UNIT 17 BIOMARKERS

Structure

17.1 INTRODUCTION

The extensive exploitation of natural resources and disposal of all types of wastes including organic waste, chemicals, industrial waste, and electronic wastes into water causes water pollution. The government from time to time has started some environmental monitoring programs in the coastal areas and most of these programs consisted only chemical and physical monitoring of the water. These measurements include the oxygen concentration, salinity, temperature, nutrients, and the presence of toxic heavy metals in the water. In some programs, the water transparency measurement using secchi disc method gave the information about the algae bloom. However, these measurements do not give information regarding the effect of contaminants on the flora and fauna in the water. One of the first development occur in 1960s when scientists find very difficult to monitor the organochlorine contaminants in water such as DDT and PCBs. These organochlorines were present in very low concentrations and concentrations were lower than detection limits or required expensive techniques and skilled analysts. This was the time when scientists felt that focus should be move to effects monitoring rather than contaminants monitoring. The researchers focused on development of novel methods that could provide the effects and warning indicators of different contaminants on flora and fauna in the marine environment. These indicators were called biomarkers.

Biomarkers have a vast area and these can be utilized in various applications in the area of science and technology. Researchers have made great developments in the area of biomarkers in the last decade due to the increase in demand to develop new drugs in the pharmaceutical industry and challenges in environmental monitoring. A deep understanding of various concepts of biomarkers is required to understand the scopes and challenges of biomarkers in the area of environmental monitoring.

17.2 OBJECTIVES

After studying this unit, you should be able to:

- define biomarkers and distinguish it from related fields such as bioindicators, COA (clinical outcome assessment);
- explain the classification of biomarkers based on several parameters;
- explain the various applications of biomarkers in science and technology;
- explain the scope of biomarkers in environmental monitoring in detail;
- explain the limitations and future prospects of biomarkers;

17.3 DEFINITION OF BIOMARKERS

Pollutants like herbicides, insecticides, and other chemicals started affecting the marine environment, this causes to focus on environmental monitoring of water rather than contaminant monitoring. Research scientists started to design new methods that can provide early warning signs for the effect of contaminants on the aquatic environment. These indicators are called biomarkers. The biomarkers can be defined in many ways and some definitions of biomarkers are discussed in this section.

Any measurable early warning indicator of any biological process, pathogenic process, or xenobiotic exposure can be termed a biomarker. Broadly, the biomarkers encompass therapeutic interventions, which can be derived from molecular, radiographic, or physiological characteristics. Some simplest examples of biomarkers used in disease diagnostics are listed in table 1.

Table 1: Some simple biomarkers used in disease diagnostics.

Biomarker	**Biological state/ disease**
Temperature	Fever
c-reactive protein (CRP)	Inflammation
Antibody	Infection
Blood pressure	Hypertension/ heart stroke

The National Academy of Sciences in the USA defined biomarkers as follows: ‘‘A biomarker is a xenobiotically induced variation in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample’’. In other words, the biomarkers are the endpoint results of an ecotoxicological test performed on living organisms. However, the term biomarker is surrounded by some confusion as different scientists view it differently. Some scientists define biomarkers just as responses at molecular, physiological, or biochemical levels occurring within the organism’s tissues, cells, or cellular fluids. While some scientists view the wider perspectives of biomarkers and consider the accumulation of toxic chemicals within the organism and responses occurring at the population level, community, or ecological level of organisms. And it is always assumed that sub-organismic responses occur first and are followed by the responses at the organismic level, population, and ecological level. These sub-organismic responses can be useful in the detection of early warning signals for various monitoring.

Depledge defined the biomarkers more comprehensively “A biomarker is defined as a change at a biochemical, cellular, physiological or behavioural level; it can be measured in tissues and/or cellular fluids and/or in the whole organism and shows the exposure and/or the effects of one or more chemical contaminants (and/or radiations)”. The above definition clearly states that the key principle of biomarkers is that the early warning signals occur long before the effects at the ecological/population level.

Difference between biomarker and bioindicator

The terms biomarker and bioindicator are a little confusing as both look like synonyms to each other. But there is a clear difference exist between the two terms. The use of the term biomarker is restricted to the response or warning signal for the biological, physiological, or molecular change in the organism (tissues, body fluids, organs) level. Thus, the biomarker is a warning sign of the toxic effects of any exposure. The term bioindicator is used for changes occurring at the population/ecological level. In short, we can say that the biomarker term is used for warning signs, and the bioindicator term is used for the endpoint effects of the exposure.

Difference between biomarker and Clinical outcome assessment (COA)

When therapy for any disease is developed the outcome assessments are used to define the endpoint efficacy of the therapy. The efficacy of therapy is defined by the clinical assessment of the patients. Thus, the clinical outcome assessment (COA) can be defined as the clinical assessment used in the clinical trial outcomes. COAs are the direct measure of a person’s feelings, behavior, or function. The biomarkers and COA are different from each other because COAs are directly important measures for the patients and COAs can be used for approval of any novel medicine or therapeutic. On other hand, a biomarker is used for different purposes and one of the uses of biomarkers is that their measurement gave a prediction of COA.

Check Your Progress 1

Notes: a) Use the Space given for your answer.

b) Check your answer with the one given at the end of this unit.

1. Define biomarkers. How biomarkers are different from bioindicators?

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17.4 CLASSIFICATION OF BIOMARKERS

Biomarkers can be classified based on different parameters such as characteristics and applications. Based on characteristics, biomarkers can be classified into imaging biomarkers (Magnetic resonance imaging, computed tomography) and molecular biomarkers. A biomarker or biological state detectable in an image is known as an **imaging biomarker**. For example, the warning signs of lung cancer can be determined by a large number of biomarkers. An x-ray, CT scan, or MRI of the lung can detect the presence of any lesion within the lung. Imaging biomarkers are cost-effective, easily available, and non-invasive diagnostic tools for screening any patient. These are readily used in cancer diagnostic, development of cancer therapeutics, chest infections, diagnosis of bone fracture etc.

Molecular biomarkers can be defined as a set of biomarkers that are detectable or measurable using genomics or proteomics technologies. A broader definition of molecular biomarkers is "All set of biomarkers existent or to be discovered, that is detectable/ measurable using the characteristics of molecules and any redesign version of these analytes. This definition of molecular biomarkers recognizes the importance of molecular design and its characteristics. Examples of molecular biomarkers are glucose, cholesterol, insulin, m-RNA genes, DNA adducts, cytochrome P4501A, etc.

On the basis of applications, biomarkers can be classified as diagnostic markers, staging of disease biomarkers, disease prognosis biomarkers, clinical monitoring biomarkers, pharmacodynamic biomarkers, and environmental monitoring biomarkers. The schematic classification of biomarkers based on different parameters is represented in figure 1. **Diagnostic markers** detect and confirm the presence of type or subtype of disease in people. Diagnostic markers not only confirm the disease

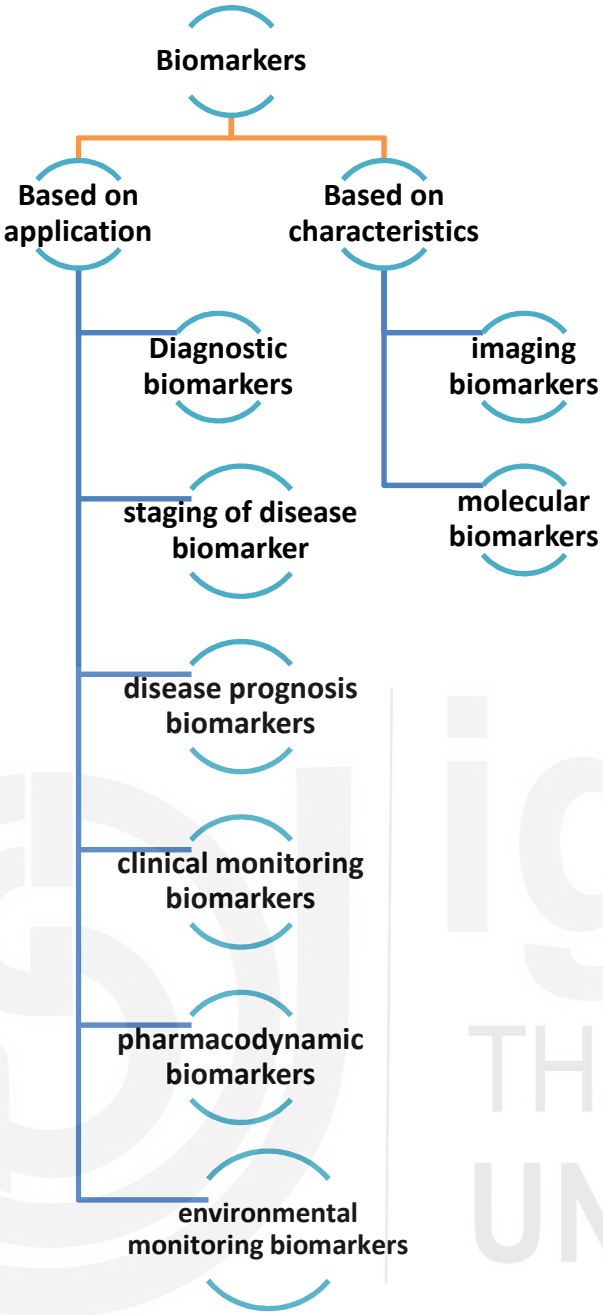

Figure 17.1: Classification of biomarkers

but also help in the classification of biomarkers. The class of diagnostic biomarkers will evolve in the future as we are moving in the era of medicinal precision. For example, earlier the cancer diagnosis depended on organ-based biomarkers but rapidly moved to molecular and imaging-based biomarkers. A biomarker that can detect the stage of a disease can be classified as a **staging of disease biomarker**. A **clinical monitoring biomarker** can be classified as a biomarker that is measured periodically to check the status of the disease or medical condition after exposure to a medical product or biological agent. For example: if a patient with high blood pressure is treated with medicines, the progress of that patient can be monitored using low-density lipoprotein (LDL) cholesterol biomarker. Likewise, a person undergoing HIV treatment can be monitored using clusters of differentiation (CD4) counts biomarker.

The biomarkers that are used to detect recurrence of disease, disease progression in a patient suffering from any disease or a state of medical condition of interest are called **prognostic biomarkers**. The prognostic biomarkers are used in clinical trials to set the trial entry and identify the higher risk population. The risk or susceptibility biomarkers deals with change from healthy state to disease and hence are different from the prognostic biomarkers. The prognostic biomarkers are also different from the predictive biomarkers as latter deals with effect of exposure to xenobiotic. In response to exposure to any medicine or environmental agent when the level of a biomarker changes, it can be known as a **pharmacodynamic biomarker**. The pharmacodynamic biomarkers are also called response biomarkers and are used in both early therapeutic development and clinical practice. For example, if a person with hypertension disease takes therapy and there is no reduction in blood pressure then it is better to give up that invention and pursue another development. Similarly, pursuing a candidate drug for any condition that does not change the key parameter of that biomarker in phase 1 trial is not worth it.

Biomarkers used in environmental monitoring and assessment to identify the exposure to xenobiotics and their effects are called **environmental monitoring biomarkers**. There are various environmental monitoring biomarkers that have been used or are under development and all of them have their strengths and weaknesses. The selection of a biomarker for a particular environmental monitoring program is done on the basis of five properties including ecological relevance, chemical specificity, ability to detect different types of chemicals and mixtures, ability to give early warning signs, and current status in environmental monitoring. Various countries perform environmental monitoring using biomarkers to detect early warning signs and to protect the ecosystem from the harmful effects of chemicals. The importance, future prospects and other important parameters of environmental monitoring biomarkers will be studied in detail in the later sections of this unit.

17.5 APPLICATIONS OF BIOMARKERS

Biomarkers have vast application areas including disease diagnosis, clinical monitoring of the patient (disease management), disease prognostic (clinical trials), therapeutic development, anti-doping test of athletes, environmental monitoring etc.

17.5.1 Disease Diagnosis

Disease diagnosis is the first challenge faced by doctors in the treatment of a disease. An accurate, fast and low-cost diagnosis of disease can help the medical industry to better the medical facilities. An early diagnosis of some of the deadly diseases can save the life of patients. A disease can be diagnosed either using a single biomarker or a set of biomarkers. For

example, diagnosis of typhoid can be done by performing a Widal test, which depends on O and H antigens. Similarly, Malaria disease can be diagnosed using various biomarkers including histidine-rich protein II (HRP II) and plasmodial lactate dehydrogenase. The early biomarkers for COVID-19 disease should include high CRP, high LDH, and high D-dimer. Diagnosis of chronic kidney disease can be done using a set of metabolites biomarkers including amino acids (valine alanine, glycine, etc.), glycolysis metabolites (glucose, lactate), tricarboxylic acid (succinate, fumarate). It was found that the development of chronic kidney disease was closely related to the dysfunction of the lipids, carbohydrates, and amino acids metabolism. This relation between metabolomics and the development of chronic kidney disease is used to diagnose the disease and in therapeutic development. Thus, we can conclude that a disease can be diagnosed by using various biomarkers or a set of them and the selection of biomarkers for disease diagnosis should be based on accuracy, fast, cost, and availability. The research to find novel biomarkers will go on to match the characteristics of an ideal biomarker.

Table 17.2: Diseases and biomarkers to diagnose these diseases.

S.No.	Disease	Biomarkers for disease diagnosis
1	Typhoid	O and H antigens
2	Malaria	histidine-rich protein II (HRP II) and plasmodial lactate dehydrogenase
3	COVID-19	high CRP, high LDH, and high D-dimer
4	Chronic kidney disease	Metabolites (amino acids, lipids, carbohydrates)
5	Alzheimer's Disease	nerve growth factor precursor protein, MRI scanning

17.5.2 Clinical Monitoring of Patient

Clinical monitoring of patients or disease management is very crucial during the treatment of any disease. This helps doctors to identify the severity of the disease and to change the medicines or therapy accordingly. For example, when dengue is treated platelets count is monitored at a regular interval. Similarly, low-density lipoprotein (LDL) cholesterol and CD4 counts are monitored during the treatment of high blood pressure and cancer treatment respectively. Monitoring of these biomarkers helps doctors in the decision-making and efficiency of drugs or therapy. The safety of human research participants can also be ensured using these monitoring biomarkers. For example, the safety of a drug with possible liver toxicity can be monitored by performing a liver function test at regular intervals.

Table 17.3: Some clinical monitoring biomarkers

S.No.	Disease	Monitoring biomarkers
1	High blood pressure	LDL-cholesterol
2	Dengue	Platelets count
3	Cancer	CD4 counts

17.5.3 Disease Prognosis

One of the important applications of biomarkers is in clinical trials to filter the trial entry and to point out the high-risk populations. Biomarkers are important to identify the risk of an event in a person so that they can be excluded from the clinical trials. Trials performed in this way are more effective and safer for humans under trials. Biomarkers are also used to identify the chances of recurrence of a particular disease and to check on the progression of a disease. This information can be very useful in determining how many days the patient will stay in the hospital or intensive care unit and thus helps in hospital patient management.

17.5.4 Therapeutic Development

Biomarkers particularly pharmacodynamic/response biomarkers help in early therapeutic development. For example, if we are developing a drug for dengue disease and we find out that after consumption of the drug there is no improvement in the number of platelets count then it is better to give up that development. So, in this case, platelets biomarker helped in developing the therapy or drug for dengue disease. Likewise, various biomarkers are used in the medical industry for the development of drugs or therapy of diseases.

Table 3: Biomarkers used in the therapeutic development of a disease

S. No.	Disease	Biomarkers for therapeutic development
1	Migraine	CGRP (calcitonin gene-related peptide) concentration
2	Chronic back pain	Nerve growth factor
3	Dengue	Platelets count
4	Inflammatory pain	TRPV (transient receptor potential cation channel subfamily V) expression

17.5.5 Anti-doping Tests of Athletes

The key challenge to sports authorities is to identify and detect the use of prohibited substances by athletes with a degree of certainty and accuracy. This detection can be done either by direct detection of prohibited substances or detection of their metabolites. Biomarkers are used in doping tests of athletes to detect prohibited substances with good accuracy. The biomarker of doping can be defined as the biological variable that indicates the effect of a

prohibited substance with a high degree of certainty. For example, primary biomarkers used in anti-doping tests may include hemoglobin concentration and the ratio of urinary testosterone to epitestosterone (T/E). the secondary doping biomarkers can not confirm the doping alone because of the low specificity of these biomarkers but can be supportive evidence. An example of secondary doping biomarker would be the immature reticulocyte fraction, which can provide evidence of altered erythropoiesis doping.

17.5.6 Environmental Monitoring

Biomarkers are also used in biomonitoring of environmental change due to xenobiotics. These uses and scope of biomarkers in environmental monitoring are discussed in section 6 of this unit in detail.

Check Your Progress 2

1. Discuss the classification of biomarkers in detail.

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2. Explain with examples the role of biomarkers in clinical monitoring of patients.

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17.6 BIOMARKERS IN ENVIRONMENTAL MONITORING

The environmental monitoring programs in the early phase consisted of only the chemical and physical monitoring of aquatic life in the coastal areas. With developments in the area of biomarkers, these programs were incorporated with biological monitoring to obtain the warning signals of ecological toxicity. The chemical analysis of aquatic water included oxygen concentration, heavy metals, and sediment concentrations. This analysis gave useful information regarding the extent of pollutants in water but did not tell about the effect of these pollutants on aquatic life. Early biomonitoring of the effect of insecticides, pesticides, and antifouling agents on biota was started in the 1960s when it was the need of the hour to focus on effects monitoring rather than contaminant monitoring. Till the 1980s there were a large number

of biomarkers suggested for biomonitoring of environmental change but there was disagreement between the scientists regarding the reliability and method. A conference was held as the Oslo conference to validate the methods and biomarkers and it was not surprising that some methods detected the contamination with high reliability and some were unable to detect the contamination. In the last decade, various biomarkers have been developed by researchers with the aim to achieve more sensitivity, reliability, and the ability to detect new effects on biota.

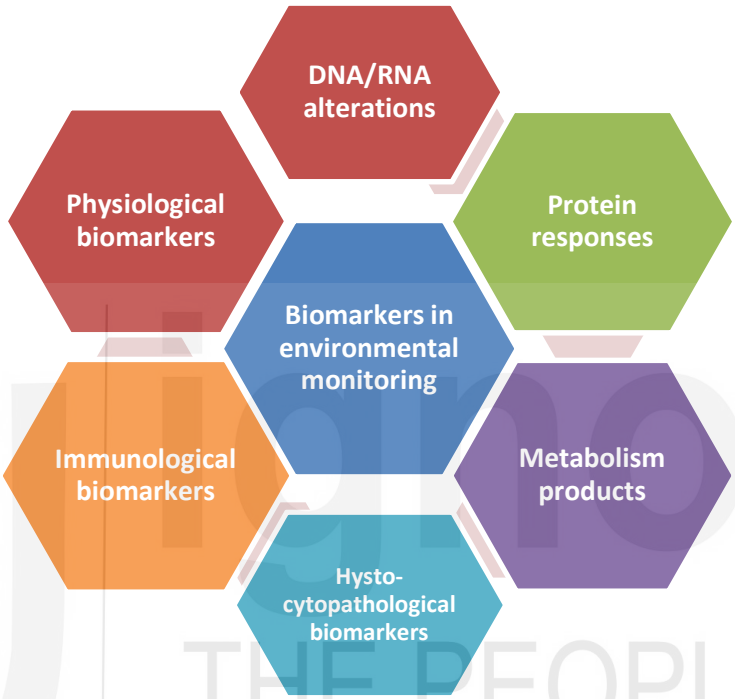

Figure 17.2: Biomarkers used in environmental monitoring

Some of the major biomarkers used in various environmental monitoring programs are discussed below:

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17.6.1 DNA-RNA Alterations

Some techniques, like mRNA fingerprinting, are used to identify the variability in gene expression for the entire genome and these techniques can also be applied to validate the presence of toxins in the field of environmental toxicology. The mRNA fingerprinting technology is based on the amplification and resolution of the 3' terminal part of the mRNA on a DNA sequencing gel where the change in the function of mRNA transcription or degradation rate is observed. The limitations of this process include more labour requirements and time-consuming.

Various techniques are employed to verify the induction of a particular gene caused by a class of contaminants or toxins. For example, the induction of gene expression in Cyprinodon variegatus (sheepshead minnows) exposed in vivo to xenoestrogens (diethylstilbestrol, estradiol, ethinylestradiol) can be measured using a designed molecular-based assay. A characteristic expression pattern of gene alterations caused by these contaminants is observed as a result of this study.

Several contaminants such as dioxins, PAH, etc. are also capable to change the genetic materials. Molecules like benzopyrene bind gradually to the double helix and formed the adducts, these adducts are reactive chemical intermediates that formed the covalent bond with the DNA bases. These adducts are used to detect warning signals for polluting events. The alteration in genes caused by genotoxicants can be very harmful because these contaminants can deform future generations. These alterations can be identified using the enzyme-linked immunosorbent assay (ELISA) method and the radiochemical technique (32P-post labelling method).

The secondary alterations are easy to measure because these are caused by adducts by fractures of the double helix of DNA. These alterations can be detected as a warning signal of environmental contamination. Generally, the secondary alterations of DNA are reversible in nature but sometimes the alterations are so significant that they can not be reversed back and this situation is called chromosomal aberrations. the tests including Sister Chromatid Exchange, chromosomal analysis, and micronucleus test can be used to evaluate biomarkers for these DNA alterations. The verification of alteration up to mutation level can be done using the oncogene activation

method.

17.6.2 Protein Responses

Protein responses are the responses of an organism when the activity of the protein is induced or inhibited by several classes of contaminants. This group of biomarkers contains the protective or adaptive detoxification mechanism of the chemical compounds and heavy metals. These biomarkers are very specific and identified the presence of a particular type of contaminant. Protein response biomarkers associated with an incident of inhibition of the enzymatic activities for example organophosphorus insecticide inhibit the blood or brain esterases. The induction of plasma protein and stress proteins are the other general biomarkers.

Metallothionein protein is another example of protein responses that are identified in approximately 50 species of crustaceans and molluscs. The function of Metallothionein is the process of metal detoxification and preservation of homoeostasis. Metallothionein is a low molecular weight but high cysteine content protein. Metallothionein has a high attraction for some metals like Cu, Ag, Cd, Zn, and Hg. Metallothionein can be detected using spectrophotometry, polarography, and radioimmunological analysis techniques. The concentrations of metallothionein in molluscs can correlate with the environmental contamination of Cu and Cd metals.

17.6.3 Metabolism Products

The metabolism of porphyrin is affected by some contaminants such as PCB, lead, and HCB in the liver and other parts of animals. As a result, there is an increase in the concentrations of intermediate products of the metabolism of porphyrins and this can act as a biomarker for some classes of contaminants. In such cases, biomarkers can be represented by the high concentration of metabolics such as uroporphyrins, protoporphyrins, and coproporphyrins in the liver. Earlier, the measurement of these porphyrins is carried out in a destructive way (in the liver) but the current research has helped us in establishing the non-destructive methods of measurement (in the excreta and biological fluids such as the blood of animals. Some scientists have established non-destructive methods for determining porphyrins in the birds and sea lions contaminated with PCBs.

17.6.4 Hysto-cytopathological Biomarkers

In particular organs, histopathological alterations can be caused by various polluting compounds. These alterations are easy to measure because they affect the structure of tissues, cells, and organs, or individual organisms. for example, the placenta of human is suggested as a good dual biomarker for monitoring contamination caused by trace elements. The maternal health of a pregnant lady can be assessed by monitoring the concentration of Pb in the placenta as a useful indicator of Pb exposure. Some scientists related the Hg concentration in the blood of fetal/maternal with eating fish in large

quantities. Thus, measuring the Hg in the blood of maternal can be a biomarker for Hg contamination in aquatic ecology. The development of male sexual characteristics in females is called Imposex. The biomarker for imposex is attributed to exposure to tributyl tin (TBT). The van deferens sequence index (VDSI) is one of the most important indices for monitoring environmental contamination caused by TBT. The degree of imposex in animals can be quantified by the sequence of formation of VDSI and the penis in TBT-exposed animals. The imposex in the population can be quantified using an index namely the relative penis length index (RPLI). This index is best suitable for the areas of low TBT contamination. Lysosomes present in the digestive system are the main sites of toxic metals and organic pollutants. These pollutants can damage and destabilize the membranes of the lysosomes as a response to stress.

17.6.5 Immunological Biomarkers

Xenobiotics act as an immune moderator and give multidimensional responses to the immune system of aquatic animals. These biomarkers can detect the effects at very low concentrations of chemical concentrations. Other advantages of immunological biomarkers are that these can provide the link between the toxic contaminants and diseases spread in fishes and aquatic animals. However, there are also some limitations like these biomarkers have low specificity and it is difficult to obtain reliable results; xenobiotics contaminating ecology must be already known, etc. some scientists have used cellular and humoral immunological responses of earthworms to test for the contamination caused by carbaryl, PCBs, and 2.4-dichloro phenoxyacetic acid. The non-specific immunity was also studied in invertebrates like earthworms and phagocytosis decreased dramatically in earthworms exposed to soil contaminants. The immunological biomarkers can be classified into three categories based on the immune system like tissues and cells involved, based on the mechanism involved, and based on the efficacy of the response in terms of disease susceptibility.

17.6.6 Physiological Biomarkers

Physiological biomarkers have not been studied on a large scale because these biomarkers require continuous measurements of the speed of the various processes involved. The activities studied in the physiological biomarker's field include the measurements of breathing, cardiac activity, growth and energy metabolism of species. The measurement of the behaviour of species like fish after exposure to contaminants is also studied in physiological biomarkers. The blood parameters including protein concentration and coagulation time are also valid to monitor toxic environments in aquatic life. The scope for growth is also one of the indexes of physiological integrity and its measurement indicates the energetic state of the organism which is highly employed in toxicological studies. Monitoring growth in organisms is one of the best ways of measurement of the physiological responses to man-induced environmental contaminations.

These physiological biomarkers can be converted into measurements of energy flow. The scope for growth depends on the various biochemical or physiological processes such as speed of food digestion, the efficiency of food consumption and nutrient absorption, and decrease or increase in the breathing speed of organisms. A high value of scope for growth value for animals is indicative of environmental contamination.

17.6.7 Behavioural Biomarkers

Environmental contamination can also be detected using the changes in the behaviour of organisms under different classes of contaminants. These types of biomarkers can not be used as an early warning signal but can be used to observe responses at a high level of contamination. Different types of measurements such as sensory measurements (i.e. chemotaxis, phototaxis, temperature preferences, larval settlement, tactile inhibition), motor activities, rhythmic activities, and interindividual responses (i.e migration, predation vulnerability, aggression, etc.). The parameters like distance covered by animals, movement frequency, and mean speed measurement can be done with the help of cameras and video recording.

Check Your Progress 3

1. DNA/RNA alterations can be used as a potential biomarker for environmental contamination. Comment.

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2. Write a short note on behavioural biomarkers in environmental monitoring.

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17.7 FUTURE OF BIOMARKERS

The induction of DNA/RNA alterations and protein response biomarkers in aquatic animals as a biomarker for xenobiotic contaminations have been extensively and with reliability in many studies. The developments in the area of capacity to store, organize and compute a large amount of information will improve the biomarker's prospects. The applications of these widespread biomarkers will increase tremendously in the future because of enhanced commercial availability of biotechnological products particularly gene probes and antibodies and specific adducts. In the future, these biomarkers will be

rediscovered with improved reliability, specificity and prognostic capabilities.

17.8 IMPORTANCE OF BIOMARKERS

Biomarkers play important role in detecting early warning signals for xenobiotics stress and these signals are useful to save ourselves from hazardous effects. Another importance of biomarkers is that biomarkers can indicate biological effects while chemical-based detectors can not do so. Different classes of biomarkers fulfill different purposes in the fields of environmental monitoring and medicinal chemistry. Biomarkers can also detect the overall toxicities caused by the mixture of contaminants. Also, a set of biomarkers can be used to verify or confirm the state of stress caused by a particular contaminant. Another advantage of biomarkers is that they are more economical than many chemical analysis of chemical contaminants. For example, chemical analysis of contaminant dioxin is very expensive and the solution to this problem is to analyze it through biomarkers.

17.9 KEYWORDS

Biomarkers, environmental monitoring, contaminants, DNA/RNA alterations, protein responses, xenobiotics.

17.10 REFERENCES/SUGGESTED FURTHER READINGS

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17.11 ANSWERS TO CHECK YOUR PROGRESS

Answers to Check Your Progress 1

1. Any measurable early warning indicator of any biological process, pathogenic process, or xenobiotic exposure can be termed a biomarker. The use of the term biomarker is restricted to the response or warning signal for the biological, physiological, or molecular change in the organism (tissues, body fluids, organs) level. The term bioindicator is used for changes occurring at the population/ecological level.

Answer to Check Your Progress 2

1. See section 4 for detailed classification of biomarkers.
2. See section 5.2 for application of biomarkers in clinical monitoring.

Answers to Check Your Progress 3

1. See section 6.1
2. See section 6.7