its skin, an amphibian's elevated skin permeability renders it more susceptible to the impacts of aquatic contaminants. Pollutants can be classified by analyzing the symptoms of poisoning they cause. One might do an initial quantitative analysis of pollution levels by considering the specific location or extent of poisoning in amphibians. Moreover, certain amphibian tadpoles reside in watery habitats. Therefore, the water quality can have a substantial effect on the growth and development of tadpoles. According to **Zhou *et al.* (2008)**, observing the physical characteristics of tadpoles might be a valuable method for precisely evaluating the quality of water.

5.3.3. Insects

Aquatic insects play a valuable role in bio-monitoring programs by helping to identify the presence of organic pollution and heavy metal contamination **(Smoulders *et al.*, 2003)**. Although most *Ephemeroptera* species have notable susceptibility to metals, chironomids, and hydropsychid caddisflies demonstrate a remarkable resistance to metals **(Winner *et al*., 1980; Clements *et al*., 2000)**. Aquatic insects possess the remarkable ability to provide precise insights about habitats and water quality, effectively reflecting alterations within the ecosystem. Therefore, they are commonly used in freshwater biomonitoring to assess the impact of human activities **(Ceneniva-Bastos *et al*., 2017)**. It is necessary to identify the specific components of metal bioaccumulation in an insect's body, as this process might vary in various trophic groups. Hence, the concentration of the metal that can be readily absorbed by insects, along with the speed and method by which the metal enters their bodies, determines the amount of metal that insects take from water and food **(Souto *et al*., 2019)**.

5.3.4. Other

Seals and sea lions, along with other marine animals, possess a remarkable capacity for bioaccumulation, which renders them valuable for monitoring marine pollution through biomonitoring. According to **Odsjo *et al*. (2004)**, findings suggest that the feathers of some seabirds can detect levels of mercury in the marine environment. Nevertheless, the widespread application of these methods for monitoring pollution in specific locations is restricted due to their significant migration **(Zhou *et al*., 2008)**.

5.4. Microbial indicators

Microorganisms are commonly employed to identify contamination in aquatic habitats. They are believed to play a crucial role in driving the productivity and nutrient cycle of most aquatic ecosystems. Due to their rapid growth, responsiveness to low levels of pollutants, and diverse range of physicochemical and biological alterations, they are easily deployable and quickly available for pollution detection. Anthropogenic factors, such as the introduction of metals, eutrophication, and faecal matter, have a higher probability of impacting the aquatic environment, including rivers, lakes, and seas **(Ma *et al*., 2022; Gouda & Taha, 2023)**.

Microorganisms, such as bacteria and fungi, can serve as reliable indicators of pollution, particularly in aquatic environments. They offer tangible evidence of the existence of contaminants in the environment **(Al-Mishrey *et al*., 2021)**. **Butterworth *et al*. (2001)** found that tracking microbiota is rather simple compared to other typical investigations. They observed that regulating microbiota can reflect improvement in their communities. In biomonitoring, the microbial consortia can readily modify their biomass, operational levels, and composition in response to environmental contaminants, as highlighted by **Uttah *et al*. (2008)**.

5.4.1. Bacterial indicators

Bacterial indicators are distinct species or clusters of bacteria that are present in an environment. Their abundance relative to other bacteria signifies exposure to contaminants. Without a doubt, bacteria play a crucial role in assessing pollution problems across different ecosystems **(Kalkan & Altuğ, 2015)**.

Bacteria are the predominant and varied microorganisms in water, with a significant number of them being detrimental. Most bacteria often reside in the human and/or animal digestive systems and are later expelled from the body through faeces. Hence, the presence of these microorganisms implies that the sample was exposed to certain conditions and is considered the primary indication of water pollution by faecal matter **(del Rosario Salazar *et al*., 2023)**.

Heavy metals are also a notable pollutant in the aquatic ecosystem **(Chaturvedi *et al*., 2015)**. The composition and diversity of microorganisms in a metal-contaminated aquatic environment can undergo substantial alterations. **Custodio *et al*. (2022)** discovered that certain types of bacteria, specifically those belonging to the genera *Deltaproteobacteria*, *Acidobacteria*, *Actinobacteria*, *Coriobacteriia*, *Chitinophagia nitrospira*, *Clostridia nitrososphaeria*, and *Betaproteobacteria*, were abundant in lake sediments containing Cd and As. Thus, these bacteria could serve as reliable bioindicators for detecting the presence of heavy metal contamination.

For instance, *Vogesella indigofera* has a quantitative response to heavy metals. Without the presence of metal contamination, a bacterial blue coloration occurs, serving as a prominent visual indication of observable morphological alteration. However, the presence of hexavalent chromium hinders the production of pigments. The correlation between the concentration of chromium and the bacterium's synthesis of blue pigmentation may be utilized to elucidate the process of pigment formation **(Oberholster *et al*., 2009; Jain *et al*., 2010; Aslam *et al*., 2012; Malik & Bharti, 2012)**. In addition, bioluminescent bacteria may be utilized to assess the presence of environmental contaminants in water. Pollutants in the water impede or disrupt the biological metabolism of bacteria, and the amount of light emissions is influenced by this factor **(Manickavasagam *et al*., 2019)**.

5.4.2. Fungal indicators

Fungi have a crucial and diverse function in ecosystems. The versatile nature of fungi allows for their effective use in both mycoremediation and biomonitoring, rendering them very promising in the fields of environmental and industrial biotechnology. Due to their wide distribution, diverse ecological roles, significant biological diversity, high sensitivity to environmental changes, and ability to survive in harsh environments, they are used as potential bioindicators (mycoindicators) to assess the quality of water, air, and soil, as presented in Table (2) **(Gerhardt, 2002; Parmar *et al*., 2016; Grossart *et al*., 2019; Soares *et al*., 2022; Warnasuriya *et al*., 2023)**.

Fungal species belonging to different taxonomic groups are found in freshwater and marine environments and play a crucial role in important biological processes. These include nutrient cycling, the decomposition of dead aquatic plants and animals, and serving as a food source for organisms higher up in the food chain **(Pascoal & Cassio, 2004)**.

Table 2. List of fungal species used for bioassays of some environmental pollutants

Fungal species	**Environmental pollutant**	**References**
Anguillospora crassa, *Tetracladium marchalianum*, *Tetrachaetum elagans*, *Articulopora tetracldia*,and *Tricladium spendens*	Polystyrene nanoparticles	**Seena *et al*. (2019)**
Gerronema viridilucens and *Neonothopanus gardneri*	2,4,6-trichlorophenol, 4-cyanophenol, 4-nitrophenol, phenol, 4-chlorophenol, 4-methoxyphenyl, phenol, 4-nitrophenol	**Ventura *et al*. (2020) and (2021)**
Rhizopus sp., *Cladosporium* sp., *Penicillium* sp., *Curvularia* sp., *Fusarium* sp., *Alternaria* sp., *Pestalotiopsis* sp., *Aspergillus* sp., *Neonothopanus gardneri* and *Trichoderma* sp.	Cd, Ni, Cu (II), Zn, Cr, and Pb	**Mahanty *et al*. (2021) and Ventura *et al*. (2021)**
Fusarium oxysporum and *Phanerochaete chrysosporum*	Polyethylene, leachates, polyethylene terephthalate leachates, and polypropylene leachates	**Li *et al*. (2022)**

Fungal communities in aquatic habitats are extensively distributed and play a crucial function as indicators **(Hyde *et al*., 2016)**. Environmental pollutants, including industrial and household wastes, fertilizers, pesticides, petroleum hydrocarbons, acid rain, and trace elements (heavy metals), can affect the composition and function of aquatic fungal communities in freshwater and marine ecosystems **(Bai *et al*., 2018)**. Consequently, a variety of fungal species that serve as indicators and can withstand certain environmental changes are employed to efficiently monitor disturbances in ecosystems.

Fungi have been extensively reported as bioindicators in many studies. **Zaghloul *et al*. (2020)** investigated several fungi commonly employed as indicators for contaminants, such as *Trichoderma* sp., *Stachybotrys* sp., *Aspergillus fumigatus*, *Aspergillus versicolor*, *Phialophora* sp., *Fusarium* sp., *Ulocladium* sp., *Penicillium* sp., *Aspergillus niger*, and specific yeasts. In addition, **Biedunkiewicz *et al.* (2013)** discovered that various yeast-like fungal genera, such as *Candida*, *Debaryomyces*, *Rhodotorula*, *Trichosporon*, *Pichia*, *Saccharomycopsis*, *Kazachstania*, *Lachancea*, *Metschnikowia*, *Meyerozyma*, and *Kluyveromyces* sp., were employed as bioindicators to assess sewage contamination and eutrophication in the Poland River and lakes. Furthermore, **Samson *et al.* (2020)** found that fungal species from the genera *Aspergillus*, *Penicillium*, *Kluveromyces*, *Lodderomyces*, and *Nakaseomyces* are used as potential bioindicators for monitoring pollution and eutrophication at river confluences.

6. Fungal adaptations to act as environmental bioindicators

Biological indicators are commonly considered to be more dependable and economical in comparison to physical and chemical indicators of environmental quality. Fungi can serve as indicators of environmental responses through processes such as bioaccumulation, adsorption, biodegradation, biotransformation, and molecular and cellular responses. These responses can manifest as changes in the morphology and physiology of the fungal community, affecting its diversity and composition. This adaptation is crucial for the fungi's overall well-being, survival, and reproductive success, as depicted in Fig. (4) **(Branco *et al*., 2022; Maurya & Pachauri, 2022; Warnasuriya *et al*., 2023)**.

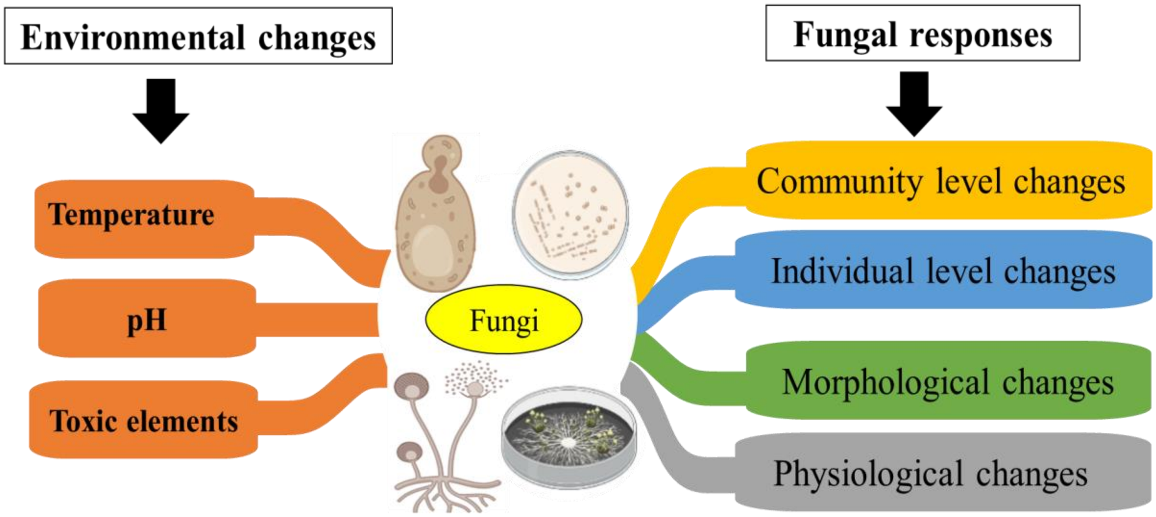

Fig. 4. Fungal responses to environmental changes

Fungi possess the ability to endure changes in their surroundings by employing a variety of processes from their internal and external enzymatic systems. This enables them to adapt to and break down a diverse array of environmental contaminants (Fig. 5). The intracellular enzymatic system serves as a detoxification mechanism for a variety of toxic substances (e.g., environmental pollutants) and plays a vital role in fungal adaptability. The system is composed of Phase I enzymes, which participate in oxidation, and Phase II enzymes, which are responsible for conjugation activities and encompass transferases. In addition, the extracellular enzymatic system breaks down complex structures and facilitates their absorption by the cell, which includes hydrolases and nonspecific oxidoreductases, which encompass laccases, unspecific peroxygenases, and class II peroxidases **(Soares *et al*., 2022)**. Fungal communities can store or adsorb various contaminants, such as pesticides, heavy metals, radionuclides, hydrocarbons, and other harmful chemicals of anthropogenic origin, inside or outside their biomass **(Gouda *et al*., 2023; Taha *et al*., 2023a)**. This process reflects the concentration of pollutants in a given ecosystem at any given time **(Maurya & Pachauri, 2022)**.

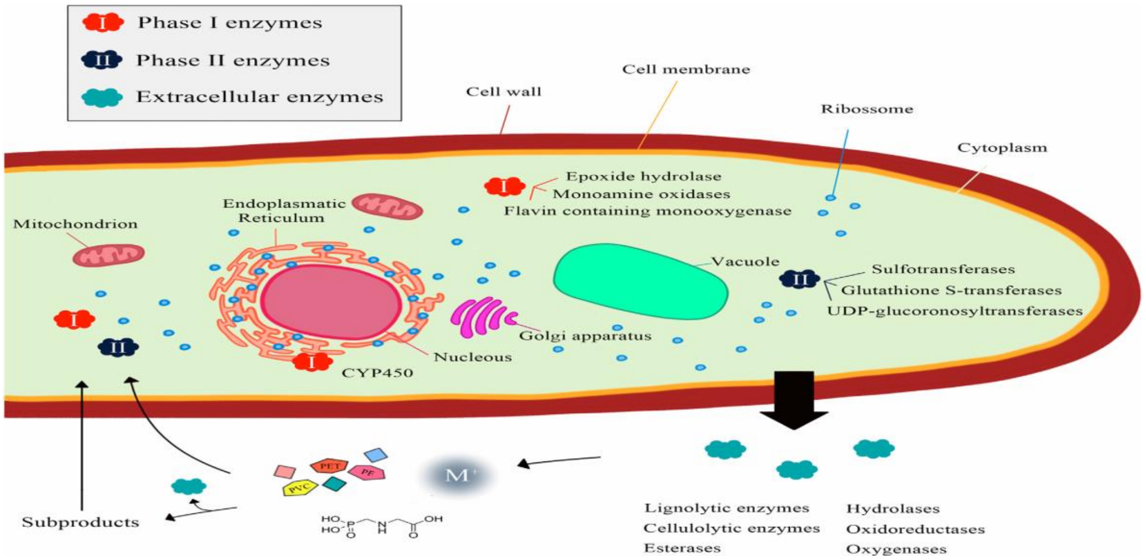

Fig. 5. Fungal adaptation to environmental pollutants through Intra- and extracellular enzymatic systems **(Soares *et al*., 2022)**

7. Fungal indicators for water pollution detection

Aquatic habitats have been polluted due to both natural phenomena and human actions, resulting in risks to the environment and public health. Fungi from different taxonomic groupings have been employed as biological indicators of pollution instead of physical and chemical indicators due to their high sensitivity to environmental pollutants. An investigation is conducted to analyze fungal species and their symbiotic relationships, such as lichens and mycorrhiza, in relation to different pollutants. The analysis includes both qualitative and quantitative methods, focusing on growth-based methods. These methods involve the development of mycelium cultures, measurement of ergosterol, assessment of enzymatic activity, evaluation of bioluminescence, and determination of plate occupation diameters, as shown in Fig. (6). Consequently, they were developed as bioindicators to detect, treat, and manage environmental pollution, as well as protecting ecological systems **(Gessner, 2020; Ventura *et al*., 2020; Baudy *et al*., 2021; Maurya & Pachauri, 2022)**.

Fig. 6. Common steps for pollution detection using fungi as bioindicators

Aquatic fungi have a response and can be used to detect pollution in water bodies. The study conducted by **Ortiz-Vera *et al*. (2018)** suggests that the impact of hazardous pollutants on aquatic ecosystems can be better understood with more precise reflection. Based on growth-based techniques, certain papers have used fungi that were isolated from contaminated waterways as bioindicators **(Soares *et al*., 2022)**.

Applications of fungal bioindicators have progressed in recent years due to the integration of genetic engineering, high-throughput DNA sequencing, and gene editing methods. Therefore, fungal indicators play a crucial role as recently developed tools for promptly, cost-effectively, and precisely identifying environmental pollutants and reducing pollution in both natural and artificial environments. The cell membrane integrity of a genetically modified yeast strain of *Saccharomyces cerevisiae*, which carries the firefly luciferase gene (luc) from *Photinus pyralis*, is compromised when it comes into contact with herbicides, diuron, and Cu ions. This exposure activates defense mechanisms that counteract the disruption by consuming ATP, thereby outcompeting the ATP-dependent bioluminescence and ultimately leading to a decrease in light emission **(Martin-Yken, 2020)**.

CONCLUSION

Ecosystems are highly complicated because they are interwoven with many living and non-living components. Therefore, bioindicator species play a crucial role in monitoring environmental changes within ecological systems. Research employing bioindicators is often characterized by its simplicity, reproducibility across various participants and environmental circumstances, and suitability for evaluating extensive geographical regions. Bioindicator species play a crucial role in ecosystem conservation by revealing the extent of pollutant exposure and the mechanisms of toxicity in the environment. In addition, they provide advanced notice of possible harm to the ecosystem and early indications of environmental restoration. Up to now, animals and plants have been the predominant indicator species due to their ease of observation and measurement. In recent times, there has been a focus on studying microorganisms, particularly bacteria and fungi, as bioindicators in many ecosystems. This is due to their heightened sensitivity to alterations in their environment. Using fungi as bioindicators might present difficulties in typical biomonitoring initiatives. The primary constraint in using fungal indicators is the insufficient number of comprehensive researches conducted. Researchers are optimistic about the potential of fungal bioindicators in several fields, including environmental monitoring, agriculture, and bioremediation. The progress in genetic engineering and biotechnology might potentially improve their ability to identify contaminants and encourage environmentally responsible actions.

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