**BIOMARKERS: A TOOL FOR ASSESSING ENVIRONMENTAL POLLUTION AND BIOREMEDIATION**

**Dr. Sonia Sethi\* (Associate Professor), MsPayal Gupta**

**Dr. B. Lal Institute of Biotechnology**

**Malviya nagar, Jaipur, pin code: 302017, India**

**\*Mail ID:** **soniakaura198@gmail.com** **(corresponding author)**

**Phone: 9833640615**

Abstract: In recent years, due to anthropogenic activities, the concentration of environmental pollutants has increased dramatically in environmental matrices give rise to deleteriousenvironment for living organisms. Interests have been developed as remonstrancemechanism for the detection of unfavourable biological responses of adulterants in both humans and wildlife. Molecular and cellular biomarkers of contaminationencounter this requirement. A biomarker alters biological response occurring at molecular, cellular or physiological levels due to the toxic effects of environmental chemicals. Risk evaluation of chemical adulterants to organisms and ecosystems is made complex by various components. Biomarkers can also be used for bioaugmentation of contaminated sites and choice of monitoring system depends upon sensitivity and specificity of detection required. The chapter discusses the recent progress in the use of biomarkers in biomonitoring and analyzing the hereafter context in the application of this tool for bridging environmental issued studies.

**Keywords:**Soil quality indicator, Bioaugmentation, Biomonitoring, Molecular markers, cellular markers

**Introduction:**

Thousands of chemicals are thought to be regularly used in industry, making them potential pollutants and contaminants of the global ecosystem. Numerous potentially dangerous chemical substances are produced by metropolitan areas, rural areas, and businesses and are frequently released into the environment. As a result, the scientific community has exhibited interest in the identification of environmental agents that threaten human health and ecosystem sustainability (Magalhes& Ferro-Filho, 2008).

The following issues confront ecotoxicologists when they try to develop an efficient management plan:

(1) The variety, toxicity, and importance of contaminants to innate biota.

(2) Speculating the distribution, final concentrations, and routes of particular chemicals in various environments.

(3) Predicting potential ecological harm that could result from the buildup of certain chemical concentrations in biota.

(4) Determining verifiable upper limits for chemical concentrations that are safe for various ecosystems.

(5) A variety of environmental factors also affect how bioavailable contaminants are.

(6) The various sensitivity of the organisms to the impacts of pollution exposure.

The specific restrictions of the current environmental management practices have been emphasized by many authors. Therefore, it has been questioned to what degree laboratory experiments can or will ever be able to anticipate the exposure to the impacts of chemical adulterants on ecosphere and their constituent parts. The inability to exploreinterconnections between adulterants, the impact of surroundings on contaminant toxicity, and pollution-induced switches in environmental connections through time are all shortcomings of present methods. Also, accumulating contaminants in the environment are not considered at all by present management techniques(Landrigan *et al.,* 2018).

Pollutants cause harm on a variety of time scales and biological organization levels, including the molecular, cellular, and physiological levels. Loss of biodiversity, destruction of habitats, and changes to common resources are a few of the effects of pollution on ecosystems. Millions of people die prematurely every year due to pollution, which is why early warning systems for identifying, estimating, and evaluating the hazards associated with environmental pollutants are becoming more and more popular. Although understanding of the chemical data on pollutant concentrations in environmental matrices over the past years has grown, it is still inadequate to accurately evaluate the hazards of contamination (Burgeot *et al.,* 2017). In light of this, anconsolidatedchemical and biological approach is required for monitoring of pollution and, also, the measurable effect of pollutants has developed.

The accumulation of difficult-to-degrade materials in large quantities in soil and water is one of the main issues, and this has significantly increased the issue of intractability to microbial decomposition. As a result, significant work has been put into developing practical, affordable strategies for sweeping up polluted sites. Bioremediation is the most effective clean-up method that is also reasonably inexpensive. For the cleanup of sites with easily degradable pollutants, the use of the local microbial community for in situ bioremediation is gaining popularity. However, specialised or engineered microbial inoculants as bioaugmentation are a suitable alternative for more recalcitrant chemicals (Alexis *et al.,* 2016).

All compounds present in the mixtures are not broken down equally is the only issue with microbial bioremediation. However, the range of substrates can be expanded through genetic engineering to include xenobiotics that are often resistant to breakdown. Different genetically modified microorganisms have been successfully built, and experimental evidence has shown that they are useful for bioremediation and have a higher degradative capability. Application of GEMs in situ is limited because of the clangers associated with proliferation and transfer of gene horizontally (Dick and Gerhard, 2020).

Alternatively, adaptation of microbes for utilization of many recalcitrant compounds as the sole carbon source andcomplete mineralization of the compound can be carried out by use of microbial consortia. Another emerging technology for cleanup of environmental pollution with hazardous substances is the use of plants i.e. Phytoremediation. Advantages of phytoremediation includelong-term applicability, cost-effectiveness and aesthetic advantages (Subhash Chandra *et al.,* 2013).

The leftovers continue to exist below the surface in the impacted locations where pollution issues still exist and negatively affect other activities even after a number of years. This fact makes it abundantly evident how important it is to create bioremediation systems to handle pollution. Therefore, it is not possible to carry out bioremediation without the consent of the local communities. Scientists who can explain to the locals the outcomes of contamination testing and microorganism tests, particularly in reference to risk assessment, should allay their worries regarding bioremediation.

**Potential Environmental Contaminants**

Different types of pollutants are chemical, biological, or physical materials. Soil and water get contaminated from chemicals used infossil fuel, from domestic and industrial waste products, mining and agriculture which significantly affect human health and safety, welfare, and the worth of the natural world. Major contaminants include petroleum products (like polychlorinated biphenyls), nitrates, insecticides, sediments and excess organic matters. Pollutants reach water bodies through leakage, improper handling and operations and application to fields. The most harmful material is plastics, to marine animals if thrown and swallowed (Tesfalem Weldeslassie *et al.,* 2018)

**Monitoringof Environmental Contaminants**

Monitoring of pollutants can be carried out by various ways, based on the intentions and objectives of a specific monitoring program. Pollutant monitoring can be achieved by chemical/physical and biological ways. A chemical-specific approach provides insufficient information about effects of pollution is as a result of the vast quantity of potentially harmful substances. And on regular basis of monitoring a very few chemical/physical parameters can be done. Also, monitoring by chemical/physical methods has not been very reliable to predict the final toxicological results.

One of the major parts of monitoring is biological monitoring, which has been crucial in the fight against pollution .It is a method for evaluating statistical knowledge about pollutants by living organisms’, which is based on analysis of an individual organism’s. Biological monitoring includes augmentation and accumulation of toxic chemicalsand detection of toxicity which are required to detect the definition of issue and intended remedies measure.

**Biomonitoring Techniques**

Biological responses at various levels of biological organisation (biomarkers and bioindicators) are used as biological monitoring techniques to detect significant environmental changes. Bioindicators are described as "an organism or biological reaction that shows the presence of the contaminants by the emergence of typical symptoms or measured responses," a phrase borrowed from environmental toxicology. By changing physiologically, chemically, or behaviorally, these creatures (or communities of organisms) provide information about changes in the environment or the amount of environmental pollutants. A trait that is objectively tested and assessed as an indicator of typical biologic processes, pathogenic processes, or pharmacologic reactions to a therapeutic intervention is referred to as a biomarker.

The techniques of biomonitoring are classified as biochemical alterations, bioaccumulation, population- and community-level approaches, morphological and behavioral observation and modeling. Alterations in biochemical pathways are due to interactions between the pollutants and biological macromolecules. Some examples of biochemical biomarkers are metallothionein, oxidative stress, and cytotoxicological responses and their selection depends on specific conditions.

Another important process through which living organisms are affected by chemicals is bioaccumulation which occurs when there is absorption of toxic substance by an organism at a greater rate than that of elimination. To study the evaluation of the balance between ecosystems, population-level (size distribution) and community-level (species-richness metrics) approaches can be used for monitoring the effect of pollution to living organisms. To study the direct effects of toxicants on the living organisms, Morphological and behavioral observations can be commonly used. These observations include histopathological techniques and ultrastructural observations which are based on the optic microscope and the electric microscope. A modelling technique that is based on experimental results or published data, from which it is feasible to create mechanistic models, can be used to comprehend the numerous biological alterations that occur under the stress of environmental pollution.

**BIOINDICATORS**

Bioindicators (biomonitoring species) are defined as species or groups of species that are used to detect harmful pollution effects. Species used as bioindicator for toxicological research are different from that of model species and the Model species are frequently absent from natural habitats. Responses in the organisms due to unfavourable outcomes of pollutants or changes in the number of species can be measurablein communities. To calculate different biological indices, different indicator species of the proportional abundances (number of species) are used. Different environment contains good bioindicator species which enables to estimate ecological health in different contexts. Bioindicator species are tolerant to variety of toxicants and can be used as ameasurable property. Also, the species population can be used as anindicator are used to indicate the environment contamination (Nkwoji et al.,2010).

For the assessment of positive and negative changes in a given ecosystem biological indicators occurring naturally are regularly used. The importance of considering environmental elements which interact with biological indicators such as temperature, light, moisture and suspended solids are emphasized (Khatri and Tyagi, 2015). In a biological system every entity/part functions as a biological indicator in its surroundings. Amasterful criterionfor the biological indicator in a given ecosystem is the correct and prompt response, targeted and able to recognise changes caused by reprehensible management, and climate changes. In a specific community, different viable species reflect different response to same pollutants and to different pollutant at same degree. Pollutants which are existing in an abnormally low concentration, identifying them requires highly sensitive equipment of high cost. As an alternative, the sensitivity of the biological indicator's range provides a picture of pollutant rates that are, regardless of how little, biologically important.

Biological markers demonstrate the biotic consequences of pollution indirectly when chemical and physical assessments unable todo so. The statement that biotas alone are the best predictor of ecosystems reaction to stressor’s occurrence is in agreement from the scientists. Also, disproportionate number of responses of divergent species is a signal of biological indication, as some species might decrease while others increase. The Indicators other than disturbance or stress, species of biological origin can be impacted which cover the mechanism of changes. The limitation of using biological indicators is their ability as scale-dependent. For instances, one indicator can fail to indicate the biodiversity response to pollutants at another community.

**Plants, animals and microorganisms as biological indicators**

To estimate the levels of pollutants in their habitat and to monitor variations in population density throughout time in ecosystem, biotas could often be usedindirectly. Biota always conveys a suggestive idea about the status of ecosystem’s health. Species are very much susceptible to pollutants present in theirecosystem, which might alter theiranatomy, physiology, or behaviour. Variousplants, animals, and microorganisms areimportant tools for indicating pollutants in a given ecosystem.

**Plants as indicators**

Higher plant species, lichens, and plankton are just a few examples of how delicately plant species can be used to predict pressures in ecosystems. Due to industrialization and urbanization, pollution of terrestrial and aquatic ecosystems had been exacerbated. Higher plants areuseful for estimation of the pollution status because of their immobility (Jain *et al.,* 2010). The pollutants affect plant in various ways ranging from changes in morphology to metabolic and/or cellular changes which are frequently noticed rather than overall impact. On the whole, the first biological indicators are external vegetative symptoms (Saber *et al.,* 2015a). Parameters such as external factors like form, color, and taste, changes in pH, changes in nitrate content and variations in the content of all soluble salts. But for quality evaluation lower plants are preferred for example, evaluation of a metal plant extraction process (Saber *et al.,* 2016a, b).

 In aquatic ecosystems, Planktons develop with chlorophyll; play a vital food supply for many small- and large-scale aquatic biotas. Planktons are able to integrate, and they are frequently employed to assess the level of pollution in a particular aquatic ecosystem. Planktons could monitor high phosphorus and nitrogen existence in the aquatic body and act as a health indicator. Cyanophyta is commonly used as bioindicators with rapideutrophication of aquatic ecosystems (Thakur *et al.,* 2013).

**Microbial indicators**

Micro-organisms, due to their rapid growth response even at low pollution rates and ability to exhibit imperative signs of changes in ecosystems, are used as pollution indicator (Khatri and Tyagi,2015). Microbial indicators are selected on six specific well-defined criteria, for example, microbial toxins and microbial counts. The Microbial Consortium has a high capacity to modify their levels of operation, biomass for managing ecosystem pollutants and is helpful in assessing the quality of a given ecosystem. Bacteria when present in any ecosystem above certain limits, indicates their contact to pollutants (Kalkan and Altuğ, 2015).

Most important bacterialbiological indicator, is to determine total bacterial counts (virtually never obtained) because it isnot that allbacteria could develop their colonies in a certain ecosystem.Bacterial counts of anaerobic mesophilic bacteria such as *Salmonella typhimurium* and *Clostridium sp*. in a given ecosystem act as a biological indicator. Fecal coliforms, which also comprise naturally occurring bacterial species found on plants and in soil, are more valuable as biological markers than total coliforms (Saber *et al.,* 2015b). Halophillic bacteria are also good biological indicators in detecting salinity problems in a given ecosystem. Other types of bacteria such as *Escherichia coli, Enterococci,Salmonella sp., Campylobacter* sp., and gastroenteritis associated bacteria are used to determine and detect pollution levels in various ecosystems. Microbial biomass depends on mineralization of C and N, breathing, biomic N2 fixation, and enzymes and out of these biomass-specific breathing, appears to be more sensitive (Aslam *et al.,* 2012).

**Fungal indicators**

Molds such as *Trichoderma sp.Penicillium sp., Aspergillus niger., Aspergillus fumigates., Aspergillus versicolor., Ulocladium sp., Exophiala sp., Stachybotrys sp., Phialophora sp., Fusarium sp.,Candida albicans*, and certain yeasts are common biological markers for pollutants since they are found in both terrestrial and aquatic habitats.

**Algal indicators**

Algae such as *Chlorella sp., Euglena sp., Scenedesmus sp., Chlamydomonas* sp., etc can be efficiently used as pollution biological indicators in aquatic ecosystems (Hosmani, 2013). Increase in algal species diversity, like *Euglena clastica, Phacustortus, and Trachelonanas*, results in marine ecosystem degradation.

**Lichens**

Mutual associations of Algae and Fungi comprise Lichens are bushy growths that appear as continuous, crusty patches on bare ground, rocks, and tree trunks. Lichens effectively respond to ecological changes particularly pollution due to high Nitrogen and sulphur oxide, therefore widely used as biological indicators in forest ecosystems.

**Enzymes**

To measure the degree of degradation in a given ecosystem, enzymatic activities are used as biological indicators because they are sensitiveto pollutants. The enzymeproduction in polluted ecosystems varies from high to low and low to high depending upon the activity of enzyme. Lysozyme improves dehydrogenase activity due to respiration inhibition therefore, the effect of certain pollutants, e.g., mercury and cyanide, could be assessed.

**Animal indicators**

Pollution in ecosystem results in harmful changes and dissimilarities in animal populations. Changes in populations of animal are related with food sources; a limited food resource means decrease in population intensity Jain *et al.,* 2010). Animals serve as biological markers, which aid in determining the level of poisons present in animal tissues.

**Assessment of Environmental Health Using Bats as Bioindicators**

Growing human population has detrimental impacts on the equilibrium between humans and other living things, which is destroying the world (Barnosky et al. 2012). Bioindicators play a vital role in attaining balanced living environment by lessening the human impact on environmental health. Among most diverse vertebrate groups, bat is sensitive to changes in land use and habitat (Fenton & Simmons, 2014). It is also cost effective, stable, sensitive to environmental stress, can be used in pollination and controlling pests in the ecosystem (Jones, 2012; Amorim *et al.,* 2015).

**Birds and fishes**

Tourism affects freshwater environment biodiversity caused by pollution and exploitation. Activities of tourist may affect birds and fishes which are bring short lived species after disturbance. Theses act as bioindicators of environmental pollution caused by human disturbance.

**Earthworms**

Presence of earthworms ecological system represents the level of pollution in that system. and as early warning system in monitoring changes as a whole, therefore they are used as effective biological indicators.Earthworms serve as significant indicators for ecotoxicology risk assessment andfor potentialpollutants which results in damage of the ecosystem.

**Frogs and toads**

For monitoring the quality and shifts in a certain environment, frogs are good biological indicators because they areaffected due to pollutantaccumulation in a given ecosystem. Anurans are more susceptible to changes in their ecology, and their skin and larval gill membranes allow them to take up hazardous compounds. Also, they are able to detoxify pesticides that they ingest, breathe in, or eat from contaminated foods, but this allows residues to build up in their biosystems. These factors allow them to use for contamination research, eco-toxicological trials, and ecosystem changesas biological indicators. Morphological changes like reduced body length, organ malformations, lower body weight, slow growth rate and limited metamorphosis are observed on exposure.

**Insects**

Insects in ecosystems are strictly and quickly affected by pollutants and can be used as aparameter of analyses about the levels of change in agiven ecosystem. There are many processes in the ecosystem for which insectsare responsible, and their loss always have negative effects on entire biological communities. Therefore, a great comprehension of pollutant and insect responses is of functional value.

Insect used as indicator should be easily apprehended and transported easily, have ecological constancy, respond to changes in ecosystem, short life cycle, sensitive for detection early environmental changes, and information is provided without interruption regarding pollution-related harm or alteration. (da-Rocha *et al.,* 2010). Insects species like Coleoptera (beetles), Homoptera(bugs), Diptera, Odonata sp. (dragonflies), Hydrophilidae (Coleoptera), families like Gyrinidae, Dytiscidae, Veliidae (Heteroptera) possess a considerable potential for adaptation as biological indicators.

Effect of Cu, Fe, Ni, Cd, and H2SO4 on different Insects species can be studied by their population, cycle length and the mortality rate of newly born larvae. Insect species like *Apis mellifera,* effective biological markers, demonstrate a high rate of ecological chemical loss and capture particles that might later be seen in the air or flowers. Ants are essential to the restoration of damaged ecosystems and Ameliorations shown high pollutant tolerance (radioactive and chemical chemicals). Bees are used to detect radioactivity after the Chernobyl disaster, hazardous pollutants, poisons in urban habitats, insecticides, and herbicides. Wasps are susceptible to the hazardous biological buildup utilised to accumulate lead at the top of the food chain.

**Zooplankton**

Zooplanktons are biological indicators and assist in determining how polluted aquatic habitats are.For the development of zooplanktons, aquatic efficiency, eutrophication,and fresh water body growth are important and any weather fluctuationsgreatly influence zooplanktons. Zooplanktons as indicators are associated with biotic and abiotic parameters e.g. predation, competitiveness, food shortage, pollutants, alkalinity, temperature and stratification. Few examples of zooplanktons include *Trichotriatetrat, Alona guttata, Moscyclopesedex, Cyclips, Aheyella, Copepods, Rotifer and Ostrocoda*.

Various bioindicators such as lichens, microorganisms, plants or animals, which produces molecular signals under environmental alterations (Posudin 2014). With the help of bioindication, which identifies distinct biological systems using straightforward data, the entire area may be fully monitored. With the use of a trustworthy bioindication process, one can evaluate how external forces affect ecosystems. Environment makes indicator species susceptible to its changes, although it is thought to be more effective and less expensive to identify an ecosystem by analysing an effective incentive of a single population.

Variations in indicator species can be identified by alterations caused due to short term or long term stress conditions like increased popularity changes in living systems, coexistence of diversity (Lindenmayer& Likens, 2011; Ahmed *et al.,* 2016).

**BIOMARKERS**

Measures of alterations can be thought of as pollution biomarkers due to pollutant exposure in a biological system in terms of its typical state. They are referred to changes which occur at biological organization (*e.g.*, molecular, cellular, physiological) but at low levels but are generally accepted as compared to those at higher levels (*e.g.*, population effects) occurred earlier. Biomarkers such as cellular and molecular give a sensitive heads-up on potential population-level toxicological effects later on (Hook *et al.,* 2014). Also, biomarkers give relevant information about the measurement of contaminants in environment and the exposure and possible negative effects of pollutants on exposed organisms' health. This accounts for the development in monitoring of the environmentand human health.

Therefore, to evaluate the type and extent of exposure, to spot changes inside an organism, and to determine an organism's underlying sensitivity, biomarkers can be used. Biomarkers expand your knowledge of the processes of chemical absorption and transformation within an organism due to alterations that occur causing toxicity at the cellular and molecular levels. Consequently, biomarkers are divided into three categories: susceptibility, effect, and biomarkers of exposure based on specific biological responses (Schettino *et al.,* 2012).

Exposure extent and occurrence of various compounds to organism provide an indication about biomarkers of exposure and are alterations to the organism's cells that are reversible and rely on the activation of cleaning processes. To learn more about the source, pathway, and route of exposure, use a biomarker of exposure. Damages, changes and adducts on proteins, DNA and Lipids molecules can be measured using exposure biomarkers. They are used to determine exposure to chemically reactive toxins like nitrosoamines, aromatic amines, polycyclic aromatic hydrocarbons and heavy metals. Examples of biomarkers of exposure are metallothionines, heat shock proteins, and antioxidant enzymes.

In particular in human biomonitoring, xenobiotic measurement in the biological system is utilised as "biomarker of internal and effective dose." The concentration of a parent substance or metabolite at the target site is referred to as the internal dose. Effective dose, on the other hand, refers to indicators found in the target tissues that show how the absorbed substance interacts with a subcellular target. A change in enzyme activity, the creation of DNA or protein adducts in circulating blood cells, or both can serve as indicators of effective dose (LadeiraandViegas,2016).

Changes in target tissues associated to biochemical processes are examples of biomarkers of effect (DNA mutations, chromosomal aberrations, induction of protein production, DNA repair enzymes, stress proteins or the inhibition of enzymes e.g. acetylcholinesterase) or physiological changes, biological effects, changes in body weight etc that are brought on by exposure and also give an evaluation of the organisms' toxicological impact and are correlated with the danger of negative health impacts. Biomarkers of vulnerability show an organism's innate or learned capacity to react to exposure to a particular contaminant (Manno *et al.,* 2010). It reflects the kinetics of the chemical methods for the analysis of microbial transition states between the stages of individuals. In reality, inter-individual biological differences may make certain people more vulnerable to diseases brought on by the environment and act as markers of susceptibility.

From highly specific biomarkers to nonspecific biomarkers, the specificity of the biomarkers to contaminants varies. Induction of metallothionein by metals (Cu, Hg, Zn, or Cd) or lead's suppression of aminolevulinic acid dehydratase (ALAD) are examples of specific biomarkers (Calisi *et al.,* 2014). Nonspecific biomarkers include DNA damage and immune system dysfunction. Combining various particular biomarkers can result in a complementarity between them that raises the level of specificity as a whole (Gonick,2011).

## There are various requirements that must be met when choosing the most pertinent indicator responses to be utilized in the multimarker method in line with the goals of each individual biomonitoring programme. Some of these factors are the biomarker's sensitivity, its dose- and time-dependent response, its biochemical memory (how long the response lasts after exposure), and its inherent variability. Biomarkers should react to a pollutant in a dose-dependent manner over a range of pollutants with environmentally plausible concentrations in order to ensure a proper toxicity evaluation. Additionally, the relationship between the biological response employed as a biomarker and significant biological functions as well as pathological outcomes is thought to be pertinent in both environmental evaluation and health assessment.

**ENVIRONMENTAL BIOMONITORING USING POLLUTION BIOMARKERS**

**Cytochrome P4501A Induction**

Cytochrome P4501A (CYP1A), a logical biomarker used for the detection of biotransformation of pollutants like dioxins, furans, polychlorinated biphenyls and polycyclic aromatic hydrocarbons. In this action, when the organisms are exposed to such pollutants, The induction of CYP1A is enhanced by the presence of aryl hydrocarbon receptor from the cytosol. For example, in case of marine bivalves (Binelli *et al.,* 2006) and Zebra mussel (*Dreissena polymorpha*), a noteworthy promotion of EROD ethoxyresorufin dealkylation (EROD) activity occurred when they exposed to PCB mixture of Arochlor 1260 and dioxin-like CB-126. The biomarker can determine the pollution status of tiny streams that are contaminated with PCBs and AhR-binding PAHs at varying levels..

**DNA resilience as a Contamination Biomarker**

DNA integrity is affected by genotoxic and exogenous agents causing DNA strand breakage, methylation loss, double strandedness synthesis of DNA adducts which may be produced during repairing of DNA. Agents like PAH such as Benzo(a)pyrene (BaP), interact with DNA to form both stable and unstable adducts with DNA which may be due to transformation of the cell. Single strand breaks are caused by transformations, which are followed by ionising radiation, an oxidation-reduction process, or a photoreaction. For instance, DNA integrity in the contaminated areas of the marine snail Planaxis sulcatus considerably deteriorated, which was related to the level of contamination of these sites by petroleum hydrocarbons released from waste products into the coastal water.

**Metallothioneins (MTs)**

Metallothioneinsare proteins rich in cysteine found in cytosoland interacts by binding sulfur atoms of cycteine residues with toxic metal ions resulting in inactivation. Through the measurement of their quantities in bivalves from polluted habitats and oxidative stress in aquatic organisms, MTs serve as biomarkers for environmental pollution. Metallothioneins act as metal-chelating agents, via oxygen free radical scavenging actions and metal binding, playing significant roles in the detoxification mechanisms and metal metabolism in aquatic species. (Andrews, 2000).This results in oxidative damage to DNA, lipids and proteins and adverse effects on the antioxidant, enzymatic and non-enzymatic, defense mechanisms of organisms.

**Pigments as Biomarkers**

Algae and plant biomarkers contain pigments, their main purpose is to serve as light-harvesting tools for photosynthesis and photo defence. There are three main groups of pigments in plants and algae: Chlorophyll, Carotenoids, Phycocyanin and Phycoerythrin. For "tagging" tumour cells and as pertinent indicators representing taxonomic specificity, pigments can be utilised extensively in cancer research. They also represent the entire phototrophic community and total primary production. Pigments get broken down to colorless compounds when exposed to pollutants resulting in breaking of double bonds (Adedeji et al.,2012).

**Lysosomal system as Biomarkers**

The lysosomal system, comprising of lysosomes, auto and heterophagic vesicles, phagosomes and residual corpuscles, capable of detecting the slightest cellular damage caused bythe exposure of the pollutants. Lysosomal compartment comprises of lysosomes (Pirmary and secondary), auto and heterophagic vesicles, possess many functions, rich in enzymes responsible for hydrolysis. Diverse components of the lysosomes are lost due to the loss of integrity of the membranes caused due to physicochemical modifications associated with cellular dysfunction, inflammatory and degenerative diseases and death. Destabilization of Lysosomal membrane (assessed by lysosomal enzyme or lysosomal dye retention) is most commonly used biomarkers in environmental biomonitoring in invertebrates (Rocco *et al.,* 2011).

**Biomarkers for oxidative stress**

When cells are exposed to pollutants, it causes oxidative stress due to an increase in reactive species and a disruption in the effectiveness of antioxidants (Regoli and Giulian,2014). GSH, a crucial intracellular free radical scavenger that works in tandem with glutathione peroxidase and glutathione reductase to neutralise peroxides, is a commonly used indicator of oxidative stress. The ratio of reduced to oxidised glutathione (GSH/GSSG) is calculated to determine the organism's oxidative stress status.

Products from the oxidative breakdown of membrane phospholipids, like lipid peroxidation, are often used indicators of oxidative stress. Additionally, by altering their activity and expression as a result of exposure to pollutants, antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase (Leomanni *et al.,* 2015) demonstrate biomarkers of oxidative stress that are suitable for evaluating the effects of pollutants in ecosystems at low concentrations and early stages.

**The lipid peroxidation biomarkers**

This is the process that has been studied the most in terms of tissue damage caused by free radicals, but because it is difficult to analyse directly, measurements are made of the secondary oxidation products (aldehydes and ketones). Malondialdehyde (MDA) production as a peroxidation product, with the thiobarbituric acid reactive substances test, is a common assay for lipid peroxidation. According to numerous research, the free radical peroxidation caused by xenobiotics raises the MDA levels in urine or tissue samples.

**Biomarkers of DNA oxidative damage occurring in vivo**

Pollutant exposure increases DNA oxidative damage, and these changes are employed as biomarkers for specific purine and pyrimidine base modifications and hydroxylations as well as damage to the deoxyribose-phosphate backbone and protein-DNA cross-links. As a biomarker for carcinogenesis, the hydroxylation by HOD of the nucleobase guanosine and its free base 8-hydroxyguanine has been measured. Thymine glycol and thymidine glycol, are formed by the oxidative damage of DNA in tissues can also be used as biomarkers for carcinogenesis.

**Biomarkers of protein oxidation**

The oxidation products of phenylalanine and tyrosine amino acids which results in the formation of dityrosine are the measure of valuable cellular and urinary marker of oxidative stress. Recently, various methods have been developed to identify oxidized amino acids in blood proteins as biomarkers of free radical damage. Oxidations of proteins forms g-Glutamyl semialdehyde and 2- amino-adipic semialdehyde, through free radical reaction mechanisms which can be identified and measured in biological samples as Biomarkers of protein oxidation caused by environmental pollutants.

**Acetylcholinesterase enzyme as biomarkers for neurotoxic pollutants**

Acetylcholinesterase gets inhibited in response to neurotoxic compounds and its monitoring can be used as biomarker of pollutant exposure in aquatic and terrestrial ecosystems. The hydrolysis of the neurotransmitter acetylcholine is catalysed by this important enzyme in the nervous system, and it is the site that pesticides are designed to block. (Calisi *et al.,* 2013). As an organophosphorus and carbamate compound's molecular target, AChE, its measurement in the blood is also acknowledged as a human biological marker and has become an important diagnostic tool in the biomedical field. Inhibition of AChE by other chemical species, besides organophosphate and carbamate pesticides, has recently been documented in humans. These chemical species include heavy metals, other pesticides, polycyclic aromatic hydrocarbons, detergents, and parts of complex combinations of pollutants. (Vioque-Fernandez*et al.,* 2007).

As a result of its interaction with the enzyme, many nanoparticle classes, including metals, oxides, and carbon nanotubes (SiO2, TiO2, Al2O3, Al, Cu, carbon-coated copper, multiwalled carbon nanotubes, and single-walled carbon nanotubes), recently demonstrated significant affinity for AChE. (Indennidate *et al.,* 2010). With IC50 values of 4, 17, 156, and 96 mgL1, respectively, Cu, Cu-C, multiwalled carbon nanotubes, and singlewalled carbon nanotubes (MWCNT, SWCNT) demonstrated a dose- responsive suppression of AChE activity.

**BIOMARKERS IN HUMAN BIOMONITORING**

In order to track biological reactions to different diseases, drug exposures, and chemical agent exposures, biomarkers have evolved into precise end points. In human biomonitoring, biomarkers from persons exposed to chemical risk factors in the workplace or general environment in the past or present are examined in human tissues and/or fluids (Manno*et al.,* 2010). Evaluating health status and preventing the negative health effects of pollution exposure are the two main goals of human biomonitoring. For instance, the brown adipose metabolic biomarker serum exosomal miR-92a was focused on, and shift workers showed a difference (Bracci *et al.,*2020). The higher brown adipose tissue activity relative to daytime employees is shown by the lower levels of miR-92a.

**Assessment of Chemicals/Metabolites as a Biomarker of Exposure**

Chemicals/Metabolites assessment in humans is a biomarker that can be used to track exposure. to those chemicals/metabolites.Benzene, toluene, and xylene levels in blood, t-muconic acid levels in urinary tract, higher amounts of polycyclic aromatic hydrocarbons and organochlorine pesticides in women's blood (PAHs) in rural children (Pathak *et al.,* 2010), Lead (Pb) (Grover *et al.,* 2010) content in urine and blood are primary biomarker used to monitor short term and prolonged exposures in humans.

**DNA Damage as an Exposure Biomarker**

Comet assay for assessment of DNA damage has been widely used in human biomonitoring as a biomarker of exposure (Valverde and Rojas, 2009). This technique can be performed in proliferating and non-proliferating cells, with few modifications that allow detection of various DNA damage as well as repair. Various pollutants containing chromium,pesticides,wood dust,coal and benzenehave demonstrated significant increase in DNA damageleading to increased risk of adverse effects in the affected population.

Use of genotoxic biomass fuels (BMF) for cooking contributes to significant DNA damage in the lymphocytes (Mondal *et al.,* 2010) of women and up-regulation of DNA repair mechanism, associated with lung cancer in women. The Comet assay is used to depicts exposure and repairable DNA damage as a biomarker of exposure.

**Biomarkers of effect**

Genotoxicity monitoring in humans, chromosomal aberrations (CA) and micronuclei (MN) are commonly used as biomarkers of effect. Studies of epidemiology suggest that chromosomal aberrations at high frequency indicates a higher risk of developing cancer (Bonassi *et al.,* 2008). High frequency of CA and MN in peripheral blood cells, due to exposure of heavy metals fumes demonstrate genotoxic risk (Vuyyuri *et al.,* 2006) has been observed. Micronucleus frequency in the lymphocytes and buccal mucosal cells of occupationally exposed individuals has been widely used as a minimal invasive tool for evaluating genetic damage due to pollutants (Sellappa *et al.,* 2010) in ambient air. These studies indicate that biomarkers of effect can be used for screening and identifying populations at risk.

**Biomarkers of susceptibility**

Gene polymorphisms related to xenobiotic-metabolizing enzymes are used as markers of susceptibility which can be detected by polymerase chain reactions (PCRs) in blood samples (Singh *et al.,* 2010). Polymorphisms in cytochrome P450 (CYPs) in combination with glutathione S-transferase (GST) M1 or T1 (Singh *et al.,* 2009), polymorphisms of N-acetylation (NAT2) gene alone or in combination with p53 increased the risk of developing lung cancer. Also, studies with polymorphisms in genes for bioactivation, detoxificationetchelps in understanding the role towards development of cancers.

**Advanced techniques: in silico technology**

In silico technologies, an advanced technique have been employed as biomarker for risk assessment, predicting toxicity endpoints, clinical effects, and ADME properties of chemicals. Thisprovide a unique platform for studying mechanism of toxicity of the chemical/metabolite with macromolecules and quantitative structure toxicity relationship (QSTR) with target proteins/enzymes. Comet assay is used to assess DNA damage exposed to benzene during petrol refilling while in silico technique can be used to assess genotoxicity of benzene, which was due to its metabolites, bezoquinone and hydroquinone.

In addition, in silico molecular docking studies showed interactions of benzene and its metabolites at the ATP binding domain of human topoisomerase II alpha enzyme (important for DNA integrity. These studies have shown the importance of using new tools along with the conventional biomarkers to clearly understand the action of toxicants and help decipher the exposure-effect relationship. Quantification of acetylcholinesterase levels in blood is used for determining the level of occupational exposure to substances with organophosphates in exposed situations. Nowadays emphasis in human biomonitoring is posed on Consequently, genotoxicity biomarkers are developed to detect pollutant exposures, forecast risk, and assess the impact of exposure to genotoxic substances.

Another mainstream marker is inflammation-related biomarkers, which are considered for determining how the body reacts inflamatorily to external stress (Stiegel *et al.,* 2017). These include cytokines /chemochines determination in blood which gets altered due to environmental exposures (Angrish *et al.,* 2016). Also, oxidative stress acts as important biomarkers in the field of human biomonitoring a result of numerous environmental exposures. Damage to DNA and lipids caused by oxidative stress are linked to the aetiology of many diseases and can be detected in cells, tissues, and biological fluids.

Nowadays interest in integrated approach in biomonitoring has stimulated which is useful for a comprehensive risk assessment perspective. As stated by numerous authorities and institutions, there is a need to enhance risk assessment and management and boost policy implementation. Health risk and environmental quality assessment are strongly related with each other and also their integration results in more accurate predictions and data collection capabilities for both research.

Biomarkers like molecular and cellular ones are helpful instruments in an integrated approach for bridging investigations relating to humans and the environment. As a result, a variety of biomarkers may be useful in an integrated strategy for intervention techniques aimed at preventing or reducing the negative health impacts of chemical contamination in both the environment and humans. Human and environmental biomonitoring are taking more notice of recent developments in molecular biology and OMIC sciences (genomics, transcriptomics, proteomics, lipidomics, epigenomics, and metabolomics, among others), which opens the door to the creation of novel and more sensitive biomarkers that can be used in an integrated approach (Suárez-Ulloa *et al.,* 2013).

**BIOMARKERS AS TOOL FOR BIOREMEDIATION / BIOMARKERS FOR MONITORING EFFICIENCY OF BIOREMEDIATION**

Bioremediation is a technique in which living organisms are employed for mineralization of pollutants, for the removal or conversion of the pollutant to a less harmful product in the area where it is present. Various microbial processes like biodegradation, volatilization, chemical transformation, dispersion, stabilization (i.e., binding and sequestration by clays and humus), dissolution, and dilution occurs in soil and groundwater. But because these processes can move very slowly, some substances may linger for a long time. Biodegradation is influenced by a number of variables connected to the physical and chemical characteristics of the environment and chemicals they are present in.

There are various ways to evaluate microbial attenuation, including microcosm investigations, analysis of the site's hydrology and subsurface geology, qualitative and quantitative pollutant biochemical profiles, and composition and activity of the microflora. The evidence of transformation activities that are taking place at a pace that is safe for both the environment and human health is necessary for an accurate assessment of microbial attenuation. Continuous monitoring using chemical, biological, microbiological, and environmental indicators is necessary to keep in mind the design of the bioremediation process, its implementation, and its efficacy.

Different approaches for assessing the bioremediation process' effectiveness and in There have been suggestions for reducing long-term environmental toxicity. It includes measurements of metabolites of dissolved or residual contaminants, Nucleic-acid-based techniques and molecular techniques which concentrate on catabolic genes that encode for particular enzymes that degrade pollutants. Biomarkers as indicators have wide applications andused as tools for monitoring efficiency of bioremediation and its choice depends on the system.

**Luciferase as biomarkers**

For monitoring bioremediation inocula, luciferase markers such as luciferase gene (luc), or bacterial luciferase genes (luxAB) can be quickly identified as markers. For example, *Pseudomonas aeruginosa,* tagged with luxAB can be can be quickly identified by counting the colonies of luminous organisms. in microcosms contaminated with oil. Similarly, *Pseudomonas cepacia*, a Colony counting was used to track a 2,4-D degrading strain that was marked with the genes for luxAB and lacZY in 2,4-D enhanced soil. Luc gene can also be used as biomarker for monitoring gasoline degrading bacteria, *Pseudomonas fluorescens strain* 935061 fused with the tac promoter and *Arthrobacter* strain tagged with luc gene, using the pAM103 vector.Using luminescence markers light output can be directly measured in luminometer which is indicating a population of cells that is not metabolically active.As cells become starved, the The amount of light produced by luciferase enzymes decreases and therefore, it is referred to as potential luminescence

**GFP as a biomarker**

The gfp gene, which codes for a protein called green fluorescent protein, is another tool for tracking the progress of bioremediation. It has the benefit of fluorescing when exposed to light and requiring no additional energy source or substrate than oxygen throughout the process of chromophore production. In bacteria Arthrobacter strains tagged with two copies of the GFP gene, the GFP gene has been utilised as a biomarker to track the breakdown of 4-chlorophenol. A p-nitrophenol-degrading strain of Moraxella and a phenanthrene-mineralizing strain of Pseudomonas were tracked in soil microcosms by counting GFP fluorescent colonies, as are other examples of the use of GFP as a biomarker for monitoring bioremediation.

**Fungal biomass as biomarker**

To monitor and regulate the efficiency of the bioremediation process, fungus biomass has been used. As a good indicator for fungal biomass in contaminated soils, biochemical approaches for assaying fungus-specific cell components such ergosterol, chitin, or phospholipid fatty acids are recommended (Barajas-Acheve *et al.,* 2002). The SIP method of monitoring in situ biodegradation of a substance is based on changes in the stable isotope composition of the target molecule. SIP includes following stable isotope atoms from certain substrates into biomarker-containing elements of microbial cells.

**SIP as Biomarker**

In environmental microbiology the biomarkersused are DNA, RNA andphospholipid fatty acids (PLFAs); each of them has its own strengths and weaknesses (Dumont and Murrell, 2005). SIP denotes in situ biodegradation ofpollutants qualitatively and quantitatively. In SIP, the most remarkable biomarker is PLFA, which is applied with the degradation of toluene by Actinomycetales in the sediment of a petroleumhydrocarbon-contaminated aquifer.

**Genetic biomarkers**

Genetic biomarkers, which can be used to monitor potential pollutant degradation, are the most potent technique employed as a biomarker. When biodegradation is dependent on a particular microbial strain, specific nucleic acid sequences, conserved regions of the 16S rRNA gene, nucleic acid hybridization using specific probes, and PCR-based systems have all been used as biomarkers to identify the presence or absence of microbial organisms. The identification of phylogenetic and catabolic genes in samples is based on a variety of genomic techniques. Probes, a dominant and active gene pool, as well as the density and frequency of particular gene lines, are needed to track the degradation of a target molecule at a place in order to ascertain the genetic diversity of microorganisms as a whole. These methods have been successful in examining several bioremediation processes, such as reductive (Lee *et al.,* 2008).

**Enyzmes as biomarkers for bioremediation**

Using BMMs, For processes involving soluble (sMMO) and particulate (pMMO) methane monooxygenase enzymes, functional genes can be targeted (McDonald *et al.,* 2008). A mixed community of methanotrophs is capable of degrading trichloroethylene (TCE) with the integration of *pmoA*gene which codes for the alpha subunit of pMMO (Shukla *et al.,* 2009). Nowadyas, several biomarkers are in application for bioremediation and monitoring of environmental contaminants (Monard *et al.,* 2013). For example, even low concentrations of MTBE (methyl tert-butyl ether) transformation by cytochrome P450 monooxygenase-encoding gene, ethB, has been utilized as an indicator for the microbial transformation (Jechalke *et al.,* 2011). Recalcitrant compounds biotransformation by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) genes associated with BMMs also play a major role. For example, THF breakdown by *Pseudonocardia tetrahydrofuranoxydans* strain K1 utilizes aldehyde and semialdehyde dehydrogenase genes, suggesting dehydrogenase genes role in biodegradation.

## Phytoremediation

## Aquatic plants in particular may benefit from biomonitoring utilising specific high metal accumulating species as a method for developing a bioremediation strategy. This will help to improve the water's quality. Plants are used in phytoremediation procedures to lessen, eliminate, degrade, or immobilise environmental pollutants (Das *et al.,* 2007). The hydroponically grown plants are then transplanted into metal-contaminated waterways, where they absorb and concentrate the metals in their roots and shoots. When the plants are saturated, they are picked for disposal.

## Due to their capacity for sequestration and ability to survive in a variety of harsh conditions, algae and aquatic plants are among the species that have the potential to serve as ecological engineers for accumulating and bioconcentrating heavy metals. (Kalin and others, 2005) It was confirmed that the duckweed (Lemna minor) is a good option for phytoremediation of low-level copper and cadmium contaminated water bodies. Because of its high accumulation potential and tolerable reaction to moderate copper exposures, the aquatic macrophyte H. verticillata (L.f.) Royle is suitable for the cleanup of moderately copper-polluted water bodies (Srivastava *et al.,* 2006).

## The following benefits make bioremedation techniques based on biomonitoring an appealing approach when compared to other methods for cleaning up aquatic metal pollution: ease of use, quick and efficient cleanup vs. natural attenuation, environmentally safe and natural treatment, ease of application and no need for protective clothing, low cost, efficiency, and long-term solutions for a balanced ecosystem. Utilizing certain biosensors or biomarkers, genomic tools may evaluate the biological potential of each habitat. For instance, enzyme-based biosensors can generate the signal by product creation, the disappearance of substrate, or co-enzyme conversion. Biosensors can monitor a biological output that can be transformed into a detectable signal. Biomarkers are particular genotypes that can be used to monitor the persistence and/or effectiveness of a certain bacterial strain during bioremediation. The luc gene, which codes for firefly luciferase, and the gfp gene, which codes for green fluorescent protein, are examples of biomarkers (GFP).

## For the bioremediation of gasoline or chlorophenols, the lucgene has been tagged with various bacteria, and the activity has been observed based on luciferase activity. Different microbes have different key metabolic processes that when combined can be used in bioremediation to remove pollutants. Molecular tools like denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism can be used to analyse the community of these microbes (T-RFLP). The separation of double stranded DNA fragments with the same length but different sequences is accomplished via DGGE analysis. The method takes advantage of the distinction

## CONCLUSION

## Pollution biomarkers are increasingly widely used in human and environmental biomonitoring as early warning systems for negative consequences. Additionally, biomarkers serve as practical instruments for combining research on humans and the environment and bridging environmental and human risk assessment. Additionally, they can advance our understanding of the relationship between environmental contamination and human health and help to bioremediation studies of contaminants.

## CURRENT & FUTURE DEVELOPMENTS

In the years to come, the field of integrated biomonitoring and integrated risk assessment should delve deeper into the research of biomarkers in human and environmental biomonitoring as well as bioremediation. Additionally, attention should be given to a productive research area for creating cutting-edge methods for using biomarkers in studies on the environment and human health.

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