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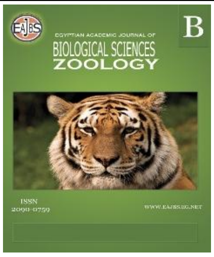

Nature's Sentinels: Harnessing Bioindicators for Environmental Health Assessment: A review

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ABSTRACT

Bioindicators serve as vital proxies for detecting pollutants and assessing potentially toxic element (PTE) concentrations, contributing to biomonitoring programs that inform environmental policies and conservation efforts by revealing disturbances from anthropogenic activities. Due to their sensitivity to various toxic elements, such as heavy metals and gases, bioindicators reflect the overall health of terrestrial and aquatic environments. A variety of organisms, including animals, plants, and microbes, have been classified as biological indicators. By functioning as proxies, their use enables non-invasive, real-time, and cost-effective pollution monitoring, providing a valuable early warning system to detect ecosystems at risk. Bioindicators are also important in the development of ecological management strategies. To better understand the role of bioindicators as proxies for toxic elements, this review focuses on scenarios that prove how effectively they are used for detecting pollution and assessing environmental health, highlighting case studies, methodological approaches, as well as species-specific sensitivities across the ecosystems.

INTRODUCTION

Environmental contamination from potentially toxic substances poses a significant international human health risk (Findorakova *et al.*, 2017; Mahanta *et al.*, 2022). Heavy metal contamination is a widespread issue resulting from urbanization and industrial development (Cunningham *et al.*, 2022). Heavy metals, which are life-threatening toxins with a mass density greater than 4 g/cm^3, include lead (Pb), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), zinc (Zn), arsenic (As), nickel (Ni), and manganese (Mn). These metals are released into the environment through industrial activities, commercial agriculture, and atmospheric deposition. Unlike many other pollutants, heavy metals are non-degradable and can bioaccumulate and biomagnify within the food chain (Kontas, 2008; Tariq *et al.*, 2024). In high concentrations, they pose serious risks to living organisms (Chandrasekaran *et al.*, 2015; Mishra *et al.*, 2019; Okereafor *et al.*, 2020; Sharma *et al.*, 2024).

Living organisms serve as biological indicators, which are used to detect contaminants in specific ecosystems (Martinello *et al.*, 2021). These indicators plants, animals, and microbes help to investigate the persistence of pollutants in ecosystems, considering past, present, and future conditions. Biological monitoring is defined as "the systematic use of living organisms and their responses to determine environmental criteria and changes" (Das and Maity, 2021; Babafemi *et al.*, 2024).

Naturally occurring biological indicators are often used to assess ecosystems and detect both positive and negative changes. A good indicator should have the following

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characteristics: numerical abundance, taxonomic soundness, high quantification and standardization potential, wide distribution, well-known ecological traits, low mobility, stability in laboratory conditions, and high sensitivity to environmental stressors (Hilty and Merenlender, 2000; Füreder and Reynolds, 2003).

This review evaluates the hypothesis that numerous biological indicator species can be used to assess pollution in both terrestrial and aquatic environments. A more comprehensive study of bioindicators, along with the incorporation of newly identified pollutants, is crucial.

1- Environmental Pollution

Environmental pollution is one of the most prominent issues affecting both terrestrial and aquatic ecosystems, primarily driven by anthropogenic activities (Bashir *et al.*, 2020; Ogidi and Akpan, 2022; Kolawole and Iyiola, 2023). The environment is highly diverse, consisting of bio-colloidal media that incorporates physical, chemical, and biological phenomena, all of which work together to maintain dynamic equilibrium. Over the past few centuries, stressors have reached unprecedented levels (Nowak *et al.,* 2010). Novel constituents, such as gases, heavy metals, organic compounds, and natural radionuclides—previously absent from ecosystems—are now being introduced at levels that exceed the tolerance thresholds of animals (Schutt, 1989; Onete *et al.,* 2010).

To mitigate the risk of harming ecosystems, no waste should be discharged into aquatic or terrestrial environments without thorough biological and chemical analysis. Potential toxic elements (PTEs), including organic pollutants and pathogens, are commonly found in significant amounts in most waste materials (Hoballah *et al.*, 2014, 2015; Saber *et al.* 2014).

2-Bioindicators:

Bioindicators, or biological indicators, are defined as biota, including bacteria, animals, plants, and plankton, that are utilized to monitor environmental health (Djamel *et al.,* 2022; De Rosario *et al.*, 2023; Gouda *et al.*, 2024). In addition, these organisms' physical responses indicate changes in the environment (Pastorino and Barceló, 2024). Their potential for qualitatively assessing environmental health and biogeographical changes in their immediate surroundings is significant (Parmar *et al.,* 2016; Ali *et al.*, 2021). Human disturbances can be assessed using biological indicators before it becomes too late to implement preventive measures (Nkwoji *et al.* ,2010). The main aim of any remediation process should encompass not only the elimination of pollutants but also the preservation of biological integrity (Zaghloul *et al.*, 2020). A wide range of biota (see Figure 2) has been identified as bioindicators to detect toxic elements in both terrestrial and aquatic environments.

2.1-Classification of Bioindicator:

Bioindicators are classified as origin of organism and mode of action (Fig. 1) (MacFarlane *et al.*, 2003).

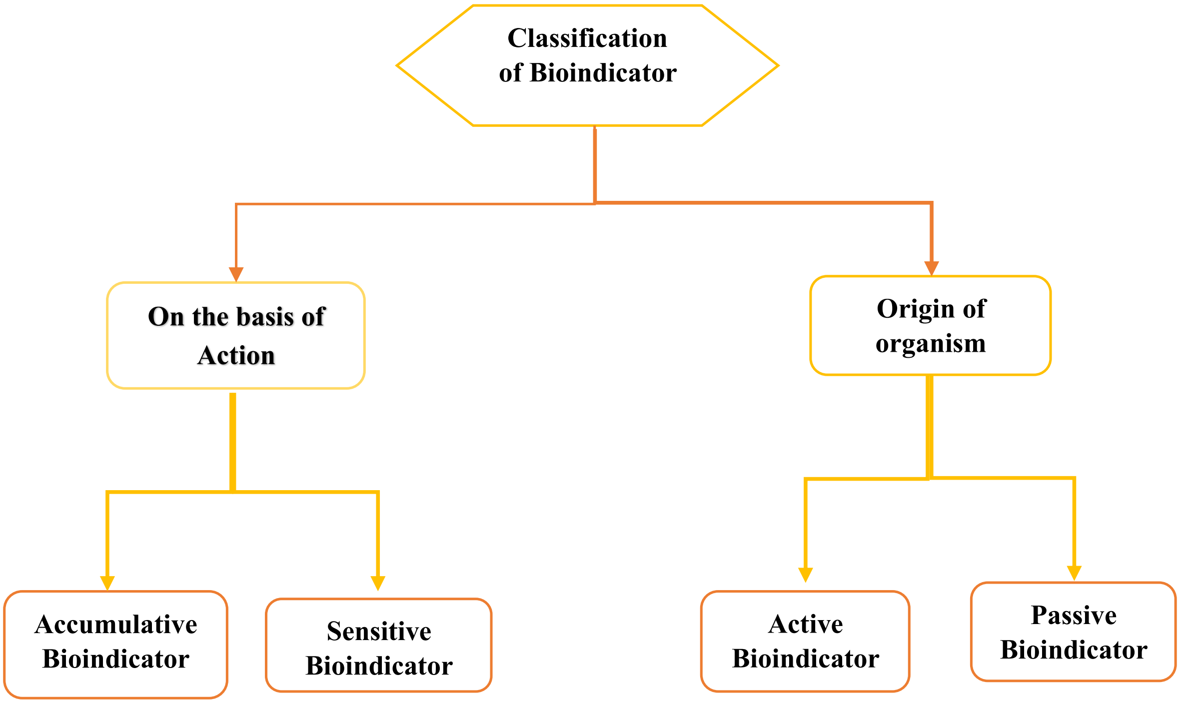

Fig. 1: Schematic illustration of classification of bioindicators (MacFarlane *et al.* 2003).

2.1.1 On the Basis of Origin of Organism:

On the basis of origin of organisms, bioindicators are classified as active and passive indicators.

Active bioindicators are organisms that are deliberately exposed to a specific environment for a predetermined duration to assess the concentration of compounds and elements. They are used to detect pollutants after intentional exposure to the environmental conditions being studied.

Passive bioindicators, on the other hand, are organisms from native ecological communities that naturally exist in the environment. They are used to assess the concentrations of substances and elements, along with their direct and indirect impacts, without any deliberate manipulation or exposure.

2.1.2 On the Basis of Mode of Action:

Accumulative bioindicators are a category of environmental bioindicators that accumulate one or more elements and chemical compounds.

Sensitive bioindicators are a subclass of bioindicators that indicate specific changes caused by exposure to chemicals or elements. These changes may pertain to the tissue, morphology, cytology, and behavior of an organism or population.

A diverse collection of plants, animals, and microorganisms are highly advantageous and serves as an effective tool for detecting pollutants within a specific ecosystem (Parmar et al. 2016) in addition (McGeoch, 1998; Stewart *et al.*, 2007) stated that there are three main types of bioindicators (Fig. 2).

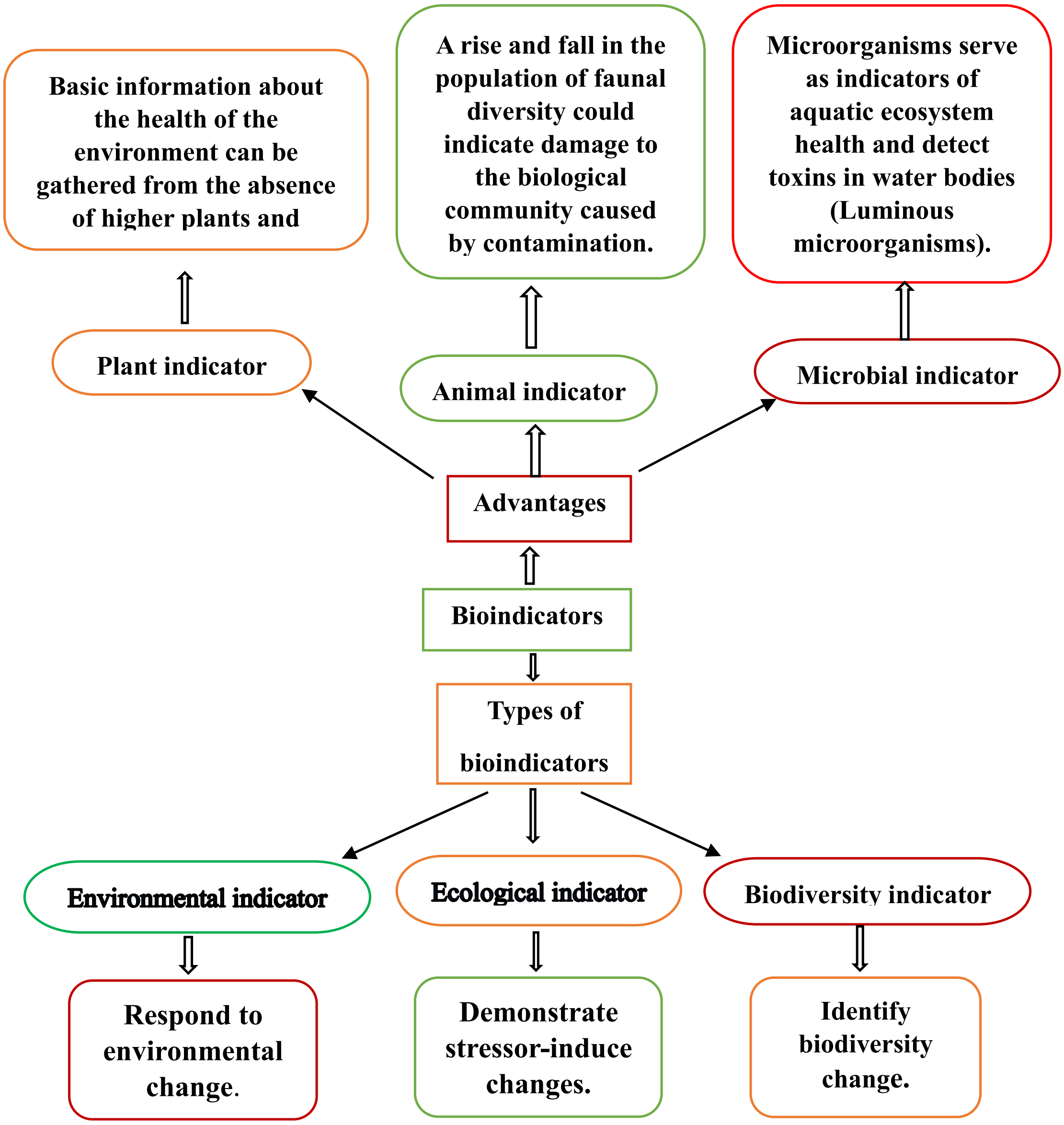

Fig. 2: Classification and Advantages of Bioindicators in Environmental Monitoring (McGeoch 1998; Stewart *et al.* 2007).

2.2. Types of Bioindicators

2.2.1. Environmental Indicator:

An environmental indicator is an organism or set of species that responds well to environmental disturbance or change in the status of the ecosystem in a predictable manner that are straightforward to observe and detect. It serves to identify modification to the environment. For example, Kunming-Montreal Global Biodiversity Framework is a significant initiative toward protecting 30% of the world's terrestrial and marine areas by 2030. It approaches biodiversity in terms of key environmental indicators-ecosystem preservation (Yale Center for Environmental Law and Policy, 2024).

2.2.2. Ecological Indicator:

A species or a group of species representing the influence of a stressor on a biological system is utilized for monitoring permanent shifts in biota caused by stressors for instance, fragmentation, climate change and habitat modification. Because arthropods are especially sensitive to changes in their environment, insects are frequently utilized as ecological indicators. As bioindicators, for instance, some insect populations have been researched to evaluate the effects of habitat loss, artificial light pollution, and climate change on ecosystems. The decline of insects is a serious indicator of the health of ecosystems, since it could lead to the extinction of forty percent of species in the next few decades (Chowdhury *et al.,* 2023). In aquatic habitats, fish diversity and community composition are commonly employed as ecological indicators. Environmental DNA (eDNA) and other DNA-based methods have been employed in recent years to check fish populations and assess the stresses that humans place on aquatic environments. This technique offers a non-intrusive way to monitor the existence of species and the health of ecosystems (Pinna *et al.,* 2023).

1.1.1 Biodiversity Indicator:

Biodiversity indicator is an array of taxa including (genus, tribe, family, order or may be a specific species group from various higher taxa) whether in terms of species richness, traits and/or endemism indicates the wide range of other taxa within a habitat. For example, species richness and abundance in forest ecosystems were measured in the study using Essential Biodiversity Variables (EBVs), such as the Biodiversity Intactness Index (BII). Researchers were able to assess the overall ecosystem integrity across forest plots globally by combining this with other variables like Net Primary Productivity (NPP), which tracks carbon inputs and ecosystem productivity, and Loss in Forest Connectivity (LFC), which measures habitat fragmentation (Dias *et al.,* 2023). Table 1, summarizes the key Bioindicators (Fig. 3).

Table 1 Key Bioindicators and Their Applications

Bioindicator	Ecosystem Type	Pollutant Detected
Lichens	Terrestrial	Nitrogen, Sulfur Dioxide
Phytoplankton	Aquatic	Phosphorus, Nitrogen
Earthworms	Terrestrial	Heavy Metals (e.g., Zinc, Copper)
Honeybees	Terrestrial	Pesticides, Heavy Metals
Cyanophyta (Algae)	Aquatic	Eutrophication, Algal Blooms
Amphibians (e.g., Frogs)	Terrestrial/Aquatic	Toxins, Contaminants
Dragonflies	Aquatic	Habitat Quality, Industrial Pollutants

3. Plants:

3.1 Higher Plants:

Various plant taxa, such as angiosperms, lichens, and phytoplankton, contribute significantly to our understanding of the health of specific ecosystems (Ogamba *et al.,* 2023). Vegetation serves as a sensitive indicator for assessing and identifying ecological stresses (Burger 2006). Most potentially toxic elements (PTEs) can be detected through specific higher plants (Malizia *et al.,* 2012). For example, certain plants exhibit biological responses to environmental contamination, such as acidophiles like common ling (Calluna vulgaris), hair grass (Deschampsia flexuosa), and round-leaved sundew (Drosera rotundifolia), which thrive under altered pH levels. Additionally, wild plants like wild barley (Hordeum murinum), French mercury (Mercurialis annua), and large nettle (Urtica dioica) are indicators of changes in nitrate content. Halophytes like glasswort (Salicornia europaea), sea aster (Aster tripolium), and sea lavender (Statice limonium) grow in response to shifts in total soluble salt content. Lower plants, such as certain lichens, are also effective indicators, particularly for assessing the quality of metal extraction procedures in plants (Hernández-Allića *et al.,* 2006).

3.2 Aquatic Plants and Plankton:

In aquatic ecosystems, a range of organisms from microscopic phytoplankton and zooplankton to complex vertebrates, such as fish, amphibians, and mammals, are used to assess elevated phosphorus and nitrogen concentrations, which promote the growth of various biotic communities (Polazzo *et al.,* 2022). Cyanophyta, a significant planktonic bioindicator, plays a key role in indicating eutrophication by forming algal blooms (Thakur *et al.,* 2013). Like terrestrial plants, phytoplankton contain chlorophyll and require sunlight for photosynthesis, typically inhabiting the upper layers of marine environments where light can penetrate (Verma *et al.,* 2012; Singh and Ahluwalia 2013). Numerous studies have shown that algal assemblages are reliable indicators of water quality, with species such as Euglena viridis, Nitzschia palea, Scillatoria limosa, and Scenedesmus quadricauda exhibiting high pollution tolerance (Chandel *et al.,* 2024). Other tolerant species include Oscillatoria tenuis, Stigeoclonium tenue, Synedra ulna, Ankistrodesmus falcatus, Pandorina morum, and Oscillatoria chlorina (Palmer 1969).

4. Microbial Indicators:
4.1 Bacteria:

In marine and coastal ecosystems, microorganisms are often utilized to assess environmental contamination (Alabssawy and Hashem, 2024). Butterworth *et al.* (2001) noted that while microbiotas are simpler to track compared to other standard studies, their regulation may only reflect changes in their populations in response to toxins. The Microbial Consortium proves a significant ability to adapt its functioning, biomass, and community structure to manage environmental contaminants (Odoh *et al.*, 2020; Yin *et al.*, 2021). Coliforms: This group mainly comprises numerous strains of E. coli types I and II, making them vital indicators of recent fecal contamination. Enterococci include Streptococcus faecalis and Streptococcus faecium. Both species have their natural habitat in the intestinal tract of humans and animals and might have a distinctive role as biological indicators of pathogens in food produced in a sewage farm (Zaghloul *et al.*, 2020).

4.2 Fungi and Algae:

A variety of fungal indicators (Molds) including Trichoderma sp., Exophiala sp., Stachybotrys sp., Aspergillus fumigatus, Aspergillus versicolor, Phialophora sp., Fusarium sp., Ulocladium sp., Penicillium sp., Aspergillus niger and Candida albicans (Hasselbach *et al.*, 2005). A composite range of algae for instance; Euglena sp., Chlamydomonas sp., Chlorella sp., and Scenedesmus sp., was successfully used to signal pollutants in aquatic ecosystems (Hosmani 2013). The significance of lichens as bioindicator is well-documented, attributable to their sensitivity to ecological pollution within forest ecosystems particularly in relation to pollutants such as nitrogen (N2) and sulfur dioxide (SO2) which can be inferred from the decline in lichen populations in affected areas (Gerhardt 2002). Lichens are esteemed as robust biological indicators because their extensive surface area enables them in capturing airborne pollutants (Holt and Miller., 2010).

5-Animal Indicators:

Fluctuations in population density may signify adverse effects on the ecological balance. Variations in population dynamics may arise from the interrelationship between species and their food sources, should these resources become limited and fail to meet the demands of the population, a subsequent decline in population numbers will ensure (Plafkin 1989; Phillip and Rainbow 1994; Jain *et al.*, 2010; Parmar *et al.*, 2016). In addition, animal indicators are utilized in assessing the concentration of toxic substances within animal tissue (Joanna 2006).

5.1 Invertebrates as Bioindicators:

Earthworms have been employed to analyze waste material treatment, reclamation of land and also used to evaluate the effects of chemical contaminants on the environment (Edward and Bater, 1992; Chen *et al.*, 2024). The primary resistance mechanism employed by earthworms against certain PTEs such as zinc (Zn), copper (Cu), lead (Pb) and mercury (Hg) are elucidating through their lipid-based antioxidative enzyme systems, which mitigate oxidative stress and facilitate the compartmentalization and immobilization of PTEs. Changes in the activity of earthworm’s nervous systems are used to quantify the rates of pollutants in terrestrial habitats. One could also infer the health of an ecosystem from the quantity of earthworm present (Gao and Luo 2005).

Macro-invertebrates serve as crucial indicators of environmental pollution due to their community dynamics which show alterations in response to variations in physicochemical parameters and the availability of habitats (Sharma and Chowdhary 2011). The ability of macro-invertebrate species to withstand adversity also makes it possible to assess the ecosystem's conditions objectively.

Amphibians, in particular anurans like frogs and toads, are often used as ecological indicators of contaminant accumulation in a specific environment (Rashid and Pandit., 2014). Anurans' epidermis and larval gill membranes allow them to absorb toxins, and they react quickly to changes in their environment.

Insects are generally thought to be the most vulnerable living things to environmental change because their sensitive bodies can detect pollution and thus serve as an excellent indicator of both aquatic and terrestrial ecosystems. A vast range of species of insects, for instance from predators to coprophages in different geographical regions offer accurate facts of quantitative data.

Insects are considered as biomarkers for pollution in any ecosystem. Since significant changes have been identified at the molecular and biochemical levels, the majority of them exhibit the rapid and reliable impact of heavy metals and thus have been considered as the most effective indicators (Nichols *et al.,* 2007; Da Rocha *et al.,* 2010) because certain species of bugs (Homoptera), true flies (Diptera) and beetles (Coleoptera) have not been used as bioindicators because of problems with taxonomy and sampling, unreliability with previously described species and taxonomic complexity especially when it comes to their larval stages. Numerous aquatic insects such as Odonata sp., (Dragonfly), order Diptera, Plecoptera, Heteroptera, Ephemeroptera and families for instance; *Gyrinidae*, *Dytiscidae, Hydrophilidae, Notonectidae, Veliidae,* have tremendous potential for adaptation as biological indicators (Hardersen 2000).

5.1.1 Major Insect Species (as a bioindicator) Are Used to Detect the Potential Toxic Elements (PTEs):

Honeybees have an extensive array of eating activities; they have been extensively studied as biological indicators of PTEs to assess the air quality of different areas (Bogdanov 2006; Martinello *et al.,* 2021). Honeybees (*Apis mellifera*) also serve as a biological indicator for assessing the quality of the ecosystem (Lim *et al.,* 2020; llyasov *et al.,* 2021). In an Italian study it is stated that honeybees are used as a bioindicator to monitor the application and spread of pesticides in Agri land (Jung *et al.,* 2018; Sajjad, 2020). Di Fiore *et al.,* (2022) stated that using honeybees as a bioindicator of metal contamination. Coleopterans for instance; ground beetles are a popular choice among researchers as an efficient bioindicator because they indicate the ecological variation caused by anthropogenic activities for instance; soil and land pollution (Avgin and Luff., 2010; Ghannem *et al.,* 2018). Carabid beetles are frequently used to assess the contaminants in soil due to their cosmopolitan distribution around the globe (Conti., 2017). *Harpalus rufipes* gives early warning indications to assess herbicides genotoxic consequences for non-target organisms living in the soil (Cavaliere *et al.,* 2019). As herbivorous insect grasshoppers serve a key role in biological accumulation and the transfer of PTEs to higher trophic levels via the food chain (Zhang *et al.,* 2009). Predators like mantis eat grasshoppers and thus in turn transfer toxic elements into other species at higher trophic levels of the food chain. Furthermore, the average concentrations of nickel, zinc, lead, iron, cadmium and chromium in grasshopper populations. (Soliman *et al.,* 2017). In terrestrial environments termites play a crucial role as decomposers (Da-Rocha *et al.,* 2010). Around 75 percent of termites, considered debris eaters and consume soil as their main source of food (Nithyatharani and Kavitha., 2018). An increase of nutrients, carbon dioxide content and clay also serve as an ecological engineer. Termites also accumulate heavy metals (lead, zinc, chromium, copper and cadmium (Alajmi *et al.,* 2019). Butterflies are extensively used as bioindicators to detect the toxic metal and contaminants in the environment near industrial states and even within urbanized districts (Da Rocha et al. 2010). (Azam *et al.,* 2015) investigated the accumulation of various toxic metals such as; Ni, Cu, Zn and Cr in butterflies (*Danaus chrysippus*) near the industrial areas of Gujarat and concluded that it is an excellent marker for heavy metal contamination. According to (Skaldina and Sorvari, pollution caused by heavy metals influences the physical and behavioral characteristics of insects for instance; aphids and butterfly larvae. (Kozlov *et al.,* 2022) also observed that the overall population of moths and butterflies (Lepidoptera) remains unchanged along the pollution gradient. Dragonflies are regarded as the most reliable ecological indicators in watersheds and aquatic ecosystems. They are vulnerable to habitat destruction (Liaqat *et al.,* 2023) and heavy metal accumulation particularly in lakes and flood drainage zones (Shafie *et al.,* 2017). The presence of dragonflies in any water body indicates that it is devoid of industrial pollutants (Azam *et al.,* 2015). Ants are often used as overly sensitive bioindicators that are crucial for the restoration of ecosystems that have been affected (Löffler and Fartmann, 2017). Because of their significant function at the ground level ants can be beneficial for monitoring areas for instance; open habitats, areas with plants and the toxicity of toxic metals in terrestrial environments (Gerlach *et al.,* 2013). Ants are becoming more utilized as biological indicators to track the health of ecosystems (Akhila and Keshamma, 2022)**.** Housefly is a mechanical transmitter for roughly 100 diseases including strains that are resistant to antibiotics (Macovei *et al.*, 2008). The presence of heavy metals, for instance lead, cadmium, zinc and copper commonly accumulates in the abdominal tissues of houseflies (Borowska and Pyza, 2011). A gastrointestinal injury has been reported to be a result of cadmium buildup in houseflies, but a low concentration of cadmium has no impact on housefly' larval development and growth (Chang-ying *et al.*, 2002). Because of their biological characteristics and feeding behavior parasitic wasps have been used as a biological marker of the wooded ecosystem (Hilszczański *et al.*, 2005). They appear higher in trophic level and have restricted host dimensions and sophisticated behavior (Skaldina and Sorvari, 2017). (Aguiar *et al.*, 2013) observed 103,000 hymenopteran different species, 70% of which were parasitic wasps that served as agricultural pests. Aphids are warning signs of pollution, despite a rise in population density as they feed on hosts that face environments that contain elevated levels of carbon dioxide (Zaghloul *et al.*, 2020).

6-Zooplanktons:

Zannatul *et al.* (2009), reported that zooplankton production is affected by abiotic (Stratification, temperature and saltiness) and biotic (predation, food scarcity and competitiveness) factors as well. They possess a strong capacity to serve as bioindicators. They additionally proposed that they were present in water bodies having elevated levels of phosphorus and PTEs. Some zooplankton species such as *Trichotria tetrat, Moscyclopesedex, Aheyella, Alons guttata* and *Cyclips* could be used as pollution indicators. Seasonal turnover in lakes shows great differences in zooplankton communities, there is a great abundance in zooplankton during the rainy season and decrease during summer due to elevated temperatures.

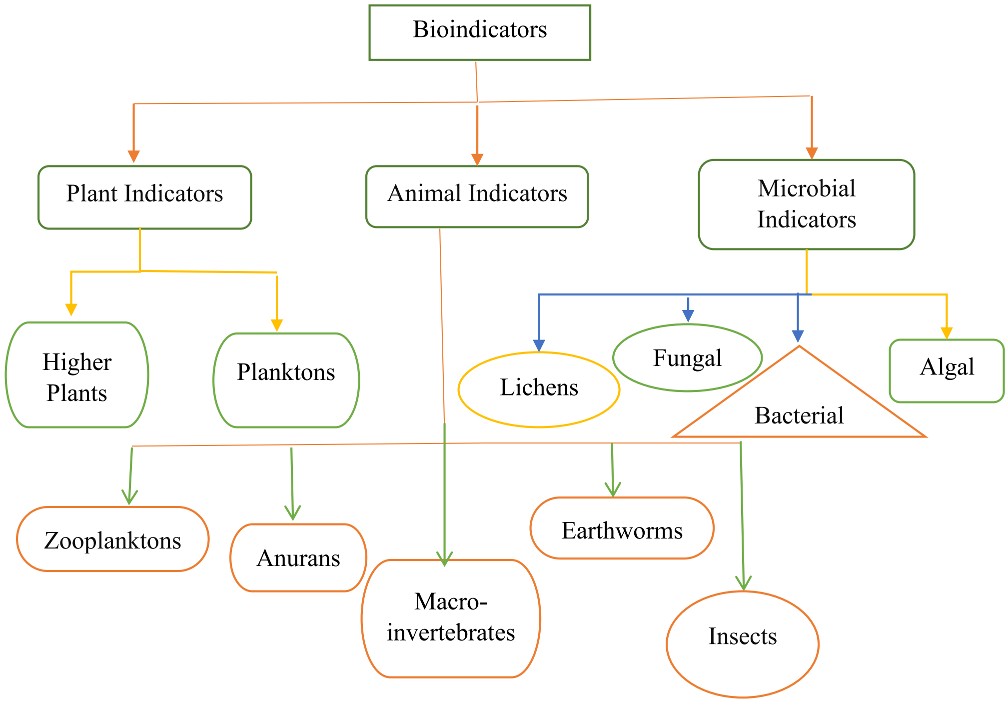

Fig. 3: Flowchart representation of a vast range of biotas.

7-Conclusions:

The review showed the significance of biological indicators for pollution monitoring in both terrestrial and aquatic ecosystems. There is a strong relationship between biotic and abiotic components of ecosystems; any alteration to them could ultimately result in the demise of a specific ecosystem. Biological indicators can assess the environmental changes as they are most reliable, vulnerable to minute environmental changes, cost-effective and easy to handle. By using biological indicators to assess PTEs, habitat conservation will be feasible. The frequency of their application across many national and international initiatives indicates the significance of bioindicators.

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