**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 generating harmful conditions for living organisms. Interests have been developed as warning tools for the detection of adverse biological responses of pollutants in both humans and wildlife. Molecular and cellular biomarkers of pollution meet this requirement. A biomarker alters biological response occurring at molecular, cellular or physiological levels due to the toxic effects of environmental chemicals. Risk assessment of chemical pollutants to organisms and ecosystems is made complex by a number of factors. 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:**

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 natural biota.

(2) Predicting 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 pollutants on ecosystems and their constituent parts. The inability to investigate interactions between pollutants, the influence of environmental conditions on pollutant toxicity, and pollution-induced changes in ecological 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 natural 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 insufficient to accurately assess the hazards of pollution (Burgeot et al., 2017). In light of this, an integrated 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 accumulation of recalcitrant compounds in soil and water at high concentrations and the problem with recalcitrance to microbial degradation is considerably enhanced. Therefore, considerable efforts for designing cheap and feasible strategies for clean-up of contaminated 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, for more recalcitrant compounds, specially adapted or designed microbial inoculants as bioaugmentation is a useful alternative (Alexis *et al*., 2016).

All components of chemical mixtures are not degraded with equal efficiency is the only difficulty with microbial bioremediation. But by genetic engineering, the range for substrates can be widened to include xenobiotics that are normally recalcitrant to degradation. Various gentically engineered microorganisms have been successfully constructed and experimentally proven to have a higher degradative capability and utility for bioremediation. Application of GEMs in situ is limited because of the risks associated with uncontrolled 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 and complete 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 include long-term applicability, cost-effectiveness and aesthetic advantages (Subhash Chandra et al., 2013).

Even after some years the residues still remain below the surface in the affected areas where contamination problems persist and negatively impact other activities. This fact indicates clearly the necessity of development of bioremediation technologies to manage pollution. Therefore, undertaking bioremediation without the agreement of local communities can-not be done. Their concerns about bioremediation, should be mitigated by scientists who can explain to the local people the results of contamination tests and microorganism tests, especially in regard to risk assessment.

**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 enormous number of potentially 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 very reliable to predict the ultimate toxicological effects.

One of the major parts of monitoring is biological monitoring which has played a most dominant role in the pollution control. 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 required to detect the nature of the problem and for corrective measure.

**Biomonitoring Techniques**

Methods of biological monitoring include biological responses at different biological organization levels (biomarkers and bioindicators) to indicate significant environmental changes. Bioindicator is a term taken from environmental toxicology and is defined as “an organism or biological response that reveals the presence of the pollutants by the occurrence of typical symptoms or measurable responses. These organisms (or communities of organisms) deliver information on alterations in the environment or the quantity of environmental pollutants by changing in one of the following ways: physiologically, chemically or behaviourally”. A biomarker is defined as “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 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. For understanding various biological alterations occurring under the stress of environ-mental pollution, modeling approach which is based on the experimental results or published data, from which it is possible to develop mechanistic models can be used.

**BIOINDICATORS**

Bioindicators (biomonitoring species) are defined as species or groups of species that are used to indicate adverse effects of contamination. Species used as bioindicator for toxicological research are different from that of model species and the model species are often not found in the natural environments. 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 measurable 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 changes in a given ecosystem biological indicators occurring naturally are regularly used. The significance of caring about the natural factors 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. 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. Pollutants which are existing in an excessively low concentration, highly sensitive technologies are required to identify them at a high cost. Alternatively, sensitivity of biological indicator’s range offers an image of pollutant rates that are biologically significant, no matter how small they are.

Biological markers exhibit the biotic effects of pollutants indirectly when chemical and physical assessments unable to do 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 species of biological origin can be affected by factors other than disruption or stress 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 track population density over time and changes in ecosystem, biotas could often be used indirectly. Biota always displays an indicative idea about the status of ecosystem’s health. Species are very much susceptible to pollutants present in their ecosystem, which might alter their anatomy, physiology, or behaviour. Various plants, animals, and microorganisms are important tools for indicating pollutants in a given ecosystem.

**Plants as indicators**

Plant species, e.g., higher plants, lichens, and planktons, are very delicate tools for the prediction of stresses in the ecosystems. Due to industrialization and urbanization, pollution of terrestrial and aquatic ecosystems had been intensified. 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 changes in total soluble salts content. 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 combined with chlorophyll; play a crucial source of food to many small and large aquatic biotas. Planktons are capable of integrating and usually used in determining the pollution status in a given 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 rapid eutrophication 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 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. Fecal coliforms are more useful as biological indicators than as total coliforms, which include bacterial specie naturally found on plant and in soil (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 detect and estimate the level of pollution 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 distributed in both terrestrial and aquatic ecosystems and are frequently used as biological indicators for contaminants.

**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**

Mutual associations of Algae and Fungi comprise Lichens occurring as crusty continuous patches of bushy growths on trunks of trees and rocks and bare ground. 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 sensitive to pollutants. The enzyme production 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). Animal as biological indicators also helps to detect the amount of toxins in animal tissues.

**Bat as Bioindicator of Environment Health Assessment**

Increasing human population is causing deteriorative effects against the balance of living entities and humans and inturn is devastating the earth (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 habitat deterioration and land use (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.

**Earthworms**

Presence of earthworms in a given ecosystems reflects the degree of pollution 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 and for potential pollutants which results in damage of the ecosystem.

**Frogs and toads**

For monitoring the quality and changes in a given ecosystem, frogs are good biological indicators because they are affected due to pollutant accumulation in a given ecosystem. Anurans are more responsive to changes to their ecosystem and ingest toxic chemicals by their skin and larval gill membranes. Also, they are capable of pesticides detoxification which they ingest, inhale, or consume from contaminated foods which permits accumulation of residues in their bio-systems. 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**

Insects in ecosystems are strictly and quickly affected by pollutants and can be used as a parameter of analyses about the levels of change in a given ecosystem. There are many processes in the ecosystem for which insects are responsible, and their loss always have negative effects on entire biological communities. Therefore, a strong understanding 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 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.

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, show a high mortality rate of ecological chemical loss and catch particles that could later be observed in air or flowers. Ants play a key role in recovery of degraded ecosystems and Ameliorations showed strong tolerance to contaminants (radioactive and chemical chemicals). Bees are used to track toxic contaminants, and toxins in urban ecosystems, radioactivity following Chernobyl accidents, pesticides, and herbicides. Wasps are vulnerable to the harmful biological accumulation at the top of the food chain and used for Pb accumulation.

**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. Few examples of zooplanktons include *Trichotria tetrat, 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). Complete monitoring of the whole area is possible by bioindication, which indicates various living systems with simple data. The effect of external factors on ecosystems can be assessed by reliable procedure of bioindication. Environment renders indicator species sensitive to its alterations, whereas detection of ecosystem by assessing an efficient incentive of a single population is believed to be more useful and cheaper.

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**

Pollution biomarkers can be defined as measures of changes due to pollutant exposure in a biological system with respect to its normal status. 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 provide a sensitive warning of more integrated toxicological effects that can occur within populations later on (Hook et al., 2014). Also, biomarkers give relevant information about the measurement of contaminants in environment and the exposure and potential impacts of pollutants on the health of the exposed organisms. This accounts for the development in monitoring of the environment and human health.

Therefore, to assess the nature and extent of exposure, to identify alterations occurring within an organism, and underlying susceptibility of an organism, biomarkers can be used. Biomarkers increase the understanding of the processes of chemical absorption and transformation within an organism due to alterations that occur at the cellular and molecular levels leading to a toxic effect. Therefore, on the basis of specific biological response biomarkers are classified into biomarkers of exposure, biomarkers of effect, and susceptibility (Schettino et al., 2012).

Exposure extent and occurrence of various compounds to organism provide an indication about biomarkers of exposure and are reversible cellular changes in the organism, which are based on the activation of detoxification mechanisms. Biomarker of exposure is used to obtain information on the route, pathway, and the source of exposure. Damages, changes and adducts on proteins, DNA and Lipids molecules can be measured using exposure biomarkers. They are used to detect exposure to various chemically reactive pollutants such as nitrosoamines, aromatic amines, polycyclic aromatic hydrocarbons and heavy metals. Examples of biomarkers of exposure are metallothionines, heat shock proteins, and antioxidant enzymes.

Xenobiotic measurement in the biological system is used as “biomarker of internal and effective dose”, particularly in human biomonitoring. Internal Dose represents the concentration of a parent compound or metabolite at the target site. On the other hand effective dose are markers measured in the target tissues that reflect the interaction of the absorbed compound with a subcellular target. Effective dose can be represented by alteration in enzyme activities or formation of DNA adducts or protein adducts in circulating blood cells (Ladeira and Viegas, 2016).

Biomarkers of effect include changes in target tissues 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 occur as result of exposure and also give an assessment of a toxicological effect on the organisms and are directly related to the risk of adverse health effects. 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 fact, inter-individual biological differences may cause some individuals to be more susceptible to environmentally induced diseases and serve as markers of susceptibility.

The biomarkers specificity to pollutants ranges from highly specific biomarkers to nonspecific. Specific biomarkers includes metallothionein induction by metal (Cu, Hg, Zn, Cd) (Calisi et al., 2014) or aminolevulinic acid dehydratase (ALAD) inhibition by lead (Gonick, 2011) and nonspecific includes DNA damage or immune system impairment. When different specific biomarkers are used together, a complementation among biomarkers can be realized that results into an overall higher degree of specificity.

The selection of the most relevant biomarker responses to be included in the multimarker approach in agreement with the objectives of each specific biomoni-toring program has to meet some criteria. Some of them include the sensitivity of the biomarker, its dose- and time dependent response, its biochemical memory (how long after exposure the response lasts), its natural variability. In order to ensure a proper toxicity assessment, biomarkers should responds to a pollutant in a dose-dependent manner over an environmentally realistic concentration range of pollutants. Moreover, the link of the biological response used as biomarker to important biological processes and to pathological consequences is considered of relevance in both environmental assessment and heath assessment.

## POLLUTION BIOMARKERS IN ENVIRONMENTAL BIOMONITORING

**Cytochrome P4501A Induction**

Cytochrome P4501A (CYP1A), a sensible biomarker used for the detection of biotransformation of contaminants like dioxins, furans, polychlorinated biphenyls and polycyclic aromatic hydrocarbons. In this action, when the organisms are exposed to such pollutants, the presence of aryl hydrocarbon receptor from cytosol enhances the induction 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 discriminate the pollution status, of small streams receiving different levels of contamination by AhR-binding PAHs and PCBs.

**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 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. Transformations generate single strand breaks proceeded by ionizing radiation, oxidation-reduction reaction or photoreaction. For example, in marine snail (*Planaxis sulcatus*) DNA integrity decreased significantly at the contaminated sites, which was attributed to the extent of contamination of these sites by petroleum hydrocarbons of waste materials into the coastal water.

**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. MTs act as biomarkers for environmental pollution by measuring their concentrations in bivalves from contaminated habitats and oxidative stress in aquatic organisms. Metallothioneins act as metal-chelating agents, thus playing important roles in metal metabolism in aquatic organisms and particularly in the detoxification mechanisms, through oxygen free radical scavenging actions and metal binding (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, whose primary function is light harvesting agents for photosynthesis and photo protection. In plants and algae, there are three basic classes of pigments- Chlorophyll, Carotenoids, Phycocyanin and Phycoerythrin. Pigments can be used as chemical "tags" with extensive use in cancer research, for "tagging" tumor cells and as relevant biomarkers representing taxonomic specificity, 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. 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. 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**

Oxidative stress is generated by the exposure to pollutants in the cells, arising from the enhancement of reactive species and perturbation of antioxidant efficiency (Regoli and Giulian, 2014). Commonly used marker of oxidative stress is GSH an important intracellular scavenger of free radicals maintains the redox balance of cells by neutralizing peroxides in combination with glutathione peroxidase and glutathione reductase. To assess the oxidative stress status of the organism, the ratio between the reduced and oxidized glutathione (GSH/GSSG) is calculated.

Products arising from the degradation of membrane phospholipids by oxidation, for example, Lipid peroxidation, represents commonly used biomarkers of oxidative stress. In addition, antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase (Leomanni et al., 2015) which get altered in their activity and expression by the exposure to the pollutants, demonstrates biomarkers of oxidative stress which are suitable for assessing effects of pollutants in ecosystems at early stages and with low concentrations.

**Biomarkers of lipid peroxidation**

In tissue injury this is the most extensively investigated process which is induced by free radicals but, its direct analysis is complicated, therefore, the levels of secondary oxidation products (aldehydes and ketones) are measured. Common assay for lipid peroxidation is malondialdehyde (MDA) formation as a peroxidation product, with the thiobarbituric acid reactive substances test. Various studies indicates that free radical peroxidation by xenobiotics increases MDA in urine or tissue samples.

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

Exposure to pollutants causes increase in oxidative damage to DNA and are used as biomarkers for specific modifications and hydroxylations of purine and pyrimdine bases and for damage to the deoxyribose–phosphate backbone and protein–DNA cross-links. Measuring the hydroxylation by HOD of the nucleobase guanosine and its free base 8-hydroxyguanine has been used as a biomarker for carcinogenesis. 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. It is a key enzyme in the nervous system, which catalyses the hydrolysis of the neurotransmitter acetylcholine and is the target site of inhibition by pesticides (Calisi et al., 2013). AChE is a molecular target of organophosphorus and carbamate compounds, so its measurement in the blood is also recognized to be a human biological marker and emerged as a diagnostic tool in the biomedical area.

Recently, the inhibition of AChE from several chemical species other than organophosphate and carbamate pesticides including heavy metals, other pesticides, polycyclic aromatic hydrocarbons, detergents, and components of complex mixtures of contaminants has been reported in humans (Vioque-Fernandez et al., 2007). Recently, different classes of nanoparticles, including metals, oxides, and carbon nanotubes (SiO2, TiO2, Al2O3, Al, Cu, carbon-coated copper, multiwalled carbon nanotubes, and single-walled carbon nanotubes), showed high affinity for AChE by interaction with enzyme (Indennidate et al., 2010). Cu, Cu–C, multiwalled carbon nanotubes, and singlewalled carbon nanotubes MWCNT, SWCNT showed a dose– response inhibition of AChE activity with IC50 values of 4, 17, 156, and 96 mgL−1, respectively.

## BIOMARKERS IN HUMAN BIOMONITORING

Biomarkers have become specific end points for monitoring cellular responses to various diseases and exposures to drugs and chemical agents. In human biomonitoring, biomarkers are measured in human tissues and/or fluids from subjects who are exposed currently or in past to chemical risk factors in the workplace and/or in the general environment (Manno et al., 2010). The main aspect of human biomonitoring is evaluating the health status and to prevent the health effects of exposure to pollutants. For example, Serum exosomal miR-92a as biomarker of brown adipose metabolism was focused and a difference was observed in shift workers (Bracci et al., 2020). The lower levels of miR-92a suggest higher brown adipose tissue activity compared with daytime workers.

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

Chemicals/Metabolites assessment in humans can be used as a biomarker to monitor the exposure to those chemicals/metabolites. Benzene, toluene, and xylene levels in blood, t-muconic acid levels in urinary tract, 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 primary biomarker used to monitor short term and prolonged exposures in humans.

**DNA Damage as a Biomarker of Exposure**

Comet assay for assessment of DNA damage has been widely used in human biomonitoring as a biomarker of exposure (Valverde and E. 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 benzene have demonstrated significant increase in DNA damage leading 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 is predictive of an increased risk to 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, 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.

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 assessing the extent of occupational exposure to organophosphate compounds in exposed environments. Nowadays emphasis in human biomonitoring is posed on carcinogens, therefore, the development of genotoxicity biomarkers are grown to measure pollutant exposures to predict the risk and monitor the effectiveness of exposure to genotoxic chemicals.

Another mainstream marker is inflammation-related biomarkers, which are considered for assessing the inflammatory response 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 caused by many different environmental exposures. Oxidative stress is related with pathogenesis of multiple diseases, damage to DNA and lipids which can be measured in cells, tissues or biological fluids.

Nowadays interest in integrated approach in biomonitoring has stimulated which is useful for an integrated risk assessment view. The need is to improve risk assessment and management and promote policy implementation declared by various agencies and institutions. 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.

In integrated approach, biomarkers like molecular and cellular represent useful tools for bridging the gap between human and environmental related studies. Therefore, a number of biomarkers can be valuable for an integrated approach addressed to intervention strategies for prevention or reduction of deleterious health effects of chemical contamination in the environment as well as in humans. Recent advances in molecular biology and OMIC sciences (genomics, transcriptomics, proteomics, lipidomics, epigenomics and metabolomics, *etc*.) are gaining increased consideration in human and environmental biomonitoring, giving the opportunity for developing novel and more sensitive biomarkers to be utilized 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. 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 different methods to assess microbial attenuation such that qualitative and quantitative pollutant biochemical profiles, analysis of the subsurface geology, and hydrology of the site, composition and activity of the microflora, and microcosm studies. Proper evaluation of microbial attenuation requires the demonstration of the transformation processes which are occuring at a rate that is protective of human health and environment. Designing the bioremediation process, its implementation and its effectiveness has to be kept in mind through continuous monitoring involving chemical, biological, microbial, and environmental indicators.

Different approaches for evaluating the efficiency of bioremediation and in lowering long-term environmental toxicity have been proposed. It includes measurements of metabolites of dissolved or residual contaminants, Nucleic-acid-based techniques and molecular techniques which focus on catabolic genes that code for specific pollutant degrading enzymes. 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 easily detected as markers. For example, *Pseudomonas aeruginosa,* tagged with luxAB can be tracked by counting luminescent colonies in microcosms contaminated with oil. 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. 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 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

**GFP as a biomarker**

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. 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**

Fungal biomass has been used to monitor and control the effectiveness of the bioremediation process. The biochemical methods for assay of fungus specific cell components like ergosterol, chitin or phospholipid fatty acids are considered a useful indicator for fungal biomass in polluted soils (Barajas-Acheve et al., 2002). This type of strategy of monitoring in situ biodegradation of a compound is known as SIP which is based on the changes in stable isotope composition of the molecule of interest. SIP involves tracking stable isotope atoms from particular substrates into components of microbial cells, referred to as biomarkers.

**SIP as Biomarker**

In environmental microbiology the biomarkers used are DNA, RNA and phospholipid fatty acids (PLFAs); each of them has its own strengths and weaknesses (Dumont and Murrell, 2005). SIP denotes in situ biodegradation of pollutants 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 petroleum hydrocarbon-contaminated aquifer.

**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. In samples, the detection of phylogenetic and catabolic genes is based on a set of different genomic approaches. To determine the overall genetic diversity among microorganism, probes, dominant and active gene pool and density and frequency of specific gene lines are required to monitor the degradation a target compound at a site. These approach has been effective in studying various bioremediation process like reductive dechlorination of chlorinated solvents by *Dehalococcoides* spp (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

## Biomonitoring using some special high metal accumulative species especially for aquatic plants may offer the approach for the establishment of the bioremediation plan to restore water quality. Phytoremediation technologies use plants to reduce, remove, degrade, or immobilize environmental toxins (Das et al., 2007). Process of phytoremediation involves rising of plants hydroponically and then transplanting them into waters containing metal where they absorb and concentrate the metals in their roots and shoots and when they become saturated plants are harvested 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. Tolerant response of aquatic macrophyte, H. verticillata (L.f.) Royle to moderate copper exposures and high accumulation potential warrants their suitability for remediation of moderately copper polluted water bodies (Srivastava et al., 2006).

## Compared to other skills for the cleanup of aquatic metal pollution, bioremedation techniques based on biomonitoring offered an appealing approach due to the advantages as follows: easy to use, effective-fast cleanup vs. natural attenuation, environmentally safe and natural treatment, easy to apply and no protective clothing required, low cost efficient and long-term solutions for balanced ecosystem.

## Genomics tools can carry out an assessment of the available biological capability of any ecosystem using specific biosensors or biomarkers. Biosensors can monitor a biological output that can be converted into a measurable signal, for example, enzyme based biosensors can generate the signal either by product formation, the disappearance of substrate, or co-enzyme conversion. Biomarkers are specified genotypes that can be used to track the survival and/or efficacy of specific bacteria in bioremediation. Examples of biomarkers include the luc gene, encoding firefly luciferase and the gfp gene, encoding the green fluorescent protein (GFP).

## The luc gene tagged with different bacteria has been used for bioremediation of gasoline or chlorophenols and the activity monitored on the basis of luciferase activity. In bioremediation, different microbes possess different key metabolic activities, which together can be set for removal of pollutants and their community analysis can be carried out using molecular tools such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism (T-RFLP). DGGE analyses are employed for the separation of double stranded DNA fragments that are identical in length, but differ in sequence. The technique exploits the difference

## CONCLUSION

In human and environmental biomonitoring, nowadays pollution biomarkers have proved their usefulness as early warning of adverse effects. Also, biomarkers represent useful tools for integrating human and environmental related studies and bridging human and environmental risk assessment. They can also contribute to bioremediation studies of pollutants and improve our knowledge on the link between environmental contamination and human health.

## CURRENT & FUTURE DEVELOPMENTS

In the coming years the study of biomarkers in human and environmental biomonitoring and as bioremediation should be explored more extensively in the area of integrated biomonitoring and integrated risk assessment. Also, a fruitful research arena for developing novel approaches in biomarker implementation in environmental and human health issued studies should be focused.

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