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Application of System Synergy in Environmental Water Monitoring

ANUNOBI, OO

Bingham University Karu, P.M.B 005, KM 26 Abuja-Keffi Expressway Kodope, Karu, Nasarawa State, Nigeria

**Corresponding Author Email: oluchukwu.anunobi@binghamuni.edu.ng*
**ORCID: https://orcid.org/0000-0003-2047-5313*
**Tel: +2347034439524*

ABSTRACT: One of the sampling techniques used in environmental monitoring is biomonitoring, which is the use of living organisms as monitoring equipment. The organisms in the examined habitat gather a lot of material from incredibly tiny concentrations in the environment because they are constantly exposed to physical, biological, and chemical influences. Changes in the health of the environment can be detected by examining the population level, physiological processes, and behavioral variations of these creatures. Bioindicators are species, or communities of organisms, that can be used to monitor environmental health because of their sensitivity to changes in their surroundings and the way their population's function. They are an important tool in traditional bioassays, which are mostly observation-based, because of their capacity to respond to changes in their surroundings. However, tests based on biotechnology are used to measure and identify the degree of environmental distress. These metabolic processes collaborate and interact with biomarker-responsive devices to provide an analysis of the environment under study. The physiological and metabolic changes that occur in a bioindicator's system in response to environmental change are translated into a format that is simple to read and quantify because biomarkers and bioindicators alone cannot be fully depended upon to analyze the environment. An instance of system synergy in environmental analysis is the combination of various processes up until the point where it is read and measured.

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Environmental analysis is a strategic tool that involves a series of processes used to identify all internal and external elements that may impact the performance of an environment. In the field of environmental biochemistry, this process involves applying the principles of biochemistry to identify, remediate, and control threats to the environment, such as managing water quality, air quality, soil quality, radiation, and bioremediation. Environmental analysis can also be used for environmental monitoring, which can help establish environmental baselines, trends, and cumulative effects, test environmental modeling processes, educate the public about environmental conditions, inform policy design and decision-making, ensure compliance with environmental regulations, evaluate the effects of human influences, or conduct an inventory of natural resources (Mitchell, 2002). Environmental monitoring can be conducted on both biotic and abiotic components of the five spheres of the earth (De Blij *et al.*, 2005) and can aid in detecting baseline patterns and patterns of change in the relationships between and within these spheres. To ensure an effective monitoring program, it is crucial to consider relevant questions, appropriate research designs, high-quality data collection and management, and careful analysis and interpretation of results. There are various sampling methods available, depending on the type of environment, the material being sampled, and the subsequent analysis of the sample. Biomonitoring is one such method that uses

**Corresponding Author Email: oluchukwu.anunobi@binghamuni.edu.ng*
**ORCID: https://orcid.org/0000-0003-2047-5313*
**Tel: +2347034439524*

living organisms as a monitoring tool. Organisms living in the environment under study are constantly exposed to the physical, biological, and chemical influences of the environment. These organisms can accumulate significant quantities of material from very low concentrations in the environment, making them useful in environmental biochemical analyses (Guerrero Aguilar *et al*., 2022). Bioindicators are biomonitoring techniques used to assess the health of the environment. Bioindicators are highly sensitive to environmental changes and can offer insight into the health of an ecosystem. The presence or increase of various markers in the environment indicates environmental stress. However, relying solely on changes in bioindicators to assess the health of an environment is insufficient; therefore, it is crucial to integrate multiple universal markers that apply to all cells of organisms. This approach reduces the likelihood of erroneous results that may arise from monitoring only one effect or process, particularly when using a single species as an indicator organism. The system approach is commonly employed in environmental analysis. A system is comprised of interconnected components that operate together as a unified whole to perform tasks that cannot be accomplished by a single component on its own. When the interaction of at least two factors, each affecting the specific performance of a system, produces a combined effect greater than the sum of their individual effects on the system, a synergistic effect is observed. The system approach is highly effective in examining environmental contaminants.

Bioindicators: Bioindicators are organisms or groups of species that can be used to assess the health of the environment in which they inhabit. These organisms' population status, behavior, and physiology can be used to predict the occurrence of environmental issues within a specific ecosystem (Mouillot *et al*., 2002). Bioindicators provide valuable information that would be difficult or time-consuming to obtain through other means. Species are considered bioindicators when their abundance and population fluctuations clearly respond to environmental changes in their specific habitat (Lindenmayer *et al.,* 2000). By monitoring specific physiological and behavioral variations in bioindicators, changes in environmental health can be identified. There are several types of bioindicators used in environmental monitoring. They include: microbial indicators like cyanobacteria that reflects the combined influence of excess phosphate and high temperatures, making it a potential indicator of nutrient imbalance (Fayissa and Kifle, 2013); plant indicators such as algal species whose diversity declines in presence of heavy metals in acidified lakes (Ranjbar-Jafarabadi, *et al.,* 2018); animal indicators such as waterbirds whose decrease in diversity can be linked to problems in water quality or degradation of their habitat. There are numerous scientific fields that employ organisms as bioindicators. Scientists can observe behavioral and demographic changes within a species, but specialized testing is required to identify physiological changes. Samples from organisms are needed for bioassays to identify environmental changes. These tests can be used to gauge the health of rivers or ensure the safety of drinking water. Bioassays can be performed using innovative techniques developed from biotechnology or in more conventional ways. In contrast to conventional techniques, biotechnology-based approaches aim to produce certain reactions that signify the existence of a particular pollutant instead of depending just on observation (Guerrero Aguilar *et al.,* 2022).

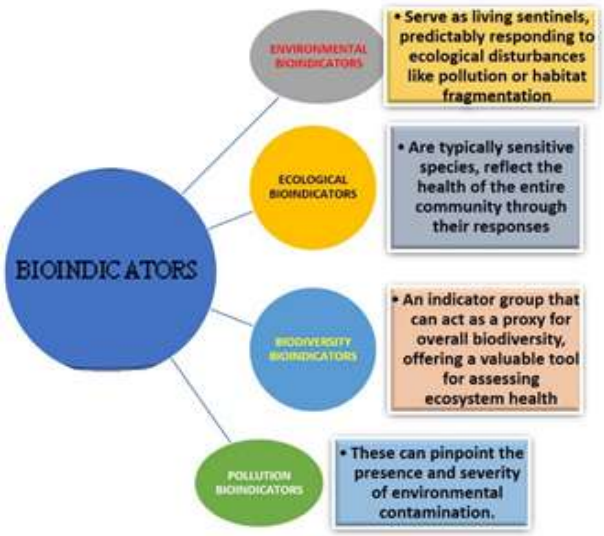

Fig 1: Classification of bioindicators based on their application (Chowdhury *et al.,* 2023)

Table 1: Bioindicators and their application in environmental monitoring

Bioindicator	Class	Application	Reference
Ground beetle, ants, butterflies	Environmental bioindicators	Monitoring heavy metal pollution in the soil	Ghannem *et al.,* 2018, Kozlov *et al.,* 2022, Akhila and Keshanma, 2022
Honey bees	Pollution bioindicator	Determination of severity of pesticide pollution	Cunningham *et al.*, 2022
Dragon flies	Pollution bioindicator	Determination of severity of pesticide pollution in water bodies	Shafie *et al.,* 2017
Parasitic wasp	Biodiversity bioindicator	Monitoring deadwood habitats ecosystem in woodlands	Brock *et al.,* 2021
Termites	Ecological bioindicator	Determination of soil fertility	Duran-Bautista *et al.,* 2020
Benthic macroinvertebrates	Biodiversity bioindicator	Assessment of ecosystem status in rivers and streams.	Juvigny-Khenafou *et al.,* 2021
meiobenthic nematodes	Environmental bioindicators	Determination of concentrations of the drug ivermectin in seawater and sediments	Essid *et al.,* 2020
Chlorophyceae and *Myxophyceae* (algal blooms)	Environmental bioindicators	Assessment of biological oxygen demand (BOD) and chemical oxygen demand (COD) levels of river water.	Patel *et al.,* 2020
Planktons	Ecological bioindicator	Monitoring the health of aquatic ecosystems	Saber *et al.,* 2016
E. coli	Pollution bioindicator	Detection of contamination of relatively recent fecal origin	Saber *et al.,* 2016
Hylocomium splendens	Environmental bioindicator	Ascertaining the levels of potential toxic elements in remote tundra ecosystems	Zaghloul *et al.,* 2020
Euglena gracilis	Pollution bioindicator	Determination of PTEs and persistent organic pollutants	Jain *et al.,* 2010
Anurans	Environmental bioindicator	Monitoring water bodies for organochlorine pesticides.	Zaghloul *et al.,* 2020
Alpine Chat, Common Stone Chat, Streaky Seedeater and Winding Cisticola (birds)	Ecological bioindicator	Monitoring effect of illegal grazing on ecosystem in Bale Mountain of Ethiopia.	Asefa and Mengesha, 2019

Application of Bioindicators in Environmental Analysis: Traditional bioassays have long served as the workhorses of environmental monitoring. These methods rely on the introduction of a "bioindicator," typically an organism or its enzymatic/cellular extracts, to environmental samples like soil or water. Biotechnology-based methods move beyond passive monitoring by actively engineering specific reactions that signal the presence of targeted pollutants or unwanted microorganisms (Chowdhury *et al.,* 2023)

Water Analysis: The number of macro-invertebrates, such as bugs, can be used to measure changes in the quality of the water; that is, the more indicators there are, the better the water quality, as pollution lowers oxygen levels. More vulnerable species would leave the area as a result of this. Water is being tested for environmental contaminants using bioluminescent bacteria, which release light when phosphorus-containing compounds undergo enzymatic-mediated chemical processes. The cellular metabolism of the bacteria is impeded or disturbed when toxins are present in the water. This has an impact on the caliber or volume of light that the bacteria release. Low dissolved oxygen concentrations are linked to a decrease in fish reproductive capacity because they disrupt yolk accumulation and change the size of oocytes as a result (Schulz and Martins-Junior, 2001; Burger *et al.,* 2005). Turtle eggs can serve as markers for the presence of polychlorinated biphenyls (PCBs), a type of pesticide. Turtle egg PCB contamination has been linked to industrial sites with significant pollution levels (King, 2008).

Air Quality Analysis: An early warning of locations at risk from air pollutant deposition is provided by the use of bioindicators in the assessment of atmospheric pollution. Air pollution is measured using a variety of techniques, including chemical analysis of mosses, frequency of epiphytic macrolichen, and community composition (Leith *et al.,* 2005). The highest amount of SO_2 that a species can withstand indicates how susceptible it is to pollution; species that are highly sensitive to SO_2 can tolerate it up to a certain point. The amazing growth of nitrophytic species—which thrive on nitrogen-rich barks with neutral to basic pH—is made possible by the decrease of SO_2 and the increase of NH_3. Thus, the primary parameters for monitoring air quality are the degree and extent of morphological alterations, such as color and form changes, behavioral adjustments made by the species,

and abundance at different levels of pollutants within a given polluted backdrop (Batzias and Siontorou, 2006).

Monitoring Biodiversity: Since biodiversity is important for environmental conservation, indicator species must be watched in the fastest changing habitat types to see if population trends align with changes in patterns and composition of the landscape. It is widely accepted that the diversity of a given taxonomic group indicates the diversity of species present in the area overall, and that the overall biodiversity may be evaluated by calculating the relative abundance of the indicator species (Hess *et al.,* 2006). Certain identifiable species, such as birds, butterflies, and mammals, are more visible to the general population, the media, and even scientists. Because of this, when they disappear or go extinct, people get concerned and the media pays greater attention. Therefore, if regional patterns of species richness are consistent across taxa, indicator species can be employed as a conservation tool to pinpoint hotspots for biodiversity (Dung and Webb, 2007).

Advantages and Disadvantages of Bioindicators: For a number of reasons, bioindicators are crucial to environmental monitoring. Pollution has a known effect on indicator species, and it is generally less expensive to apply (Spiegel, 2002). Furthermore, bioindicator-based research typically requires straightforward methods that are easily replicable by other people on a periodic basis.

The approach is appropriate for evaluating vast regions and works well in a variety of habitats (Gerhardt, 2002). Since they are simpler to understand and less confusing than directly sampling and evaluating every plant and animal community present in a particular ecosystem, indicator species are crucial for environmental monitoring (Dale and Beyeler, 2001).

Notwithstanding their benefits, bioindicators have limitations when it comes to their use in environmental monitoring. According to McGeoch *et al*. (2002) and Mouillot *et al.* (2002), indicator species must be widespread throughout the locality under study, have a sufficient number of individuals at the designated locality, and have a well-understood physiological mechanism for absorbing and retaining toxic substances or environmental contaminants (Burger and Gochfeld, 2001).

Biosensors: A biosensor is a self-contained integrated device that consists of a transduction element (an analytical device that may be a chemical sensor) in direct contact with a biological recognition element (an enzyme, antibody, receptor, or microorganism). Together, these elements transform the biological recognition event into a useful output signal and respond to a chemical species in a concentration-dependent manner in a reversible manner (Rodriguez-Mozaz *et al.,* 2005).

Associated signal processors present the outcomes in an easy-to-read format. A change in one or more physicochemical properties (pH change, electron transfer, mass change, heat transfer, uptake or release of gases/ions) is the result of the specific binding of the target analyte to the complementary biorecognition material. This specific interaction is then detected and measured by the transducer and converted to an electronic signal, which is a function of the analyte's concentration and allows for both quantitative and qualitative measurements in real time (Batzias and Siontorou, 2007).

In the process of developing biosensors, immobilizing the biological component at the transducer surface is a crucial stage. Immobilization ensures that the biomaterial is stabilized and that it is in close proximity to the transducer. Physical adsorption at a solid surface, cross-linking between molecules, covalent attachment to a surface, and entrapment within a membrane surfactant matrix, polymer, or microcapsule are the immobilization techniques most frequently used (McConnell *et al.,* 2020).

Classification of Biosensors: Biosensors are often categorized into a number of fundamental classes based on the basis of bio-recognition or the signal transduction mechanism. Therefore, depending on the transducing element, biosensors can be classified as electrochemical, optical, piezoelectric, or thermal sensors.

Similarly, biosensors that use bio-recognition principles in their design or that perform a particular mode of signal transduction can be classified as immunochemical, enzymatic, non-enzymatic receptor, whole-cell, or DNA biosensors. According to Justino *et al.* (2017), the biological recognition element can also be divided into two categories: bio-complexing recognition element and biocatalytic recognition element (made of one or more biocatalytic elements). An immobilized molecule catalyzes a reaction in a biocatalytic device, continuously consuming and releasing substrate or product (analyte). The consumption of the analyte is then tracked by measuring the amount of co-substrate consumed or the amount of reaction product formed (Upadhyay and Verma, 2015).

Table 2: Classes of biosensors applied in environmental monitoring

Class of biosensor	Component		Detection process	LOD	Application	Reference
	Analyte	***Enzyme***				
Enzyme based biosensor	Chromium	Glucose oxidase	Amperometry	0.05ppm	Detection of Cr(IV) in water	Dabhade et al., 2021
	Mercury	Urease	Voltammetry	0.02µM	Detection of mercury in river water samples	Gumpu et al., 2017
	Catechol	Laccase	Amperometry	0.085µM	Detection of catechol in water samples	Palanisamy et al., 2017
Microbial biosensor	***Analyte***	***Microorganism***				
	Manganese (Mn^{2+})	*E. coli*	Optical	0.01 µM	Quantification of manganese in soil samples	Jeon et al., 2022
	Lead (Pb^{2+})	Inactivated *E. coli*	Square wave voltammetry	0.13µg/L	Quantification of lead in wastewater effluent	Sabah and Fehti,2022
	Cadmium (Cd^{2+}), Zinc (Zn^{2+})	*Bacillus megaterium*	Fluorescent	1.42×10^{-4} 2.42×10^{-4}	Detection of cadmium and zinc in soil samples	Rathnayake et al., 2021
Optical biosensor	***Analyte***	***Chromophore***				
	Lead (Pb^{2+})	dithizone, 1-(2-pyridylazo) 2-naphthol and 4-(2-pyridylazo)-resorcinol)	Colorimetry	0.1ppm	Detection of lead in river water samples	Low et al., 2022
DNA-based sensors	***Analyte***	***Sensor***				
	Carbendazum	aptasensor on gold electrode	Farradaic electrochemical impedance spectroscopy	8.2pg/ml	Detection of herbicide in agricultural products	Eissa and Zourob, 2017
	Atrazine	ssDNA aptamer with gold nanoparticles	Colorimetry	-	*Invitro* experiment	Abraham et al., 2018
Aptamer-based biosensor	Lead (Pb^{2+})	aptasensor with screen-printed carbon electrode	Voltammetry	0.096 µg/L	Detection of lead both polluted water and soil sample	Ding et al., 2020
Immunosensor	***Analyte***	***Sensing material***				
	Okadaic acid	Graphene	Impedimetric	0.05 ng mL^{-1}	Detection of Okadaic biotoxin in sea water	Antunes *et al.*, 2018, Zhou *et al.*, 2021

LOD: limit of detection

Application of Biosensors in Environmental Analysis: Biosensors for Pesticides Determination: Many man-made chemicals and by-products from industrial or combustion activities have been released into the environment and continue to do so as a result of technology and human advancement. Certain compounds, such pesticides, heavy metals, and polychlorinated biphenyls (PCBs), are widely acknowledged pollutants that have been shown to have an impact on environmental quality (Ivanov *et al.*, 2000). Various enzymatic biosensors have been developed for organophosphorous and carbamate pesticides (Bahner *et al.*, 2018). Some of these biosensors rely on the activity of choline oxidase and the inhibition of acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) (Tunç *et al*., 2015). Some are aptamer-based biosensors that rely on ability of the target molecule to interact with the aptamer (Berlina et al., 2019). Pesticides are typically determined by enzyme analysis using specific enzymes such cholinesterase, acid phosphatase, tyrosinase, ascorbate oxidase, acetolactate synthase, and aldehyde dehydrogenase, which are inhibited in their activity. Certain enzymes can create stable complexes with certain substances. This is because the pesticides' shapes mimic those of the substrate, obstructing the enzyme's active core and reducing its activity. There must be a substrate present for this inhibition to occur.

Biosensors for Heavy Metal Determination: As a result of their high toxicity and capacity to accumulate in living things, heavy metals including copper, cadmium, mercury, and zinc represent a concern to the ecosystem (Rodriguez-Mozaz *et al*., 2006). Metals including mercury, cadmium, chromium, lead, and copper salts are commonly detected utilizing heavy metal biosensors that use immobilized urease and

glucose oxidase (Abu-Ali *et al.,* 2019). By creating a physiologically sensitive membrane through the crosslinking of urease with bovine serum albumin, an enzyme was rendered immobile. The transducer was a gold electrode that had been interdigitated. After soaking the electrodes in heavy metal ion sample solutions, the urease activity was measured to assess the sensor's responsiveness to different concentrations of heavy metal ions. Because mercury has a stronger affinity for urease's cysteine residue, it can be found at concentrations as low as 10 nM (Tsai and Doong 2005). A straightforward fluorescence-based approach was used by Ravikumar *et al.* (2018) to create a "turn-on" aptamer biosensor for the detection of arsenic in environmental water samples. An arsenic-binding aptamer that was fluorophore-terminally tagged was used in their design. Pan *et al.* (2018) described the development of an ultrasensitive aptamer-based biosensor for arsenic detection in environmental water samples. This biosensor utilized a triple-helix molecular switch, enzyme-based signal amplification, and fluorescence.

Biosensors for Biochemical Oxygen Demand: According to Abdulghani and Jaffrezic-Renault (2001), biochemical oxygen demand (BOD) is a metric that is frequently used to quantify the quantity of organic material that can decompose in water. The BOD values represent the quantity of carbonaceous demand, or biodegradable organic material, and the amount of oxygen required to oxidize inorganic materials such ferrous iron and sulphides. The majority of BOD sensors that have been published are whole-cell microbial sensors of the biofilm type, which measure the rate at which bacteria respire in close proximity to an appropriate transducer (Moraskie *et al*., 2021; Liu *et al.,* 2022). These sensors all share the same biological recognition element, which is a microbial film encased in a porous cellulose membrane and a gas-permeable membrane.

Biosensors for microorganisms' contamination: Apt-5 is a single-stranded DNA aptamer specifically designed to bind to the lipopolysaccharide (LPS) molecule on the outer membrane of *E. coli* forming a complex which gives off a fluorescent signal measured by a fluorimeter if the aptamer is labelled with a fluorescent dye (Zou *et al*., 2018). Apt-5 can also be immobilized on an electrode surface. The binding of *E. coli* changes the electrical properties of the electrode, which can be measured using an electrochemical detector. The presence of *E. coli* in the sample causes a visible line to appear on the strip, indicating a positive result (Zou *et al.,* 2018). The intensity of the generated signal is directly proportional to the concentration of *E. coli* cells in the sample. By comparing the signal intensity to a calibration curve, the concentration of *E. coli* can be accurately quantified.

Advantages and Disadvantages of Biosensors: The capacity to quantify contaminants in complicated matrices with little sample preparation, as well as the possibility of portability and on-site operation, are the key advantages that biosensors offer over traditional analytical procedures (Huang *et al*., 2023). It has been demonstrated that some enzymes are inhibited by hazardous metals found in the environment. poor sensitivity, poor selectivity, interference from ambient matrices, and non-metal inhibitors have all been mentioned as limitations to the possible application of enzyme biosensors (Naresh and Lee, 2022).

Biosensors in environmental water analysis: Given that biosensors have such high sensitivity and selectivity, they are essential for monitoring contamination in both fresh and marine water. Numerous contaminants, including as heavy metals, organic pollutants, and bacterial infections, can be found with these instruments. With low limits of detection (LOD), cell-based biosensing systems, for example, have been effectively used to monitor heavy metal pollution in water sources, such as Cu^{2+}, As^{3+}, and Hg^{2+} (Coronado-Apodaca *et al.,* 2023). Furthermore, biosensors have been created to identify bacterial infections that are transmitted through water, offering a useful instrument for tracking water contamination (Wu *et al*., 2021). Additionally, to address important environmental and health concerns, biosensors have been used to detect specific chemicals like fluoride and uranium (Thavarajah *et al*., 2019). For the purpose of monitoring water quality, a number of biosensor technologies have been investigated, including microbial fuel cell biosensors, paper-based biosensors, and enzyme-based electrochemical biosensors. A potential remedy for this problem is the use of enzyme-based biosensors, which have demonstrated promise in the electrochemical detection of pharmaceutical residues in wastewater (Campaña *et al*., 2019). Paper-based biosensors have been used to identify a variety of water contaminants in ambient and wastewater samples, such as microorganisms, medications, and heavy metals (Peixoto *et al*., 2019). Furthermore, microbial fuel cell biosensors have been created specifically to monitor copper in aquatic settings, offering a quick and easy way to provide early warning in developing nations (Zhou *et al*., 2020). Biosensors have been presented as a useful tool for environmental surveillance, providing not only specific contaminant detection but also continuous monitoring of contaminated areas (Goradel *et al*., 2017). Additionally, it has been determined that

biosensors are necessary for on-site water quality monitoring, indicating their potential for broad use in this regard (Thavarajah *et al.,* 2020).

Biomarkers: A modification at a cellular, physiological, behavioral, or biochemical level is referred to as a biomarker (Depledge, 1994). Biomarkers are employed as early warning systems to indicate potentially dangerous circumstances. The biological response should ideally take place between the start of the anthropic event, which is the ideal state, and the commencement of the organism under observation's deadly conditions. With biomarkers, the mode of action itself is monitored rather than just monitoring all chemicals exhibiting the said mode of action (Hanson *et al.,* 2013). Two categories of biomarkers exist: exposure biomarkers and impact biomarkers. The responses of an organism to exposure to a chemical substance or group of chemical compounds are known as exposure biomarkers, and they occur at many levels of structural organization. A quantifiable change in an organism's biochemistry, physiology, or behavior that may be linked to a known or suspected illness or impairment is known as a biomarker of effect. Biomarkers of susceptibility reveal an organism's innate or learned capacity to react to a particular environment. There are two further categories for biomarkers: generic and specific. All of an organism's responses at different levels (genetic, molecular, cellular, physiological, and behavioral) that aren't solely brought on by a single class of pollutants are referred to as general biomarkers. These reactions show how stressed out the species in the examined ecosystem are (Conti, 2008). Elevated ATP and acetylcholine levels, changes in DNA or mRNA, the presence of oxidative stress, heat-shock proteins, and specific responses to metals, neurotoxic pollutants and genotoxic substances are examples of biomarkers used in environmental monitoring (Lionetti *et al.,* 2019).

Role of Biomarkers in environmental monitoring: As markers of the integrity and health of ecosystems, biomarkers are essential to environmental monitoring. These indicators, which might be molecular, cellular, or biochemical, are used to evaluate the existence and effects of environmental pollutants on living things and their environments. A thorough grasp of the physiological processes and adaptation mechanisms used by organisms to deal with a variety of environmental situations is made possible by the ongoing validation and application of biomarkers (De Almeida *et al.* 2019). The intricate relationships between seasonal fluctuations and contamination levels, indicators of oxidative stress have been used to evaluate the effects of environmental contamination on species in estuarine systems (Sardi *et al.,* 2017). Additionally, biomarkers have been acknowledged as useful instruments for evaluating the effects of pollutants on living things, offering perceptions into the reactions of cells and subcellular structures to chemical pollutants. A recent development in environmental monitoring is the measurement of these reactions, or biomarkers, which provide a way to identify and analyze the effects of contaminants on living things (Lionetto *et al.,* 2021). Fish biomarkers have proven useful in environmental surveillance by helping to track the effects of diffuse pollution and the ongoing introduction of novel chemicals in the setting of aquatic ecosystems (Hanson, 2009). Their critical significance in preserving the integrity and health of the environment is highlighted by their capacity to record dynamic responses, evaluate the effectiveness of corrective efforts, and provide guidance for monitoring programs.

System synergy: Systems emerge from the interdependent interaction of multiple components, collectively accomplishing functions beyond the reach of each constituent part. This synergistic effect underscores the fundamental principle that the whole is greater than the sum of its parts. This suggests that measuring only one effect for example CO_2 production, or lipid peroxidation may not give accurate result of the toxic effect of a pollutant in an ecosystem. Integration of multiple markers that are universal to all cells will reduce erroneous results likely to be obtained in monitoring only one effect or process such as CO_2 production, especially when a single species is being used as an indicator organism

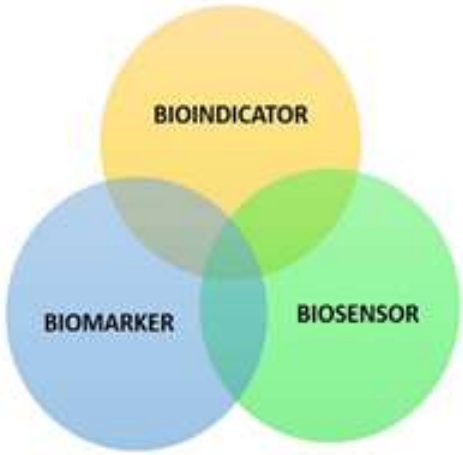

Fig 2: Schematic representation of system synergy of three biomonitoring indices

Application of system synergy in environmental water analysis: Use of bioluminescent bacteria to determine biochemical oxygen demand: Microorganisms are commonly affected by pollution and environmental

disruptions, making them valuable as bioindicators in disturbed environments. Traditional methods for assessing environmental disturbances using microorganisms typically involve incubating samples in culture media for 2 to 7 days to obtain contamination results due to the time required for stressed microorganisms to grow visible colonies (Griffiths and Philippot, 2013). Biochemical oxygen demand (BOD) is widely used to gauge the biodegradable organic material in water, reflecting the carbonaceous and nitrogenous demand, as well as the oxidation of inorganic materials (Vanwonterghem and Webster, 2020).

The sensitivity of microorganisms to environmental disturbances has been well-documented, with studies highlighting their rapid and specific responses to such disruptions (Shetty *et al*., 2019). Furthermore, the use of microorganisms for water quality assessment and their complex relationships with climate change and other stressors has been emphasized (Michán *et al.*, 2021).

The relevance of microorganisms as bioindicators is further supported by their vulnerability to ionic imbalance, osmotic stress, and reactive oxygen species, particularly in photosynthetic microalgae (Shetty *et al*., 2019). While the conventional BOD test serves as a mainstay for evaluating wastewater treatment plant performance, biosensors offer a faster alternative. These sensor systems leverage the metabolic activity of microbes, often measuring dissolved oxygen depletion or light emission as indicators of BOD.

Physical transducers convert these biological responses into electrical or optical signals, enabling rapid quantification of biodegradable material. Adenosine triphosphate (ATP) is an excellent biomarker for feasibility and cellular contamination because it is found in all living organisms. Bioluminescence is a visible light produced by living organisms.

Bioluminescent species are found in large numbers across the animal phyla but majority of them dwell in marine habitat (Shakeel and Prabhu, 2017). When levels of ATP are increased, it is indicative of presence of microorganisms reacting to an environmental stress. The most common use for ATP-luminescence technology detection of ATP is to replace conventional methods and drastically shorten detection times without sacrificing accuracy. The luminometer measures the amount of bioluminescence light, which is then expressed in Relative Light Units (RLU). According to AIB International's Director of Microbiology and Food Safety Education (2013), RLU numbers are directly correlated with ATP levels.

The primary disadvantage, which is the length of time required to obtain microbiological data, has led to the potential for quick results with ATP-bioluminescence based on the luciferine/luciferase reaction. Sludge or sewage serves as a substrate for bioluminescent bacteria, which are generally capable of decomposing organic materials and producing CO_2, H_2O, and ATP in the process. Using the readily available molecular oxygen in the water, the bacteria oxidize the glucose in the organic matter (Vanwonterghem and Webster, 2020).

$$C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O + ATP$$

Molecular oxygen, which comes from the external cellular environment, is essential to the biochemistry of bacterial bioluminescence. Luminous bacteria cannot produce light unless they receive molecular oxygen (Lin and Meighen, 2009). Living things that produce light are abundant in nature and come in a variety of forms. According to Cholet and Ribault (2012), luciferase catalyzes the oxidation of luciferin by molecular oxygen, which transforms the molecule into an excited form known as oxyluciferin.

After emitting visible light, oxyluciferin returns to its ground state. The luciferase enzyme-based bioluminescence mechanism is a multi-step process that primarily needs ATP, oxygen (O_2), magnesium cation (Mg^{++}), and luciferin substrate (Seliger, 1989). The luciferine/luciferase method of ATP-bioluminescence depends on the luciferine being oxidized by the enzyme, and the integrated light intensity is directly correlated with the amount of ATP present. D-luciferin is converted by luciferase into the equivalent enzyme-bound luciferil adenylate when ATP and magnesium are present. Oxyluciferin is the end product of an oxidative process that starts with the luciferil adenylate complex. The oxyluciferin molecule undergoes a rapid transition from an excited state to a stable state, leading to the emission of light.

$$\text{D-luciferin + luciferase + ATP} \xrightarrow{Mg^{++}} \text{Luciferil adenylate complex +PPi}$$

$$\text{Luciferil adenylate complex} \xrightarrow{O_2} \text{Oxyluciferin + AMP+ } CO_2 \text{ + light}$$

The genetic sequences responsible for encoding the proteins involved in the light-emitting system are known as the lux genes. These luminescent bacteria, containing the Lux-AB gene, have the potential to emit light through the catalytic action of the luciferase enzyme. This enzyme facilitates the reaction involving three substrates: Flavin mononucleotide ($FMNH_2$), oxygen (O_2), and long-chain aldehyde (RCOH). As a result of this reaction, flavin (FMN), long-chain fatty acids (RCOOH), and water (H_2O) are released, accompanied by the liberation of excess free energy in the form of blue-green light at a wavelength of 490nm. Bacterial luciferase serves as the key enzyme responsible for catalyzing light emission in the process of bacterial bioluminescence. However, the catalytic mechanism involved in the sustained production of light in luminous bacteria encompasses not only bacterial luciferase but also the enzymes involved in providing and regenerating the substrates required for bacterial luciferase. These substrates include reduced flavin mononucleotide ($FMNH_2$), molecular oxygen, and long-chain fatty aldehyde. The surplus energy released from the oxidation of $FMNH_2$ and aldehyde, concurrent with the reduction of molecular oxygen, manifests as blue/green light emission (with a maximum at approximately 490 nm).

$FMNH_2 + RCHO + O_2 \rightarrow FMN + RCOOH + H_2O +$ Light (490nm)

ATP-bioluminescence based systems and kits, such as the Milliflex rapid microbiological detection and enumeration system and the Clarity Luminescence Microplate Reader, are employed for assessing contamination in soil and water bodies. These systems utilize high precision reagent injectors in combination with an ultra-sensitive photon counting photomultiplier tube (PMT) detector (Chollet and Ribault, 2012).

Application of immobilised microorganism to determine amount of dissolved oxygen: The use of a dissolved oxygen microsensor has allowed for the measurement of the amount of dissolved oxygen in biofilms, indicating the intensity of microbial metabolic activity and correlating the results with biofilm thickness (Tomazinho *et al.,* 2014). The majority of biochemical oxygen demand (BOD) sensors are microbial sensors of the biofilm type, which rely on assessing the bacterial respiration rate in close proximity to a suitable transducer. These sensors typically feature a microbial film positioned between a porous cellulose membrane and a gas-permeable membrane, serving as the biological recognition element. The immobilized microbial population within this film can bio-oxidize the organic substrate targeted for quantification. The response generated by these sensors typically involves a change in the concentration of dissolved oxygen (DO). The DO diffuses from the aerated phosphate buffer through the dialysis membrane into the immobilized cell layer, where a portion of the oxygen is consumed by the immobilized microorganisms. The remaining oxygen then diffuses through the gas-permeable Teflon membrane and is detected by the oxygen electrode. Upon introducing a wastewater sample into the sensor system, assimilable organic substrates diffuse through the dialysis membrane and are taken up by the immobilized bacteria, leading to an increase in the bacterial respiration rate and oxygen consumption for bio-oxidation processes. Consequently, less oxygen is able to diffuse through the Teflon membrane and be detected by the oxygen electrode. This results in a decrease in the current until a new equilibrium value for oxygen is attained. Upon reintroducing the buffer into the system, the residual wastewater sample is diluted and flushed out. As the respiration rate of the microorganisms diminishes, the endogenous respiration rate gradually returns. As the process is governed by substrate diffusion, the sensor signal is directly proportional to the concentration of easily biodegradable organic substrates present in the sample (Liu and Mattiasson, 2002). Additionally, the development of a biochemical oxygen demand (BOD) sensor using immobilized microbial consortium in an alginate-based matrix has enabled the rapid detection of river water pollution, showcasing the practical application of immobilized microorganisms for environmental monitoring (Hussin *et al*., 2012). As immobilized microbe carriers, sodium alginate gel spheres made using the N, N-methylene bisacrylamide cross-linking technique have been employed in measuring dissolved oxygen in urban domestic wastewater (Li *et al.,* 2022).

Application of heat shock proteins in water quality monitoring: Heat shock proteins (HSPs) are recognized as valuable biomarkers in environmental monitoring due to their role as molecular chaperones that respond to cellular stress and environmental perturbations (Jeyachandran *et al.,* 2023). The presence and expression of HSPs, particularly HSP70 and HSP60, have been utilized as indicators of cellular stress and damage in response to various environmental stressors, including temperature fluctuations, chemical exposure, and pathogen invasion (Kilemade and Mothersill, 2001). In environmental monitoring framework, the upregulation of HSPs in aquatic organisms, such as fish and invertebrates, has been linked to exposure to environmental pollutants, thermal stress, and other adverse conditions (Mukhopadhyay 2003). According

to de Jong (2008), the induction of HSPs functions as a defense mechanism to help with protein folding, repair, and degradation, hence promoting cellular homeostasis and survival in stressful environments. The detection and quantification of HSPs in environmental samples provide valuable insights into the impact of environmental stressors on the health and resilience of aquatic organisms, making HSPs important biomarkers for assessing environmental quality and ecosystem health (Kilemade and Mothersill, 2001). Moreover, the assessment of sublethal effects of environmental stressors on aquatic organisms has involved the study of heat shock proteins (HSPs) in the context of environmental toxicology and ecotoxicology. This has been exemplified in the evaluation of the sensitivity of HSPs, particularly HSPs and Hb genes, in the larvae of the aquatic midge *Chironomus tentans* (Lee, 2006). In amphipod species from the Lake Baikal region, the induction of cellular stress response systems, heat shock protein hsp70/Hsp70, and multixenobiotic transporter abcb1 by cadmium chloride ($CdCl_2$) was investigated and it was discovered that genes for both proteins were upregulated in the presence of severe toxic stress (Protopopova *et al.,* 2020). When gill and liver tissues of Zacco platypus, a pale chub found in urban rivers and reservoirs were examined, transcriptional responses of genes involving cellular homeostasis (heat-shock protein 70, HSP70; heat-shock protein 90, HSP90), metal detoxification (metallothionein, MT), and antioxidation (superoxide dismutase, SOD; catalase, CAT) were found to be upregulated, suggesting low water quality in Singal Lake (Kim *et al.,* 2022). According to Maresca *et al*., (2020) *Conocephalum conicum* was able to alter biological parameters in response to cadmium stress, such as the generation and distribution of reactive oxygen species, the activity of antioxidant enzymes, and the stimulation of Heat Shock Protein 70 expression. Cadmium bioaccumulation in these Liverworts were linked to the biological reactions observed.

Application of acetylcholinesterase inhibition to determine level of organic pollution in water: This is applied either using real-time environmental monitoring, or toxicology *invitro* analysis (Costa-Silva *et al*., 2015). While carrying out real-time monitoring, a synergy of biosensors and biomarkers are commonly employed while a synergy of bioindicators and biomarkers are commonly paired in toxicology *invitro* assays. Acetylcholinesterase (AChE) activity can be inhibited by various environmental pollutants and chemicals. The mechanisms through which AChE activity is inhibited by pesticides have been investigated using biosensing techniques and enzymatic platforms (Carvalho dos Santos *et al.,* 2022). For instance, the inhibitive action of metals such as arsenic has been shown to affect AChE enzyme activity (Sanllorente *et al.* 2010). Moreover, the ultrasensitive detection of organophosphorus pesticides has been achieved through the measurement of conductance changes when AChE activity is inhibited (Dong *et al.,* 2023). Additionally, the exposure to pollutants in water bodies has been associated with the inhibition of AChE activity, as evidenced by the significant inhibition of AChE activities in zooplankton communities due to water quality variations (Özhan-Turhan and Gökçe, 2022). Furthermore, the integrated use of biomarkers has indicated that concentrations of certain pollutants, such as organophosphate pesticides, can significantly inhibit AChE activity (Führer *et al*., 2012). Moreover, different types of pesticides, including organophosphorus, carbamates, and organochlorine, have been identified as inhibitors of AChE enzymatic reactions (Pino *et al.,* 2015). Additionally, environmental pollutants have been linked to the biochemical response of organisms, such as amphipods, with implications for AChE activity, catalase, and glutathione transferase (Schvezov and Amin, 2011). *Aulacomya ater*, the ribbed mussel, exhibits heightened sensitivity to low concentrations of the organophosphate pesticide Lorsban 4E in its environment. This sensitivity manifests as a substantial inhibition of acetylcholinesterase (AChE) activity, resulting in a concomitant increase in ammonia excretion. Ribbed mussels employ valve closure as a defensive strategy to mitigate their exposure to the pesticide. Principle for use of acetylcholinesterase inhibition stems from ability of some organic pesticides and heavy metals to inhibit activity of acetylcholinesterase, thus diminishing the thiocholine product such that the higher the contaminant, the lower the product and in effect the activity of acetylcholinesterase (Sanllorente-Méndez *et al.,* 2010). A screen-printed carbon electrode (SPCE) fitted with immobilized acetylcholinesterase (AchE) was utilized for the determination of arsenate (III) (AsO_3^{3-}) in water samples. The method relies on the enzyme's inhibition by AsO_3^{3-}, leading to a decrease in thiocholine production from the enzymatic reaction between Ach and acetylthiocholine iodide. As thiocholine is electroactive and undergoes anodic oxidation, the resulting current provides a quantifiable signal for AsO_3^{3-} determination. The As(III)-mediated inhibition of acetylcholine activity is reflected in the diminished thiocholine production, consequently decreasing the oxidation current. This decrease in current is directly proportional to the AsO_3^{3-} concentration. The extent of As(III) inhibition is quantitatively evaluated by measuring the difference

between the steady-state oxidation currents in the absence (I_0) and presence (I) of As(III) (Sanllorente-Méndez *et al*., 2010).

$$Acetylthiocholine\ iodide + H_2O \overset{AchE}{\rightleftarrows} Thiocholine\ iodide + acetic\ acid$$

Application of fluorescence in-situ hybridization in water monitoring: Fluorescent in-situ hybridization (FISH) is also a valued tool for monitoring water contamination due to its ability to detect and identify specific microorganisms in water samples. FISH has been successfully applied in various water monitoring technologies, including hand-held sensing devices, for the real-time detection of contaminants such as microorganisms, pesticides, heavy metal ions, and organic components (Zulkifli *et al*., 2018). This technique has been used to specifically detect viable *Escherichia coli* in water samples, proving to be a valuable tool for monitoring microbial communities in water bodies (Yamaguchi *et al.,* 2015; Kenzaka *et al.,* 2006). Additionally, FISH has been used to identify biological pollution of surface water by evaluating the dispersive phases of parasites; this approach has shown to be more sensitive, quicker, and less expensive than traditional approaches (Nowosad *et al.,* 2006). Moreover, FISH has been utilized for the fluorescent labeling of *Cryptosporidium parvum* oocysts in water samples, demonstrating its potential in detecting specific pathogens in water (Vesey *et al*., 1998). The technique has also been employed to investigate the abundance and diversity of archaea in heavy-metal-contaminated soils, showing its versatility in assessing microbial populations in different environmental matrices (Sandaa *et al*., 1999). Additionally, FISH has been used to identify microorganisms in hydrocarbon-contaminated aquifer samples, highlighting its applicability in assessing microbial communities in contaminated environments (Schattenhofer *et al*., 2014). FISH has also been employed to monitor changes in microbial communities in response to contamination, as evidenced by its use in monitoring the reaction of sulfate-reducing bacteria in marine sediments to a man-made oil spill (Suárez-Suárez *et al.,* 2011). The use of FISH in water analysis involves sample collection, fixation, hybridization with fluorescent probes, visualization, image analysis, data interpretation, and reporting. These steps enable the specific detection and quantification of microorganisms and genetic material, contributing to the assessment of water quality and pollution monitoring. Specific oligonucleotide probes, known as gene probes, are meticulously designed to complement targeted gene sequences or metabolic products of the organism or population under investigation. These probes are conjugated with fluorescent dyes, enabling the emission of detectable fluorescent signals when binding to their complementary target sequences within cells. This fluorescence can be visualized using microscopy techniques such as epifluorescence microscopy or alternatively, quantified using flow cytometry.

Table 3: Summary of system synergy components reported in this paper

Bioindicator	Biosensor	Biomarker	Toxicant	System synergy target
Photorhabdus spp	Photomultiplier tube detector	ATP	Sewage/sludge	Use Bioluminescent Bacteria to Determine Biochemical Oxygen Demand
Anaerobic denitrifying bacteria like *Aridibacter*	Biofilm-type microsensor	-	Waste water	Application of immobilised microorganism to determine amount of dissolved oxygen
Liver worts, Pale chub	-	Heat shock proteins	Cadmium	Application of heat shock protein in water quality monitoring
Aulacomya ater	Screen-printed carbon electrode	Acetylcholinesterase	Organophosphorus pesticides	Application of acetylcholinesterase inhibition to determine level of organic pollution in water
Autochthonous deltaproteobacteria	Oligonucleotide probes	Reduced sulfate	Oil spill and naphthalene contamination	Application of fluorescence in-situ hybridization in water monitoring

Conclusion: System synergy is effective in environmental monitoring. Whether it is a synergy of bioindicators and biomarkers or biosensors and biomarkers, a combination of at least two systems effectively helps quantify level of pollution in a particular water environment and help ensure water quality for vitality.

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