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

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Abstract: In recent years, due to anthropogenic activities, the concentration of environmental pollutants has increased dramatically in environmental matrices creating settings that are hazardous to living things. The rise of passions as early-warning indicators of the harmful biological effects of pollution on both people and wildlife. Molecular and cellular biomarkers of pollution meet this requirement. Biomarker alters biological response occurring at molecular, cellular or physiological levels due to the toxic effects of environmental chemicals. Numerous factors contribute to the complexity of the risk assessment of chemical pollutants to organisms and ecosystems. 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 developments in the use of biomarkers in biomonitoring and analyzing the future perspectives in the application of this tool for bridging environmental issued studies.

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

**Introduction:**

Numerous of compounds are thought to be regularly used in industry, making them probable contaminants and harmful substances of the global ecosystem (Maugh 1978). Numerous potentially dangerous chemical substances are produced by metropolitan areas, rural areas, and businesses and frequently discharged into the surrounding environment. Because of this, the scientific community has demonstrated interest in the identification of chemical and biological agents that threaten human well-being and ecosystem sustainability (Magalhães & Ferrão-Filho, 2008).

Ecotoxicologists are faced with the following issues encountered when striving to develop a successful management plan:

(1) The diversities and toxicity of pollutants and their ranking to natural biota.

(2) predicting the distribution, fate, and final concentrations 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 (Cairns and McCormick 1992).

The specific restrictions of present sustainable development practises have been emphasised by several authors. Therefore, it has been questioned to what degree laboratory experiments can or will ever be able to anticipate the exposure to the impacts caused by chemical contaminants on ecological systems and their constituent parts (Depledge 1992). The inability to investigate interactions between pollutants, the influence of environmental conditions on pollutant toxicity, and pollution-induced variations in environmental relationships through time are all shortcomings of present methods. Additionally, existing management practises give no consideration to environmental toxins that have accumulated over time.

Pollutants exert the negative effects at different time-scales and at several tiers of biological structure which includes molecular, cellular and physiological levels. Some of the impact of pollution on ecosystems includes loss of biodiversity, loss and degradation of habitats, and alterations of natural resources. Pollutants are also accountable for human diseases and death even premature death of millions of humans which explains the increasing curiosity in preventative measures for identifying, estimating, and evaluating the risks caused by environmental pollutants(Landrigan et al.  2018). The chemical data of concentrations of pollutants in environmental matrices of last years have developed awareness but are inadequate to accurately determine the possible risks of pollution (Burgeot et al. 2017). Therefore, in this regard, an amalgamated chemical and biological approach is required for monitoring of pollution and, also, the measurable effect of pollutants has developed.

One of the major problems is the a buildup of stubborn substances in soil and water at high concentrations and the recalcitrance to microbial decomposition is a significant concern. Therefore, considerable efforts for designing cheap and feasible strategies for clean-up of contaminatated sites have been done. The best promising and relatively cheap clean up strategy is Bioremediation. Use of indigenous microbial population for in situ bioremediation is an increasingly popular option for clean-up of sites with readily degradable contaminants. However, specialised or planned inoculants containing microbes such as bioaugmentation are an acceptable replacement for more recalcitrant chemicals (Vogel 1996).

The only issue with microbial bioremediation is that not all of the elements in chemical mixtures are broken down equally. The diversity of substrates, thereby however, can be expanded through genetic engineering to include xenobiotics that are often resistant to breakdown (Erb et al. 1997). Different genetically modified microorganisms have already been efficiently built, with evidence from experiments demonstrating their greater utility for bioremediation processes and degradative capabilities (Furukawa 2003). Application of GEMs in situ is limited because of the risks associated with uncontrolled proliferation and transfer of gene horizontally (Velkov 2001).

Alternatively, adaptation of microbes for utilization of many recalcitrant compounds as the sole carbon source and complete mineralization of the compound can be carried out by use of microbial consortia. Another emerging technology for cleaning up ecological blight with dangerous materials is the use of plants i.e. Phytoremediation. Advantages of phytoremediation include long-term applicability, cost-effectiveness and aesthetic advantages (Subhash Chandra et al. 2013).

In the afflicted locations, where contamination issues still exist and significantly influence other operations, residues continue to exist below the surface 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 findings of contamination testing and microorganism tests, particularly with reference to risk assessment, should allay their fears regarding bioremediation (Harayama et al. 1999).

**Potential Environmental Contaminants**

Different types of pollutants are chemical, biological, or physical materials. Soil and water get contaminated from chemicals used in fossil fuel, from domestic and industrial waste products, mining and agriculture which have considerable implications for human health and safety, welfare and the value of nature. 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)

**Monitoring of Environmental Contaminants**

Monitoring of pollutants can be performed by various ways, depending on the reasons and the objectives of a particular 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 due to the unlimited number of probably polluting 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 particularly trustworthy to predict the absolute toxicological effects.

One of the major parts of monitoring is biological monitoring has been the most important factor a part in combating pollution. It is a scientific technique for assessing environmental exposure to pollutants by living organisms’, which is based on analysis of an individual organism’s. Biological monitoring includes augmentation and accumulation of toxic chemicals and detection of toxicity which are essential to identify the issue's genesis and take appropriate action..

**Biomonitoring Techniques**

Biological reflexes at various levels of biological organisation (biomarkers and bioindicators) are used as physiological tracking methods to detect significant alterations in the environment. Bioindicators are described as "an organism or biological response that reveals the presence of the pollutants by the occurrence of typical symptoms or measurable 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. According to the definition of a biomarker, it is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."

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 understand 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. For understanding a number of biochemical changes occurring under the stress of environ-mental pollution, modeling approach which is feasible to create computational models based on findings from experiments or publicly available data.

**BIOLOGICAL INDICATORS**

Species or groups of species used to identify negative impacts of contamination are referred to as bioindicators (biomonitoring species). Species used as bioindicator for toxicological research are different from that of model species and the modelling creatures are frequently absent from natural habitats. Responses in the organisms due to adverse effects of pollutants or changes in the number of species can be measurable in 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 ecosystem health in various instances. Bioindicator species are tolerant to variety of toxicants and can be used as a measureable property. Also, the species population can be used as an indicator are used to indicate the environment contamination (Nkwoji et al. 2010).

For the assessment of positive and negative biological indicators of a certain ecosystem's modifications 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). Every component of a biological system serves as a biological indication in the environment. A masterful criterion for the biological indicator in a given ecosystem is the correct and prompt response, targeted and able to detect changes caused by depraved management, and climate changes. In a specific community, different viable species reflect different response to same pollutants and to different pollutant at same degree. Highly sensitive technologies are needed to discover toxins at a high cost since their concentrations are too low. As an alternative, the level of sensitivity of the ecological indicator's range provides a picture of pollutant rates that are, regardless of how little, biologically important.

When chemical and physical analyses are unable to show the biotic consequences of pollution, biological markers do so. The scientists all concur that the biota alone can best forecast how an ecosystem would respond when a stressor appears. Additionally, a marker of biological indication is an abnormally high number of reactions from divergent species, since some species may experience a decline while others see an increase. The biologically derived indicator species may be impacted by elements other than disruption or stress that affect the mechanism of change. Utilising biological markers is constrained by the fact that they are scale-dependent. For example, one indicator could not accurately reflect the biodiversity response to contaminants in a different group.

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

To estimate the levels of pollutants in their habitat and to chart the evolution of population density and changes in ecosystem, biotas could often be used indirectly. Biota always conveys a suggestive idea about the status of ecosystem’s health. In their ecosystem, contaminants have a significant impact on species, which might alter their anatomy, physiology, or behaviour. Various plants, animals, and microorganisms are important instruments for identifying contaminants in a specific ecosystem.

**Plants as indicators**

Plant species, e.g., higher plants, lichens, and planktons, are extremely delicate instruments for predicting pressures in ecosystems. Urbanisation and industrialization have increased environmental contamination in both terrestrial and aquatic environments. Higher plants are useful for estimation of the pollution status because of their immobility (Jain et al. 2010). The pollutants affect plant in various ways ranging from morphological fluctuations to biochemical and/or cellular alterations 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, review of an extraction method at a metal factory (Saber et al. 2016a, b).

Planktons grow in conjunction with chlorophyll in aquatic environments and are a vital source of nourishment for both large and tiny aquatic biotas. Because of their ability to integrate, planktons are frequently employed to assess the level of pollution in a particular aquatic ecosystem. Planktons could serve as an indicator of wellness and measure the presence of high phosphorus and nitrogen in an aquatic body (Thakur et al. 2013). Cyanophyta is commonly used as bioindicators with rapid eutrophication of aquatic ecosystems (Thakur et al. 2013).

**Microbial indicators**

Micro-organisms, due to their rapid growth response even if weak pollution rates and ability to show clear evidence of ecological changes, 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 capacity of Microbial Consortium is considerable to modify their levels of operation, biomass for managing ecosystem pollutants and is helpful in assessing the quality of a given ecosystem. Bacteria show a contact with pollutants when they are present in any ecosystem above a specific threshold (Kalkan and Altuğ 2015).

Most important bacterial biological indicator, is to determine total bacterial counts (virtually never obtained) because it is not that all bacteria 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. In comparison to total coliforms, which also comprise naturally occurring bacterial species on plants and in soil, faecal coliforms are more effective as biological markers (Saber et al. 2015b). Additionally useful as biological markers for identifying salt issues in a particular habitat are halophillic bacteria. Various kinds of microorganisms including Salmonella spp., Campylobacter spp., Escherichia coli, Enterococci, and bacteria linked to gastroenteritis, are used to identify and gauge the degree of contamination in different habitats. The biomass of microbial organisms depends on breathing, biomic N2 fixation, enzymes, and the mineralization of C and N, and among these, biomass-specific breathing tends to be more responsive (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 distributed in both terrestrial and aquatic ecosystems and are a common practise for biological pollutants indicators (Hasselbach et al. 2005).

**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, Phacus tortus, and Trachelon anas*, results in marine ecosystem degradation.

**Lichens**

Lichens, which appear as crispy contiguous clusters of thick growths on tree trunks, rocks, and bare ground, are one of the mutual connections between algae and fungi. Lichens effectively respond to ecological changes particularly pollution due to high Nitrogen and sulphur oxide, therefore widely used as biological indicators in forest ecosystems (Gerhardt 2002).

**Enzymes**

Enzymatic processes are utilised as biological indicators because they are sensitive to contaminants and can be used to gauge the level of degradation in a specific ecosystem. Depending on the activity of the enzyme, the level of enzyme production ranges from high to low and from low to high in polluted habitats. Lysozyme increases dehydrogenase activity by inhibiting respiration; as a result, the impact of some contaminants, such as mercury and cyanide, may be measured.

**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). The use of animals as biological markers aids in determining the presence of poisons in animal tissues (Joanna 2006).

**Assessment of the Environment's Health Using Bioindicators: Bat**

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 susceptible to changing land use and ecosystem conditions (Fenton & Simmons 2014). It is also cost effective, stable, responsive to environment stress, can be used in pollination and pest control 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 (Newsome et al. 2004).

**Earthworms**

Earthworms are utilised as an efficient biological indicator because their presence in a specific ecosystem may be used to gauge pollution levels and as an early warning system to track broader changes (Gao and Luo 2005). Earthworms serve as significant indicators for ecotoxicology risk assessment and for potential pollutants which results in damage of the ecosystem.

**Frogs and toads**

For monitoring the attributes and changes in a given ecosystem, frogs are good biological indicators because they are affected due to pollutant accumulation in a given ecosystem. Anurans have skin and larval gill membranes that can absorb hazardous compounds, making them more sensitive to changes in their ecology. Additionally, they have the ability to detoxify pesticides that they ingest, inhale, or consume from contaminated foods, allowing residues to build up in their biosystems. These factors allow them to use for contamination research, eco-toxicological trials, and ecosystem changes as biological indicators. Morphological changes like reduced body length, organ malformations, lower body weight, slow growth rate and limited metamorphosis are observed on exposure.

**Insects**

As a parameter of assessments regarding the levels of change in a particular environment, insects can be utilised because they are rigorously and quickly impacted by contaminants in ecosystems. There are many processes in the ecosystem for which insects are responsible, and every time they disappear, every aspect of biological community suffers. Therefore, a strong understanding of pollutant and insect responses is of functional value (Nichlsa et al. 2007).

Insect used as indicator ought to be simple apprehended and transported easily, have ecological constancy, respond to changes in ecosystem, short life cycle, sensitive for detection early changes in ecosystem, and provide information without any interruption in damage or alteration caused by pollutants (da-Rocha et al. 2010). Insects species like Coleoptera (beetles), Homoptera (bugs), Diptera, Odonata sp. (dragonflies), Hydrophilidae (Coleoptera), families like Gyrinidae, Dytiscidae, Veliidae (Heteroptera) have high adaptive capacity potential as biological indicators (Hardersen 2000; Nummelin 2007).

Effect of Cu, Fe, Ni, Cd, and H2SO4 on different Insects species can be studied by their population, cycle duration, and newly hatched larval mortality rate. Insect species like Apis mellifera, effective biological markers demonstrate a high rate of ecological chemical loss and capture particles that may later be seen in the air or flowers (Ghini et al. 2004). Ants are essential to the restoration of damaged ecosystems, and Ameliorations have demonstrated a high level of resistance to pollutants (radioactive and chemical compounds). Bees are utilised to detect radioactivity after Chernobyl accidents, hazardous pollutants, and poisons in urban habitats, as well as pesticides and herbicides (Urbini et al. 2006). Wasps are utilised to accumulate lead and are susceptible to the detrimental biological buildup at the top of the food chain.

**Zooplankton**

Zooplanktons are biological indicators and help to assess the levels of contamination of aquatic ecosystems. For the development of zooplanktons, aquatic efficiency, eutrophication, and fresh water body growth are important and any weather fluctuations greatly influence zooplanktons. Zooplanktons as indicators are associated with biotic and abiotic parameters e.g. predation, competitiveness, food shortage, pollutants, alkalinity, temperature and stratification (Ramchandra et al. 2006).Few examples of zooplanktons include *Trichotria tetrat, Alona guttata, Moscyclopesedex, Cyclips, Aheyella, Copepods, Rotifer and Ostrocoda* (Zannatul and Muktadir 2009).

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. An effective bioindication approach can be used to evaluate how external variables affect ecosystems (Markert 2008). Environment makes indicator species sensitive to its changes, however it is thought that detecting an ecosystem by evaluating the effectiveness of an incentive in a single population is more effective and less expensive (Spellerberg 2005).

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).

**BIOLOGICAL MARKERS**

When compared to a biological system's normal state, pollution biomarkers are measurements of the alterations brought on by exposure to pollutants. According to Dagnino et al. (2008), these are changes that occur at lower levels of biological organization (such as molecular, cellular, or physiological) yet are commonly acknowledged in comparison to earlier changes that happened at higher levels (such as population impacts). Cellular and molecular biomarkers give populations a sensitive early warning of more comprehensive toxicological consequences that may happen later (Hook et al. 2014). Additionally, biomarkers provide pertinent data regarding the measurement of environmental contaminants as well as the exposure to pollutants and any potential negative effects on the health of creatures exposed to such pollutants. This explains how environmental monitoring has advanced.

Biomarkers can therefore be used to determine the type and extent of exposure, the modifications taking place inside an organism, and the underlying vulnerability of an organism. Due to changes that take place at the cellular and molecular levels that result in a hazardous effect, biomarkers improve our understanding of the processes of chemical absorption and transformation within an organism. As a result, biomarkers are categorised as biomarkers of exposure, biomarkers of effect, and susceptibility based on the specific biological response (Schettino et al. 2012).

Exposure extent and occurrence of various compounds to organism provide an indication about biomarkers of exposure and are organismal cellular alterations that are reversible, which are in accordance with the activation of detoxifying 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 employed to identify exposure to numerous chemically reactive contaminants, such as heavy metals, polycyclic aromatic hydrocarbons, and nitrosoamines. Biomarkers of exposure include things like heat shock proteins, antioxidant enzymes, and metallothionines (Kaegi, 1991; Ryan and Hightower, 1996).

In particular with respect to human biological surveillance, xenobiotic assessment in the biological system is utilised as "biomarker of internal and effective dose." (Ladeira and Viegas, 2016). Internal Dose is a measure of the amount of a parent substance or derivative present at the target site. Applicable dose, on the other hand, refers to indicators that are detected in the tissues that are targeted and reflect the interaction of the absorbed substance with a subcellular target. Alteration in enzyme activity, DNA or protein adduct formation, or change in enzyme activity can all serve as indicators of effective dose in circulating blood cells (Ladeira and Viegas 2016).

Changes in the target tissues are examples of biomarkers of impact related to biochemical (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 come from being exposed, provide an evaluation of the organisms' toxicological impacts, and are inversely associated to the risk of negative health effects (de la Torre et al. 2007). Biomarkers of susceptibility indicate an inherent or acquired ability of an organism to respond to specific pollutant exposure (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 surroundings and act as indications of vulnerability.

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. 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 ((Lionetto et al. 2001; Calisi et al. 2014; Gonick 2011)

## There are various requirements that must be met when choosing the most pertinent biomarker responses to be incorporated in the multimarker technique in line with the goals of each individual biological surveillance programme. The biomarker's sensitivity, its dose- and time-dependent response, its biochemical memory (how long the response persists after exposure), and its inherent variability are a few of them (Hagger et al. 2006). Biomarkers should react to a toxin in a dose-dependent way 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 realistic biomarker used for the detection of pollutants that are transformed by biology like dioxins, furans, polychlorinated biphenyls and polycyclic aromatic hydrocarbons (Sarkar et al. 2006). In this action, when the organisms are exposed to such pollutants, the induction is enhanced by the cytosolic presence of the aryl hydrocarbon receptor of CYP1A. For example, in case of marine bivalves (Binelli et al. 2006) and Zebra mussel (*Dreissena polymorpha*), a significant induction of EROD ethoxyresorufin dealkylation (EROD) activity occurred when they exposed to PCB mixture of Arochlor 1260 and dioxin-like CB-126. The biomarker can distinguish between the amounts of pollution in tiny streams that are contaminated with PCBs and AhR-binding PAHs.

**DNA integrity as a Biomarker of Pollution**

DNA integrity is affected by genotoxic and exogenous agents inducing DNA strand breaks, loss of methylation, double strandedness and formation of DNA adducts (Sarkar et al. 2006) which may be produced during repairing of DNA. Agents like PAH such as Benzo(a)pyrene (BaP), cooperate with DNA to create both stable and unstable DNA adducts, which may be the result of cellular change (Behrens and Segner 2005). 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 marine snails (Planaxis sulcatus) considerably deteriorated at contaminated sites, which was related to the amount of these sites' pollution by petroleum hydrocarbons released from waste items into coastal water (Sarkar et al. 2006).

**Metallothioneins (MTs)**

Metallothioneins are proteins rich in cysteine found in cytosol and interacts by binding sulfur atoms of cycteine residues with toxic metal ions resulting in inactivation (Amiard and Cosson 1997). MTs measure their amounts in bivalves from polluted habitats and oxidative stress in aquatic species to serve as indicators for environmental pollution. Metallothioneins act as metal-chelating agents, hence, through oxygen free radical scavenging actions and metal binding, plays significant roles in metallic metabolism in aquatic species and specifically in the elimination mechanisms (Andrews GK 2000). This has negative impacts on the antioxidant, enzymatic, and non-enzymatic defensive systems of organisms and causes oxidative damage to DNA, lipids, and proteins.

**Pigments as Biomarkers**

Algae and plant biomarkers contain pigments, whose light-harvesting organisms' main purposes are photosynthesis and photo defense. In plants and algae, there are three basic classes of pigments- Chlorophyll, Carotenoids, Phycocyanin and Phycoerythrin. Pigments can serve as useful biomarkers for taxonomic specificity and are frequently utilised as chemical "tags" in cancer research, for "tagging" tumour cells, and in other cancer-related applications (Leavitt and Hodgson 2001), and hold the representation of the entire phototrophic community and overall 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 by the exposure of the pollutants (Köhler et al. 2002). Lysosomal compartment comprises of lysoosomes(Pirmary and secondary), auto and heterophagic vesicles, multifunctional, rich in hydrolytic enzymes. 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 (van Nierop et al., 2006). 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).

**Oxidative stress as biomarkers**

Pollutant exposure causes oxidative stress in cells, which is caused by an increase in reactive species and a disruption in the effectiveness of antioxidants (Regoli and Giulian 2014). Commonly used marker of oxidative stress is GSH (Dalle-Donne et al. 2006) an important intracellular scavenger of free radicals by neutralising peroxides in conjunction with glutathione peroxidase and glutathione reductase, maintains the redox equilibrium of cells. The ratio of reduced to oxidised glutathione (GSH/GSSG) is calculated to determine the organism's oxidative stress status.

Lipid peroxidation, for instance, is a typical indicator of oxidative stress that results from the oxidative destruction of membrane phospholipids. In addition, antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase, whose activity and expression are altered by exposure to pollutants (Leomanni et al. 2015), exhibit biomarkers of oxidative stress that are suitable for evaluating the effects of pollutants in ecosystems at an early stage and with low concentrations.

**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 (Draper et al. 1993). According to numerous research, xenobiotic-induced free radical peroxidation raises the MDA levels in urine or tissue samples (Di Pierro et al. 1992).

**DNA oxidative damage biomarkers in vivo**

In addition to being utilised as biomarkers for particular modifications and hydroxylations of purine and pyrimidine bases, damage to the deoxyribose-phosphate backbone, and protein-DNA cross-links, exposure to pollutants increases the amount of oxidative damage to DNA. Measuring the hydroxylation by HOD of the nucleobase guanosine and its free base 8-hydroxyguanine has been used as a biomarker for carcinogenesis (Lodovici et al. 2000). 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 (Daneshvar et al., 1997).

**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 is also recognised as a biological marker of humans and has become a diagnostic tool in the biomedical field.

## Inhibition of AChE by a number of chemical species, besides organophosphate and carbamate pesticides, has recently been documented in humans (Vioque-Fernandez et al. 2007). These chemical species include heavy metals, other pesticides, polycyclic aromatic hydrocarbons, detergents, and components of complex mixtures of contaminants. As a result of their interactions with the enzyme, many kinds of nanoparticles, 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. 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

Biomarkers have evolved into precise end points for tracking cellular reactions to diverse diseases, pharmacological exposures, and chemical agent exposures. Biomarkers are detected in human tissues and/or fluids from persons who have recently or historically been exposed to chemical risk factors at work or in the general environment as part of human biomonitoring (Manno et al. 2010). Human biomonitoring's primary goals are to assess each individual's health and to guard against any negative health impacts that may result from exposure to contaminants (Manno et al. 2010). For instance, the biomarker of brown adipose metabolism serum exosomal miR-92a was focused on, and shift workers showed a difference (Bracci et al. 2020). Compared to daytime workers, the brown adipose tissue activity may be higher given 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 (Pandey et al. 2008), t-muconic acid levels in urinary tract (Raghavan and Basavaiah 2005), elevated levels of organochlorine pesticides in blood of women and polycyclic aromatic hydrocarbons (PAHs) in rural children (Pathak et al. 2010), Lead (Pb) (Grover et al. 2010) content in urine and blood are the main biomarkers that humans employ to evaluate both short-term and long-term exposures.

**DNA Damage as a Biomarker of Exposure**

As a biomarker of exposure, the comet assay for DNA damage assessment has been widely employed in human biomonitoring (Valverde and E. Rojas 2009). With a few tweaks, this technique may be applied to both proliferating and non-proliferating cells and allows for both the detection and repair of different types of DNA damage. Multiple pollutants, including those containing chromium, pesticides, wood dust, coal, and benzene, have shown a considerable rise in DNA damage, increasing the likelihood of negative repercussions in the population.

Cooking with genotoxic biomass fuels (BMF) causes considerable DNA damage in women's lymphocytes and an upregulation of DNA repair mechanisms, which are linked to lung cancer in women. The Comet assay is used as a biomarker to show exposure and repairable DNA damage(Mondal et al., 2010).

**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 is predictive of an increased risk to cancer (Bonassi et al. 2008). Due to exposure to heavy metal vapours, there is a high frequency of CA and MN in peripheral blood cells, which indicates a genotoxic risk (Vuyyuri et al. 2006) has been observed. The frequency of micronuclei in lymphocytes and buccal mucosal cells of people who have been exposed to pollutants at work has been frequently utilised as a minimally intrusive approach to assess genetic damage caused by pollutants in ambient air (Sellappa et al. 2010). These studies show that populations at risk can be screened for and identified using biomarkers of effect.

**Biomarkers of susceptibility**

Polymerase chain reactions (PCRs) can discover gene polymorphisms related to xenobiotic-metabolizing enzymes in blood samples and are employed as markers of susceptibility (Singh et al. 2010). Lung cancer risk was enhanced by polymorphisms of the N-acetylation (NAT2) gene alone or in combination with p53, as well as polymorphisms of the cytochrome P450 (CYPs) gene in combination with glutathione S-transferase (GST) M1 or T1 (Singh et al. 2009). Also, studies with polymorphisms in genes for bioactivation, detoxification etc helps 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. This provide 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 (Pandey et al. 2009).

Additionally, in silico molecular docking studies revealed interactions between benzene and its byproducts at the human topoisomerase II alpha enzyme's ATP binding domain (critical for DNA integrity) (Pandey et al. 2009). These research have demonstrated how crucial it is to combine novel methods with traditional biomarkers in order to fully comprehend the action of toxicants and unravel the exposure-effect relationship. When determining the level of occupational exposure to organophosphate compounds in exposed situations, blood levels of acetylcholinesterase are quantified. Carcinogens are currently the focus of human biomonitoring; as a result, genotoxicity biomarkers are being developed to assess pollutant exposures, predict risk, and track the efficacy 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 can be detected in cells, tissues, and biological fluids, and it is linked to the development of many diseases.

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 (Hagger et al.2008). Health risk and environmental quality assessment are strongly related with each other and also their integration produce more realistic results and predictive capability for obtaining data in both studies (Galloway 2006).

In an integrated approach, biomarkers like molecular and cellular ones serve as helpful instruments for bridging investigations relating to humans and the environment. Therefore, a variety of biomarkers may be useful for an integrated strategy addressing intervention options for prevention or mitigation of harmful health impacts of chemical contamination in both humans and the environment. Recent developments in molecular biology and OMIC sciences (genomics, transcriptomics, proteomics, lipidomics, epigenomics, and metabolomics, among others) are receiving more attention in the field of environmental and human biomonitoring, providing the chance to create new and more sensitive biomarkers that can be used in an integrated approach (Suárez-Ulloa et al. 2013, 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. However, these processes can be very slow, and therefore, certain chemicals may persist for years. Biodegradation depends on various factors related to chemical and physical properties of environment and chemical in which they are present.

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.

Numerous methods for assessing bioremediation effectiveness and reducing long-term environmental toxicity have been put forth. Molecular approaches that concentrate on catabolic genes that code for particular pollutant-degrading enzymes, nucleic acid-based techniques, and assessments of the metabolites of dissolved or residual pollutants are also included. The use of biomarkers as indicators and instruments for gauging the effectiveness of bioremediation depends on the system (Jansson et al. 2000).

**Luciferase as biomarkers**

For monitoring bioremediation inocula, luciferase markers such as luciferase gene (luc), or bacterial luciferase genes (luxAB) can be easily detected as markers. For example, *Pseudomonas aeruginosa,* tagged with luxAB can be tracked by counting luminescent colonies in microcosms contaminated with oil (Flemming et al. 1994). Similarly, *Pseudomonas cepacia*, a 2,4-D degrading strain, tagged with lacZY and luxAB genes, was monitored by colony counting in 2,4-D amended soil (Masson et al. 1993). Luc gene can also be used as biomarker for monitoring gasoline degrading bacteria, *Pseudomonas fluorescens strain* 935061 fused with the tac promoter (MoÈller and Jansson 1998) and *Arthrobacter* strain tagged with luc gene, using the pAM103 vector (Westerberg et al. 1999). Using luminescence markers light output can be directly measured in luminometer (Rattray et al., 1990) which is indicative of a metabolically active population of cells. As cells become starved, the light production from luciferase enzymes declines and therefore, it is referred to as potential luminescence (Meikle et al., 1994)

**Biomarker using GFP**

Another marker for monitoring of bioremediation is the gfp gene, encoding green Fluorescent protein having an advantage of fact that the protein fluoresces upon illumination with light and no requirement of other energy source or substrate, other than oxygen during initial formation of the chromophore (Tombolini and Jansson 1998). GFP gene has been used as a biomarker for monitoring 4-chlorophenol degradation in bacteria *Arthrobacter* strain tagged with 2 copies of gfp gene. Additional examples of the use of GFP as a biomarker for monitoring bioremediation includes a p-nitrophenol degrading strain of Moraxella and a phenanthrene mineralizing strain of *Pseudomonas* were tracked in soil microcosms by counting of GFP fluorescent colonies.

**Fungal biomass as biomarker**

To monitor and regulate the efficiency of the bioremediation process, fungus biomass has been used. According to Barajas-Acheve et al. (2002), biochemical techniques for analysing fungus-specific cell components such ergosterol, chitin, or phospholipid fatty acids are regarded to be a useful indication for fungal biomass in polluted soils. SIP, a technique for tracking in-situ chemical biodegradation, bases its analysis 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**

DNA, RNA, and phospholipid fatty acids (PLFAs) are the biomarkers employed in environmental microbiology; each has advantages and disadvantages (Dumont and Murrell 2005). SIP stands for in situ qualitative and quantitative biodegradation of pollutants. The most notable biomarker for SIP is PLFA, which is used in conjunction with toluene breakdown by Actinomycetales in the sediment of an aquifer contaminated with petroleum hydrocarbons (Pelz et al. 2001).

**Genetic biomarkers**

The most powerful tool used as biomarker are Genetic biomarkers that can be used for potential contaminant biodegradation. Detection of specific nucleic acid sequences, conserved regions of the 16S rRNA gene, nucleic acid hybridization using specific probes, PCR based system has been used as biomarkers to identify the presence or absence of microbial organisms when biodegradation is dependent on a specific microbial strain. 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 monitor the degradation of a target molecule at a site in order to ascertain the genetic diversity of microorganisms as a whole (Steffan and Atlas 1991). The reductive dechlorination of chlorinated solvents by Dehalococcoides spp. has been successfully studied using this methodology (Lee et al. 2008).

**Enyzmes as biomarkers for bioremediation**

Using BMMs, functional genes can be targeted for processes that involve soluble (sMMO) and particulate(pMMO)methane monooxygenase enzymes (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 used 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 an important 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. According to Das et al. (2007), phytoremediation technologies use plants to decrease, eliminate, degrade, or immobilise environmental contaminants. Plants are grown hydroponically, transplanted into metal-contaminated waters, where they absorb and concentrate the metals in their roots and shoots, and when they become saturated with the metals, the plants are picked for disposal.

## Among organisms, algae and aquatic plants are potential ecological engineer for accumulating and bioconcentrating heavy metals because of their ability of sequestration and can live under many extreme environments. (Kalin et al. 2005). The duckweed (Lemna minor) was corroborated to be a suitable candidate for the phytoremediation of low-level copper and cadmium contaminated water body (Hou et al. 2007). According to Srivastava et al. (2006), the aquatic macrophyte H. verticillata (L.f.) Royle's tolerance to mild copper exposures and high accumulation potential make them suitable for the rehabilitation of moderately copper-polluted water bodies.

## 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.

## Utilising certain biosensors or biomarkers, genomic technologies can 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 the green fluorescent protein (GFP), are examples of biomarkers.

## The bioremediation of petrol or chlorophenols has used the luc gene tagged with different bacteria, and the activity has been assessed on the basis of luciferase activity. With the help of molecular tools like denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism (T-RFLP), it is possible to analyse the community of microbes involved in bioremediation and determine which of their key metabolic activities can be used to remove pollutants. Double stranded DNA fragments that are equal in length but have different sequences are separated using DGGE analysis.

## CONCLUSION

## Pollution biomarkers have recently demonstrated their value as early indicators of negative impacts in human and environmental biomonitoring. 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, a profitable research area for creating novel methods for implementing biomarkers in studies of the environment and human health should be concentrated.

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