BIOMARKERS: A TOOL FOR ASSESSING ENVIRONMENTAL POLLUTION AND BIOREMEDIATION

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. This requirement is met by molecular and cell based biomarkers of pollution. Biomarkers are indicators that reflect alterations in biological responses resulting from the harmful impact of environmental chemicals, operating at molecular, cellular, or physiological levels. The intricate nature of assessing the risks posed by chemical pollutants to organisms and ecosystems is influenced by various factors. Additionally, biomarkers can be employed for the bioaugmentation of polluted sites, with the selection of the monitoring system contingent upon the necessary levels of sensitivity and specificity for detection. 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

Author

Dr. Sonia Sethi
Associate Professor
Dr. B. Lal Institute of Biotechnology
Jaipur, Rajasthan
India.
soniakaura198@gmail.com

Ms. Payal Gupta
Dr. B. Lal Institute of Biotechnology
Jaipur, Rajasthan
India.

I. 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 research community has demonstrated fascination in the identification of chemical and biological agents that threaten human well-being and ecosystem sustainability (Magalhães & Ferrão-Filho, 2008).

Environment toxicologists encounters the following when striving to develop a successful management plan:

1. The diversities and toxicity of pollutants and their ranking to indigenous flora and fauna.
2. Forecasting the dispersion, destiny, and ultimate levels of specific substances across diverse 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). Current methodologies suffer from limitations, including an inability to study interactions between pollutants, the impact of environmental conditions on pollutant toxicity, and changes in environmental relationships over time due to pollution. 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, habitat loss and degradation, 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 induced by nature based adulterants (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 affordable and viable methods for the

remediation of polluted sites have been done. The best promising and relatively cheap clean up strategy is Bioremediation. Use of native microbial population for in situ bioremediation is a growingly favored choice for remediating sites containing easily 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 biological cleanup 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 exclusive 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 capable of elucidating contamination test results and microorganism assessments, particularly in the context of risk assessment, can help alleviate the concerns of local residents regarding bioremediation (Harayama et al. 1999).

1. **Potential Environmental Contaminants:** Various categories of pollutants encompass chemical, biological, and physical substances. The contamination of soil and water results from the introduction of chemicals derived from fossil fuels, domestic and industrial waste, mining, and agricultural activities. This contamination poses significant implications for human health, safety, well-being, and environmental integrity. Prominent pollutants comprise petroleum-derived substances, such as polychlorinated biphenyls, as well as nitrates, insecticides, sediments, and excessive organic materials. The introduction of pollutants into aquatic ecosystems occurs through mechanisms like leakage, improper handling, operational lapses, and the application of these substances to agricultural fields. Among these contaminants, plastics present a particularly detrimental hazard to marine animals when improperly disposed of and ingested (Tesfalem Weldeslassie et al. 2018).

2. **Monitoring of Environmental Contaminants:** The monitoring of pollutants can be executed through diverse methods, contingent upon the motivations and goals of a specific monitoring initiative. 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.

3. **Biomonitoring Techniques:** Physiological tracking methods, employing biological responses at different levels of biological organization, such as biomarkers and bioindicators, are employed to identify notable environmental changes. Bioindicators are defined as "organisms or biological responses that manifest the presence of pollutants through the display of characteristic symptoms or measurable reactions," a terminology originating from the field of environmental toxicology. Through biological, chemical, or action oriented modifications, these creatures (or habitat consortia) provide information about changes in the environment or the amount of nature based adulterants. As per the biomarker definition, it is "an objectively measurable characteristic assessed to indicate normal biological processes, pathological processes, or the pharmacological reactions to therapeutic interventions."

Biomonitoring techniques are categorized into biochemical changes, bioaccumulation, methods at population and community levels, morphological and behavioral observations, as well as modeling. Biochemical pathway modifications arise from interactions between pollutants and biological macromolecules. Specific conditions dictate the choice of biochemical biomarkers, with examples including metallothionein, oxidative stress, and cytotoxic responses.

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 cellular pathological techniques and submicroscopic 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.

II. 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 life indicators such as temperature, light, moisture and suspended solids are emphasized (Khatri and Tyagi 2015). Every component of a living 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. Extremely responsive technologies are needed to discover toxins at a high expense 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.

1. **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 result in changes to their bodily, physiological, or behavioural attributes. Various plants, animals, and microorganisms are important instruments for identifying contaminants in a specific environmental milieu.

2. **Plants as indicators:** Plant species, such as flora and microflora, 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). Pollutants influence plants in diverse ways, encompassing changes in morphology, as well as biochemical and cellular modifications, which are often more readily observed than assessing their 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).

3. **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 distinct and precisely outlined 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 when evaluating the integrity of a specific 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. function as a biological indicator within a particular ecosystem. 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 carbon and nitrogen mineralization, with biomass-specific respiration, typically demonstrate a higher level of responsiveness (Aslam et al. 2012).

4. **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).

5. **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 deterioration of marine ecosystem.

6. **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).

7. **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. Contingent upon the enzyme's activity, 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.

8. **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 scarcity of food resources results in a reduction in population density (Jain et al. 2010). The use of animals as biological markers aids in determining the presence of poisons in animal tissues (Joanna 2006).

9. **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).

10. **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).

11. **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 forewarning 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.

12. **Frogs and toads:** For monitoring the attributes and transformations in a given environmental milieu, frogs are good biological indicators because they are affected due to pollutant buildup 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. Furthermore, they possess the capacity to metabolize pesticides that they ingest, breathe, or acquire from tainted foods, enabling the accumulation of residues in their biological 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.

13. **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, Highly responsive to detecting early ecosystem changes, they furnish uninterrupted information about the harm or modifications resulting from pollutants without interruption (da-Rocha et al. 2010). Insects species like Coleoptera (beetles), Homoptera (bugs), Diptera, Odonata sp. (dragonflies), Hydrophilidae (Coleoptera), families like Gyrinidae, Dytiscidae, Veliidae (Heteroptera) exhibit significant potential for adaptability as biological indicators (Hardersen 2000; Nummelin 2007).

    The influence of copper (Cu), iron (Fe), nickel (Ni), cadmium (Cd), and sulfuric acid (H2SO4) on various insect species can be investigated through the analysis of their population dynamics, life cycle duration, and the mortality rate of newly hatched larvae. Notably, insect species such as Apis mellifera serve as effective ecological indicators, exhibiting a heightened capacity for capturing and retaining chemical substances, which may subsequently become evident in the surrounding atmosphere or on 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.

14. **Zooplankton:** Zooplankton species serve as valuable biological indicators for evaluating the extent of contamination within aquatic ecosystems. The growth and development of zooplankton populations are intricately linked to factors such as aquatic productivity, eutrophication levels, and the expansion of freshwater bodies. Additionally, zooplankton are significantly responsive to fluctuations in weather patterns, making them highly sensitive to environmental changes and thereby contributing to their role as effective indicators. 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).

III. 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). The concept of "Internal Dose" quantifies the quantity of the parent substance or its derivative found at the designated target site. In contrast, "Applicable Dose" pertains to markers detected within the specific tissues being studied, providing insight into the interaction between the absorbed substance and 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 (Genetic mutations, deviations in chromosomal structure, the initiation of protein synthesis, DNA repair enzyme activity, stress protein expression, or the suppression of enzymes such as 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 vulnerability signify an organism's innate or acquired capacity to react to particular pollutant exposures. (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)

When selecting the most appropriate biomarker responses for inclusion in a comprehensive biomarker method within the context of individual biological surveillance programs, several essential criteria must be considered. These criteria encompass the biomarker's sensitivity, its responsiveness in a response correlation with exposure level and duration, its biochemical persistence (the duration of the response after exposure), and its inherent variability (Hagger et al., 2006). It is crucial that biomarkers exhibit a response proportionate to the dosage or dose-related reaction, to toxins across a spectrum of pollutants at environmentally relevant concentrations. This is imperative to ensure a comprehensive evaluation of toxicity. Furthermore, establishing the relevance of the biological response used as a biomarker to significant biological functions and pathological outcomes is considered critical for both ecological evaluation and wellness assesment.

IV. ENVIRONMENTAL BIOMONITORING USING POLLUTION BIOMARKERS CYP1A

1. **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 when the zebra mussel (Dreissena polymorpha) was exposed to a mixture of PCB Arochlor 1260 and dioxin-like CB-126, a substantial increase in EROD (ethoxyresorufin dealkylation) activity was observed. The biomarker can distinguish between the amounts of pollution in tiny streams that are contaminated with PCBs and AhR-binding PAHs.

2. **DNA integrity as an indicator of environmental contamination:** DNA integrity is compromised by genotoxic and external factors that lead to DNA strand damage, methylation loss, double-strand disruptions, and the creation 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 showed degradation at polluted sites, a condition linked to the extent of pollution from petroleum hydrocarbons discharged into coastal waters due to waste items. (Sarkar et al. 2006).

3. **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 scavenging of oxygen free radicals and binding with metals, plays significant roles in metallic metabolism in aquatic species and specifically in the elimination mechanisms (Andrews GK 2000). This has negative impacts on the free radical scavenging, catalytic, and non-catalytic defensive systems of organisms and causes oxidative damage to DNA, lipids, and proteins.

4. **Pigments as indicators in biomonitoring:** Phytoplankton and plant biomarkers contain pigments, whose light-harvesting organisms' main purposes are photochemical assimilation and photo defense. Within plants and algae, three primary categories of pigments exist: 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 depiction of the entire phototsynthetic 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).

5. **Lysosomal system as Biomarkers:** The lysosomal system, comprising of Lysosomes, autophagic and heterophagic vesicles, phagosomes, and residual bodies, capable of detecting the slightest cellular damage caused by the exposure of the pollutants (Köhler et al. 2002). Lysosomal compartment comprises of lysosomes (Pirmary and secondary), auto and heterophagic vesicles, multifunctional, abundant in hydrolases. Diverse components of the lysosomes are lost due to the deterioration of membrane integrity caused due to physicochemical modifications linked to cellular malfunction, inflaming and degenerative ailments, and mortality (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).

6. **Oxidative stress as biomarkers:** Pollutant exposure induces reactive oxygen species (ROS) stress in cells, characterized by an elevation in reactive species and a disruption in the effectiveness of antioxidants (Regoli and Giulian, 2014). A commonly utilized biomarker of reactive oxygen species (ROS) stress is glutathione (GSH) (Dalle-Donne et al., 2006), a crucial intracellular scavenger of free radicals that neutralizes peroxides in coordination with enzymes like glutathione peroxidase and glutathione reductase, thereby maintaining the cellular redox balance. The assessment of the ratio between reduced and oxidized glutathione (GSH/GSSG) is employed to determine the organism's reactive oxygen species (ROS) stress status.

   As an example, lipid peroxidation is a common marker of oxidative stress, arising from the oxidative breakdown of membrane phospholipids. Furthermore, antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase, whose activity and expression are modulated by pollutant exposure (Leomanni et al., 2015), serve as reliable indicators of oxidative stress. These biomarkers are well-suited for early-stage assessments of the effects of pollutants on ecosystems, even at low concentrations.

7. **The lipid peroxidation biomarkers:** This is the process that has been studied the most in terms of tissue harm inflicted by free radicals but because it is difficult to analyse directly, measurements are made of the oxidation derivatives (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).

8. **DNA oxidative damage biomarkers in vivo:** Besides serving as biomarkers for specific adjustments and hydroxylations of purine and pyrimidine bases, as well as damage to the deoxyribose-phosphate backbone and protein-DNA cross-links, exposure to pollutants escalates the level of oxidative harm inflicted upon DNA. The measurement of nucleobase guanosine and its free base 8-hydroxyguanine's hydroxylation through 8-hydroxydeoxyguanosine (8-OHdG) has been employed as an indicator for carcinogenesis (Lodovici et al., 2000). Furthermore, the formation of thymine glycol and thymidine glycol resulting from oxidative damage to DNA in tissues can also be employed as biomarkers for carcinogenesis.

9. **Biomarkers of protein oxidation:** The oxidation byproducts derived from the amino acids phenylalanine and tyrosine, leading to the production of dityrosine, serve as valuable indicators for oxidative stress, detectable both in cell identifiers and urological indicators. Recently, a range of methodologies has been developed for the identification of oxidized amino acids in blood proteins, serving as biomarkers for damage caused by free radicals. Protein oxidation gives rise to g-Glutamyl semialdehyde and 2-amino-adipic semialdehyde through free radical reactions, which can be detected and quantified in biological samples. These compounds act as biomarkers for protein oxidation resulting from exposure to nature based adulterants (Daneshvar et al., 1997).

10. **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.

Beyond organophosphate and carbamate pesticides, various chemical agents have been recently observed to inhibit acetylcholinesterase (AChE) activity in humans (Vioque-Fernandez et al., 2007). These chemical agents encompass heavy metals, alternative pesticides, polycyclic aromatic hydrocarbons, detergents, and constituents of complex contaminant mixtures. Furthermore, numerous types of nanoparticles, including metals, oxides, and carbon nanotubes (e.g., SiO2, TiO2, Al2O3, Al, Cu, carbon-coated copper, multiwalled carbon nanotubes, and single-walled carbon nanotubes), have recently exhibited significant affinities for AChE. Cu, Cu-C, multiwalled carbon nanotubes, and single-walled carbon nanotubes (MWCNT, SWCNT) demonstrated dose-dependent inhibition of AChE activity, with IC50 values of 4, 17, 156, and 96 mg/L, respectively.

V. 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.

1. **Evaluation of Chemicals or Metabolites as Exposure Biomarkers:** 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), heightened concentrations of organochlorine pesticides were detected in the sanguineous fluid in women, while rural children exhibited elevated levels of polycyclic aromatic hydrocarbons (PAHs). (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.

2. **DNA Injury as an Exposure Biomarker:** 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 single-cell gel electrophoresis assay is used as a biomarker to show exposure and repairable DNA damage(Mondal et al., 2010).

3. **Biomarkers of effect:** Genotoxicity monitoring in humans, chromosomal aberrations (CA) and micronuclei (MN) are routinely used as biomarkers of effect. Surveys of epidemiology suggest that chromosomal aberrations at high frequency is predictive of an escalated susceptibility 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 mutagenic 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.

4. **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.

5. **Advanced techniques: Virtual modeling:** Virtual modelling, an advanced technique have been utilized as biomarker for risk assessment, predicting toxicity endpoints, clinical impacts, and ADME (Absorption, Distribution, Metabolism, and Excretion) characteristics 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, Computational molecular docking investigations (or 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 toxicant mechanism and unravel the exposure-impact connections. When determining the degree of workplace 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 damage can be detected in cells, tissues, and biological fluids, and it is associated with the onset of numerous illnesses.

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 generate more accurate outcomes and enhanced predictive capacity 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. Hence, a range of biomarkers can prove invaluable in implementing a comprehensive strategy aimed at intervention options for preventing or mitigating the adverse health effects of chemical contamination in both human populations and the environment. The recent advancements in molecular biology and OMIC sciences, including genomics, transcriptomics, proteomics, lipidomics, epigenomics, and metabolomics, among others, are gaining increasing significance in the realm of environmental and human biomonitoring. These developments offer the opportunity to develop novel and highly sensitive biomarkers that can be incorporated into an integrated approach (Suárez-Ulloa et al. 2013, 2013).

VI. 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, evaporation, chemical alteration, dissemination, immobilization (i.e., adsorption and retention by clay minerals and organic matter), disintegration, and dilution occurs in soil and groundwater. However, these processes may proceed at a sluggish pace, and hence, specific chemicals might endure for extended periods. Biodegradation relies on a multitude of factors associated with the environmental and chemical properties in which they are found.

There are various ways to evaluate microbial attenuation, including microcosm investigations, analysis of the site's hydrology and subsurface geology, biochemical profiles of pollutants, both qualitative and quantitative, as well as the 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 are essential for this process for particular enzymes responsible for pollutant degradation, 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).

1. **Luciferase as biomarkers:** For monitoring bioremediation inocula, luciferase markers such as luciferase gene (luc), or bacterial luciferase genes (luxAB) can be readily identified 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). Likely, Pseudomonas cepacia, a 2,4-D degrading strain, marked with lacZY and luxAB genes, was tracked through colony counting in soil treated with 2,4-D (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 signifies a population of cells that are metabolically active. As cells become starved, the light production from luciferase enzymes declines and therefore, it is referred to as potential luminescence (Meikle et al., 1994)

2. **Biomarker using GFP:** An additional marker for bioremediation monitoring is the gfp gene, which encodes Green Fluorescent Protein. It offers the benefits of fluorescing when exposed to light without the need for any additional energy source or substrate, apart from oxygen, during the initial chromophore formation (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. Further instances of utilizing GFP as a biomarker for monitoring bioremediation involve the tracking of a p-nitrophenol degrading strain of Moraxella and a phenanthrene mineralizing strain of Pseudomonas in soil microcosms through the enumeration of GFP fluorescent colonies.

3. **Fungal biomass as biomarker:** To assess and manage the effectiveness of the bioremediation process, fungal biomass has been employed. According to Barajas-Acheve et al. (2002), biochemical techniques for analysing components specific to fungi such ergosterol, chitin, or phospholipid fatty acids are regarded to serve as a valuable marker for estimating fungal biomass in contaminated soils. SIP, a technique for tracking in-situ chemical biodegradation, bases its analysis on variations in the stable isotope composition of the target molecule. Stable Isotope Probing (SIP) involves tracing stable isotope atoms from particular substrates into biomarker-containing elements of microbial cells.

4. **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).

5. **Genetic biomarkers:** The most powerful tool used as biomarker are Genetic biomarkers that can potentially be employed for the biodegradation of contaminants. 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 for identifying the presence or absence of microbial organisms, especially when biodegradation relies on a particular 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).

6. **Enyzmes as biomarkers for bioremediation:** With the help of Biomarker Molecular Methods (BMMs), it is possible to focus on functional genes associated with processes that encompass both 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 utilized as an indicator of microbial conversion (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.

7. **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), green remediation utilize plants to reduce, eliminate, degrade, or immobilise nature based contaminants. Plants are grown hydroponically, transplanted into metal-contaminated waters, in this process, they absorb and accumulate metals in their roots and shoots, and once they reach a saturation point with the metals, the plants are harvested for disposal.

   Among organisms, algae and aquatic plants are potential ecological engineer for gathering and biomagnifying heavy metals because of their ability of sequestration and can live under many extreme environments. (Kalin et al. 2005). Duckweed (Lemna minor) has been validated as a viable option for the phytoremediation of water bodies

contaminated with low levels of copper and cadmium (Hou et al. 2007). According to Srivastava et al. (2006), the aquatic macrophyte *H. verticillata* (L.f.) Royle's ability to withstand mild copper exposure and their high accumulation capacity render them well-suited for the restoration of water bodies moderately polluted with copper.

Bioremediation techniques grounded in biomonitoring offer several advantages compared to alternative methods for addressing aquatic metal pollution: Ease of use, Swift and efficient cleanup in contrast to natural attenuation, Environmentally safe and natural treatment, Simple application without the need for protective clothing, Cost-effectiveness, Effectiveness, Long-term solutions for fostering a balanced ecosystem.

By employing specific biosensors and biomarkers, genomic technologies can assess the biological potential of each habitat. For example, enzyme-driven biosensors can prompt a signal through product formation, substrate disappearance, or cofactor transformation. Biosensors have the capability to monitor a biological result that can be converted into a detectable signal. Biomarkers refer to specific genotypes that can be utilized to monitor the persistence and/or effectiveness of a particular bacterial strain during bioremediation. Examples of biomarkers include the luc gene, responsible for firefly luciferase, and the gfp gene, responsible for the green fluorescent protein (GFP).

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.

VII. CONCLUSION

Contaminant biomarkers have recently demonstrated their value as early indicators of negative impacts in biological and ecological 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 comprehension of the relationship among nature based contamination and well being 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.

REFERENCES

[1] Adedeji OB, Okerentugba PO, Okonko IO (2012) Use of molecular, biochemical and cellular biomarkers in monitoring environmental and aquatic pollution. Nature and Science 10: 83-104
[2] Ahmed AHS, Aaron ME, Alison O, Claudia VL, Matthew KL (2016) How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in Ecological Indicators. Ecological Indicators 60: 223-230
[3] Amiard JC, Cosson RP (1997) Les métallothionéins: Biomarqueurs en Ecotoxicologie, Aspects Fondamantaux. Masson editors, Paris
[4] Amorim F, Mata VA, Beja P, Rebelo H (2015) Effects of a drought episode on the reproductive success of European free-tailed bats, Tadarida teniotis, Mammalian Biology.
[5] Andrews GK (2000) Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem Pharmacol 59: 95-104
[6] Angrish MM, Pleil JD, Stiegel MA, Madden MC, Moser VC, Herr DW (2016) Taxonomic applicability of inflammatory cytokines in Adverse Outcome Pathway (AOP) development. J Toxicol Environ Health A 79(4): 184-96
[7] Aslam M, Verma DK, Dhakerya R, Rais S, Alam M, Ansari FA (2012) Biological indicators: a comparative study on uptake and accumulation of heavy metals in some plant′s leaves of M.G. road, Agra City, India. Res J Environ Earth Sci 4: 1060–1070
[8] AVioque-Fernandez EA, de Almeida J, Ballesteros ´T, Garc´ıa-Barrera JL, Gomez-Ariza, opez-Barea JL (2007) "Donana National Park survey using crayfish (˜ Procambarus clarkii) as bioindicator: esterase inhibition and pollutant levels," Toxicology Letters 168(3):260–268
[9] Barajas-Aceves M, Hassan M, Tinoco R, Vazquez-Duhalt R (2002) Effect of pollutants on the ergosterol content as indicator of fungal biomass. J Microbiol Methods 50:227–236
[10] Barnosky AD, Hadly EA, Bascompte J, Berlow EL, Brown JH, Fortelius M, Getz WM, Harte J, Hastings A, Marquet PA, Martinez ND, Mooers A, Roopnarine P, Vermeij G, Williams JW, Gillespie R, Kitzes J, Marshall C, Matzke N, Mindell, D.P., Revilla, E. and Smith, A.B. (2012). Approaching a state shift in Earth's biosphere. Nature, 486, 52-58.
[11] Behrens A, Segner H (2005) Cytochrome P4501A induction in brown trout exposed to small streams of an urbanised area: results of a five-year-study. Environ Pollut 136: 231-242
[12] Binelli A, Ricciardi F, Riva C, Provini A (2006) New evidences for old biomarkers: effects of several xenobiotics on EROD and AChE activities in Zebra mussel (*Dreissena polymorpha*). Chemosphere 62: 510-519.
[13] Bonassi S, Norppa H, Ceppi M, Stromberg U, Vermeulen R, Znaor A, Cebulska-Wasilewska A, Fabianova E, Fucic A, Gundy S, Hansteen IL, Knudsen LE, Lazutka J, Rossner P, Sram RJ, Boffetta P (2008) Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: Results from a pooled cohort study of 22 358 subjects in 11 countries. Carcinogenesis 29:1178
[14] Bracci M, Elexpuru-Zabaleta M, Tartaglione MF, Ledda C, Rapisarda V, Santarelli L (2020) Exosomal miR-92a Concentration in the Serum of Shift Workers. Appl. Sci 10: 430
[15] Burgeot T, Akcha F, Ménard D, *et al.* (2017) Integrated monitoring of chemicals and their effects on four sentinel species, *Limanda limanda, Platichthys flesus, Nucella lapillus and Mytilus sp.*, in Seine Bay: A key step towards applying biological effects to monitoring. Mar Environ Res 124: 92-105
[16] Cairns J Jr, McCormick PV (1992) Developing and ecosystem-based capability for ecological risk assessments. Environ. Profession 14:186-96
[17] Calisi A, Lionetto MG, De Lorenzis E, Leomanni A, Schettino T (2014) Metallothionein induction in the coelomic fluid of the earthworm Lumbricus terrestris following heavy metal exposure: a short report. In: BioMed Res Intern. Article ID 109386
[18] Calisi A, Zaccarelli N, Lionetto MG, Schettino T (2013) Integrated biomarker analysis in the earthworm Lumbricus terrestris: application to the monitoring of soil heavy metal pollution. Chemosphere 90(11): 2637-44
[19] da Rocha J, Almeida J, Durval A (2010) Insects as indicators of environmental changing and pollution: a review of appropriate species and their monitoring. HOLOS Environ 10:1519–8634
[20] Dagnino A, Sforzini S, Dondero F, *et al.* (2008) A "weight of evidence" approach for the integration of environmental "triad" data to assess ecological risk and biological vulnerability. Integr Environ Assess Manag 4(3): 314-26
[21] Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A (2006) Biomarkers of oxidative damage in human disease. Clin Chem 52(4): 601-23

[22] Daneshvar B, Frandsen H, Autrup H, Dragsted LO (1997) g-Glutamyl semialdehyde and 2-amino-adipic semialdehyde: biomarkers of oxidative damage to protein. Biomarkers 2:117–123
[23] Das M, Maiti SK (2007) Asian J. Water Environ. Pollut 4:169
[24] de la Torre FR, Salibián A, Ferrari L (2007) Assessment of the pollution impact on biomarkers of effect of a freshwater fish. Chemosphere 68(8): 1582-90
[25] Depledge MH, Amaral-Medes JJ, Daniel B, Halbrook R, Kloepper-Sams P, Moore MN, Peakall DB (1992) The conceptual basis of the biomarker approach. In Peakall, D.B. and Shugart, L.R. eds. Biomarkers: research and application in the assessment of environmental health, NATO ASI Series H: Cell Biology 68:15-29. Berlin, Heidelberg: Springer-Verlag
[26] Di Pierro D, Tavazzi B, Lazzarino G, Giardina B (1992) Malondialdehyde is a biochemical marker of peroxidative damage in the isolated reperfused rat heart. Mol. Cell Biochem 116:193–196
[27] Draper HH, Squires EJ, Mahmooch H, Wu J, Agarwal S, Handley M (1993) A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radicals Biol. Med 15:353–363
[28] Dumont MG, Murrell JC (2005) Stable isotope-probing-linking microbial identity to the function. Nat Rev, Microbiol 3:499–504
[29] Erb RW, Eichner CA, Wagner-DoÈbler I, Timmis KT (1997) Bioprotection of microbial communities from toxic phenol mixtures by a genetically designed pseudomonad. Nat. Biotechnol. 15:378± 382
[30] Fenton MB, Simmons NB (2014) Bats: a world of science and mystery. University of Chicago Press, Chicago.
[31] Flemming CA, Leung KT, Lee H, Trevors JT, Greer CW (1994) Survival of lux-lac-marked biosurfactant-producing *Pseudomonas aeruginosa* UG2L in soil monitoried by nonselective plating and PCR. Appl. Environ. Microbiol 60:1606±1613
[32] Furukawa K (2003) 'Super bugs' for bioremediation. Trends Biotechnol 21:187–190
[33] Galloway TS (2006) Biomarkers in environmental and human health risk assessment. Mar Pollut Bull 53(10-12): 606-13
[34] Gao Y, Luo Y (2005) Earthworms as biological indicators of soil pollution and their potential for remediation of contaminated soils. EPA, NCEA, HERO 42: 140-148
[35] Gerhardt A (2002) Biological indicators species and their use in bio monitoring. Environmental Monitoring I. Encyclopedia of life support systems. UNESCO ed. Oxford (UK): Eolss Publisher.
[36] Ghini S, Fernandez M, Pico Y, Marin R, Fini F, Manes J, Girotti S (2004) Occurrence and distribution of pesticides in the province of Bologna, Italy, using honeybees as bioindicators. Arch Environ ContamToxicol 47:479–488
[37] Gonick HC (2011) Lead-binding proteins: A review. J Toxicol Article ID 686050
[38] Grover P, Rekhadevi PV, Danadevi K, Vuyyuri SB, Mahboob M, Rahman MF (2010) Genotoxicity evaluation in workers occupationally exposed to lead. Int. J. Hyg. Environ. Health 213:99.
[39] Hagger JA, Jones MB, Leonard DR, Owen R, Galloway TS (2006) Biomarkers and integrated environmental risk assessment: are there more questions than answers? Integr Environ Assess Manag 2(4): 312-29
[40] Hagger JA, Jones MB, Lowe D, Leonard DR, Owen R, Galloway TS (2008) Application of biomarkers for improving risk assessments of chemicals under the Water Framework Directive: A case study. Mar Pollut Bull 56(6): 1111-8
[41] Harayama S, Kishira H, Kasai Y, Shutsubo K (1999) Petroleum biodegradation in marine environments. J Molec Microbiol Biotechnol 1:63–70
[42] Hardersen S (2000) The role of behavioural ecology of damselflies in the use of fluctuating asymmetry as a bioindicator of water pollution. Ecolog Entomol 25:45–53
[43] Hasselbach L, Ver Hoef JM, Ford J, Neitlich P, Crecelius E, Berryman S, Wolk B, Bohle T (2005) Spatial patterns of cadmium and lead deposition on and adjacent to National Park Service lands in the vicinity of red dog mine, Alaska. Sci Total Environ 348:211–230
[44] Hook SE, Gallagher EP, Batley GE (2014) The role of biomarkers in the assessment of aquatic ecosystem health. Integr Environ Assess Manag 10(3): 327-41
[45] Hosmani SP (2013) Fresh aquatic algae as indicators of aquatic quality. Univers J Environ Res Technol 3:473–482
[46] Hou WH, Chen X, Song GL, Wang QH, Chang CC (2007) Plant Physiol. Biochem 45:62
[47] Indennidate L, Cannoletta D, Lionetto F, Greco A, Maffezzoli A (2010) "Nanofilled polyols for viscoelastic polyurethane foams," Polymer International 59(4):486–491

[48] Jain A, Singh BN, Sl S, Singh HB, Singh S (2010) Exploring biodiversity as biological indicators for aquatic pollution National Conf. On biodiversity, development and poverty alleviation; Uttar Pradesh. Uttar Pradesh State Biodiversity Board, Lucknow (India)
[49] Jansson JK, BjoÈ rkloÈ f K, Elvang AM, Jùrgensen KS (2000) Biomarkers for monitoring efficacy of bioremediation by microbial inoculants Environmental Pollution 107:217±223
[50] **J**echalke S, Rosell M, Martinez-Lavanchy PM, Perez-Leiva P, Rohwerder T, Vogt C, Richnow HH (2011) Linking low-level stable isotope fractionation to expression of the cytochrome P450 monooxygenaseencoding *ethB* gene for elucidation of methyl *tert*-butyl ether biodegradation in aerated treatment pond systems. Appl. Environ. Microbiol 77: 1086–1096
[51] Joanna B (2006) Biological indicators: types, development, and use in ecological assessment and research. Environ Bio-indicators 1:22–39
[52] Jones G (2012) What bioindicators are and why they are important. In: Flaquer, C., Puig-Montserrat, X. (Eds). Proceedings of the International Symposium on the Importance of Bats as Bioindicators. Museum of Natural Sciences Edicions, Granollers, Pp: 18-19
[53] Kaegi JHR (1991) Overview of metallothionein. In: Riordan, J.F.,Vallee, B.L. (Eds.), Methods in enzymology, metallobiochemistry (Part B: Metallothionein and related molecules), Academic Press, San Diego 205:613–626
[54] Kalin M, Wheeler WN, Meinrath G (2005) J. Environ. Radioactivity 78:151
[55] Kalkan S, Altuğ G (2015) Bio-indicator bacteria & environmental variables of the coastal zones: the example of the Güllük Bay, Aegean Sea, Turkey. Mar Pollut Bull 15(95):380–384
[56] Khatri N, Tyagi S (2015) Influences of natural and anthropogenic factors on surface and ground aquatic quality in rural and urban areas. Front Life Sci 8:23–39
[57] Köhler A, Wahl E, Söffker K (2002) Functional and morphological changes of lysosomes as prognostic biomarkers of toxic liver injury in a marine flatfish (Platichthys flesus). Environ. Toxicol. Chem 21(11): 2434-2444
[58] Ladeira C, Viegas S. (2016) Human Biomonitoring – An overview on biomarkers and their application in Occupational and Environmental Health. Biomonitoring 3: 15-24
[59] Landrigan PJ, Fuller R, Acosta NJR, *et al. (2018)* The Lancet Commission on pollution and health. Lancet 391(10119): 462-512
[60] Leavitt PR, Hodgson DA (2001) Sedimentary pigments: Developments in paleoenvironmental research, Tracking environmental changes using lake sediments: terrestrial algal and siliceous indicators. Kluwer Academic Publishers, Dordrecht
[61] Lee PKH, Macbeth TW, Sorenson KS, Deeb RA, Alvarez-Cohen L (2008) Quantifying genes and transcripts to assess the in situ physiology of "*Dehalococcoides*" spp. in a trichloroethene-contaminated groundwater site. Appl. Environ. Microbiol 74**:**2728–2739
[62] Leomanni A, Schettino T, Calisi A, *et al.* (2015) Antioxidant and oxidative stress related responses in the Mediterranean land snail *Cantareus apertus* exposed to the carbamate pesticide Carbaryl. Comp Biochem Physiol C Toxicol Pharmacol 168(Part C): 20-7
[63] Lindenmayer DB, Likens GE (2011) Direct measurement versus surrogate indi-cator species for evaluating environmental change and biodiversity loss. Ecosystems 14:47–59
[64] Lionetto MG, Giordano ME, Caricato R, Pascariello MF, Marinosci L, Schettino T (2001) Biomonitoring of heavy metal contamination along the Salento coast (Italy) by metallothionein evaluation in Mytilus galloprovincialis and Mullus barbatus. Aquatic Conser: Mar Freshw Ecosyst 11: 305-10
[65] Lodovici M, Casalini C, Cariaggi R, Michelucci L, Dolara P (2000) Levels of 8-hydroxydeoxyguanosine as a marker of DNA damage in human leukocytes. Free Radicals Biol. Med 28:13–17
[66] Magalhães DP, Ferrão Filho AS (2008) A ecotoxicologia como ferramenta no biomonitoramento de ecossitemas aquáticos. Oecologia Brasiliensis 12(3):355-381
[67] Manno M, Viau C, Cocker J, et al (2010) Biomonitoring for occupational health risk assessment (BOHRA). Toxicol Lett 192(1): 3-16
[68] Manno M, Viau C, Cocker J, *et al.* (2010) Biomonitoring for occupational health risk assessment (BOHRA). Toxicol Lett 192(1): 3-16
[69] Markert B (2008) From biomonitoring to integrated observation of the environment – the multi-markered bioindication concept. Ecological Chemistry and Engineering 15(3):315–330
[70] Masson L, Comeau Y, Brousseau R, Samson R, Greer C (1993) Construction and application of chromosomally integrated lac-lux gene markers to monitor the fate of a 2,4-dichlorophenoxyacetic acid-degrading bacterium in contaminated soils. Microbiol. Releases 1:209±216
[71] Maugh TH (1978) Chemicals: how many are there? *Science* 199, 162

[72] McDonald IR, Bodrossy L, Chen Y, Murrell JC **(**2008). Molecular ecology techniques for the study of aerobic methanotrophs. Appl. Environ. Microbiol 74:1305–1315
[73] Meikle A, Glover LA, Killham K, Prosser JI (1994) Potential luminescence as an indicator of activation of genetically-modified *Pseudomonas fluorescens* in liquid culture and in soil. Soil Biol. Biochem 26:747±755
[74] MoÈller A, Jansson JK (1998) Detection of Firefly luciferase-tagged bacteria in environmental samples. In LaRossa, R. (Ed.). Methods in Molecular Biology, Vol. 102: Bioluminescence Methods and Protocols. Humana Press, Totowa, NJ 269±283
[75] Monard C, Martin-Laurent F, Lima O, Devers-Lamrani M, Binet F (2012) Estimating the biodegradation of pesticide in soils by monitoring pesticide-degrading gene expression. Biodegradation 24**:**203–213
[76] Mondal NK, Mukherjee B, Das D, Ray MR (2010) Micronucleus formation, DNA damage and repair in premenopausal women chronically exposed to high level of indoor air pollution from biomass fuel use in rural India. Mutat. Res 697:47
[77] Newsome D, Lewis A, Moncrieff D (2004) Impacts and risks associated with developing, but unsupervised, stingray tourism at Hamelin Bay, Western Australia. International Journal of Tourism Research, 6(5):305–323
[78] Nichlsa E, Larsen BT, Spectora S, Davise AL, Escobar CF, Favilad M, Vulinece K (2007) Global dung beetle response to tropical forest modification and fragmentation: a quantitative literature review and meta-analysis. Biol Conserv 137:1–19
[79] Nkwoji JA, Igbo JK, Adeleye AO, Obienu JA (2010) Implications of biological indicators in ecological health: study of a coastal lagoon, Lagos, Nigeria. Agric Biol J Noth Am 1:683–689
[80] Nummelin M, Lodenius M Tulisalo, E Hirvonen H, and Alanko T (2007). Predatory insects as bioindicators of heavy metal pollution. Environ Pollution 145: 339– 347
[81] Pandey AK, Bajpayee M, Parmar D, Kumar R, Rastogi SK, Mathur N, Thorning P, de Matas M, Shao Q, Anderson D, Dhawan A (2008) Multipronged evaluation of genotoxicity in Indian petrol-pump workers. Environ. Mol. Mutagen 49:695
[82] Pandey AK, Gurbani D, Bajpayee M, Parmar D, Ajmani S, Dhawan, A (2009) In silico studies with human DNA topoisomerase-II alpha to unravel the mechanism of in vitro genotoxicity of benzene and its metabolites. Mutat. Res 661:57
[83] Pathak R, Mustafa M, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD (2010) Association between recurrent miscarriages and organochlorine pesticide levels. Clin. Biochem 43:131
[84] Pelz O, Chatzinotas A, Zarda-Hess A, Abraham W-R, Zeyer J (2001) Tracing toluene-assimilating sulphate-reducing bacteria using 13C-incorporation in fatty acids and whole-cell hybridisation. FEMS Microbiol Ecol 38:123–131
[85] Posudin Y (2014) Bioindication, in Methods of Measuring Environmental Parameters. John Wiley & Sons, Inc., Hoboken, NJ, USA. Proceeding of XXXIX-th Apimondia International Apicultural congress, 21-26 August 2005, Dublin, Ireland, Pp. 145 – 146
[86] Raghavan SB, Basavaiah K (2005) Biological monitoring among benzene-exposed workers in Bangalore city, India. Biomarkers 10:336
[87] Ramchandra TV, Rishiram R, Karthik B (2006) Zooplanktons as Biological Indicators: Hydrobiological investigation in selected Bangalore Lakes. Technical report 115
[88] Rattray EAS, Prosser JI, Killham K, Glover LA(1990) Luminescence-based nonextractive technique for in situ detection of *Escherichia coli* in soil. Appl. Environ. Microbiol 56: 3368±3374
[89] Regoli F, Giuliani ME (2014) Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar Environ Res 93: 106-17
[90] Rocco A, Scott-Fordsmand J, Maisto G, Manzo S, Salluzzo A, Jensen J (2011) Suitability of lysosomal membrane stability in Eisenia fetida as biomarker of soil copper contamination. Ecotoxicol Environ Saf 74(4): 984-8
[91] Ryan JA, Hightower LE (1996) Stress proteins as molecular biomarkers for environmental toxicology In: Feige, U., Morimoto, R.I., Yahara, I., Polla, B. (Eds.), Stress-inducible cellular responses Birkauser Verlag, Basel
[92] Saber M, Abouziena HF, Hoballah E, Soad El-Ashry and Zaghloul AM (2015b). Phytoremediation of potential toxic elements in contaminated sewaged soils by Sunflower (Helianthus annuus) and Corn (Zea mays L.) plants. 12th Inter Phytotechnology Conf., Manhattan, Kansas, USA, 27-30 September
[93] Saber M, Soad El-Ashry, Nizinski J, Montoroi JP, and Zaghloul AM (2015a) Chemical characterization of sewage effluent repetitively used in arid soils irrigation. 12th Inter Phytotechnology Conf, Manhattan, Kansas, USA, 27-30 September

[94] Sarkar A, Ray D, Shrivastava AN, Sarker S (2006) Molecular Biomarkers: their significance and application in marine pollution monitoring. Ecotoxicology 15: 333-340
[95] Schettino T, Caricato R, Calisi A, Giordano ME, Lionetto MG (2012) Biomarker approach in marine monitoring and assessment: new insights and perspectives. Open Environ Sci 6: 20-7
[96] Sellappa SP, Parthyumnan S, Keyan KS, Joseph S, Vasudevan S, Sasikala K (2010) Evaluation of DNA damage induction and repair inhibition in welders exposed to hexavalent chromium. Asian Pac. J. Cancer Prev 11:95.
[97] Shukla AK, Vishwakarma P, Upadhyay SN, Tripathi AK, Prasana HC, Dubey SK (2009) Biodegradation of trichloroethylene (TCE) by methanotrophic community. Bioresour. Technol. 100:2469–2474
[98] Singh AP, Shah PP, Ruwali M, Mathur N, Pant MC, Parmar D (2009) Polymorphism in cytochrome P4501A1 is significantly associated with head and neck cancer risk. Cancer Invest. 27:869
[99] Singh SS, Srivastava NK, Singh G, Omics MP (2010) In mechanistic and predictive toxicology. Toxicol. Mech. Methods 20:355
[100] Spellerberg IF (2005) Monitoring Ecological Change. Cambridge University Press, Cambridge
[101] Srivastava S, Mishra S, Tripathi RD, Dwivedi S (2006) Aquat. Toxicol 80:405
[102] Steffan RJ, Atlas RM (1991) Polymerase chain reaction: application in environmental microbiology. Annu Rev Microbiol 45:137–161
[103] Stiegel MA, Pleil JD, Sobus JR, Stevens T, Madden MC (2017) Linking physiological parameters to perturbations in the human exposome: Environmental exposures modify blood pressure and lung function via inflammatory cytokine pathway. J Toxicol Environ Health A 80(9): 485-501
[104] Suárez-Ulloa V, Fernández-Tajes J, Manfrin C, Gerdol M, Venier P, Eirín-López JM (2013) Bivalve omics: state of the art and potential applications for the biomonitoring of harmful marine compounds. Mar Drugs 11(11): 4370-89
[105] Subhash Chandra & Richa Sharma & Kriti Singh & Anima Sharma (2013) Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon Ann Microbiol 63:417–431
[106] Tesfalem Weldeslassie, Huma Naz, Balwant Singh, Mohammad Oves (2018) Chemical Contaminants for Soil, Air and Aquatic Ecosystem Modern Age Environmental Problems and their Remediation, Springer International Publishing AG
[107] Thakur RK, Jindal R, Singh UB, Ahluwalia AS (2013) Plankton diversity and aquatic quality assessment of three fresh aquatic lakes of Mandi (Himachal Pradesh, India) with special reference to planktonic indicators. Environ Monit Assess 185:8355–8373
[108] Tombolini R, Jansson JK (1998) Monitoring of GFP tagged bacterial cells. In LaRossa, R. (Ed.). Methods in Molecular Biology, Vol. 102: Bioluminescence Methods and Protocols. Humana Press, Totowa, NJ285±298
[109] Urbini A, Sparvoli E, Turillazzi S (2006) Social paper wasps as bioindicators: a preliminary research with Polistes dominulus (hymenoptera: Vespidae) as a trace metal accumulator. Chemosphere 64:697–703
[110] Valverde M, Rojas E (2009) The Comet assay in Human biomonitoring, edied by A. Dhawan and D. Anderson, The Comet Assay in Toxicology, Royal Society Of Chemistry, Cambridge 227–266
[111] van Nierop K, Muller FJ M, Stap J, van Noorden CJF, van Eijk M, de Groot C (2006) Lysosomal destabilization contributes to apoptosis of germinal center B-lymphocytes. J. Histochem. Cytochem 54(12):1425-1435
[112] Velkov VV (2001) Stress-induced evolution and the biosafety of genetically modified microorganisms released into the environment. J Biosci 26:667–683
[113] Vogel TM (1996) Bioaugmentation as a soil bioremediation approach. Curr. Opinion Biotechnol 7:311±316
[114] Vuyyuri SB, Ishaq M, Kuppala D, Grover P, Ahuja YR (2006) Evaluation of micronucleus frequencies and DNA damage in glass workers exposed to arsenic. Environ. Mol. Mutagen 47:562
[115] Westerberg K, ElvaÈng AM, Jansson JK (1999) Biodegradation of high concentrations of 4-chlorophenol: isolation and characterization of *Arthrobacter chlorophenolicus* sp. nov. (submitted).
[116] Zannatul F, Muktadir AKM (2009) A review: potentiality of zooplankton as biological indicators. Am J Appl Sci 6:1815–1819

