**Prof. Dr. Reyam Naji Ajmi Lec 3**

**Bioindicators for ecosystem management with special reference to freshwater systems**

Bioindicators for ecosystem management provide information on the occurrence of ecological processes and structures. Furthermore, bioindicators provide information on the ecosystem condition by comparing the ecosystem with a reference level of good ecological functioning and on cause-effect relationships within an ecosystem. The bioindicators are developed in four steps in Table below :

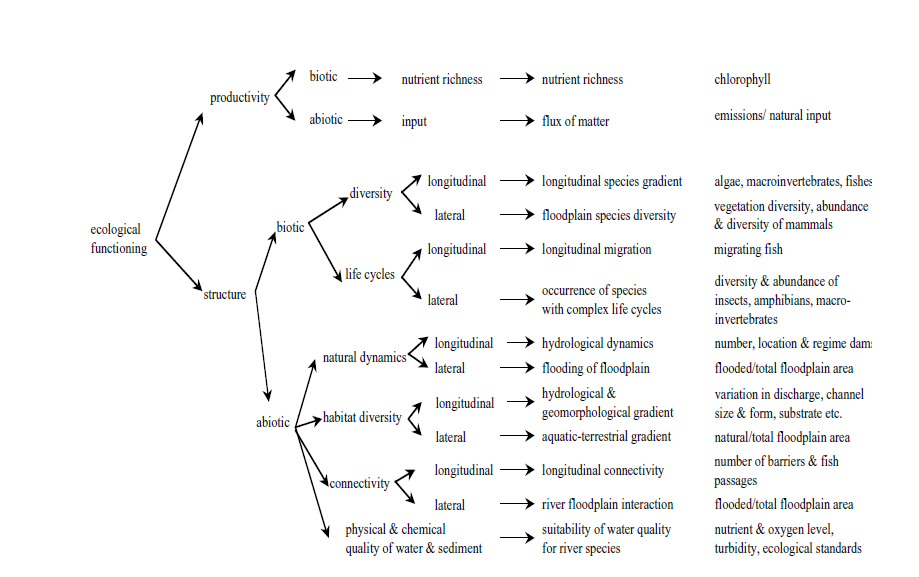
In the first step ecosystem functioning is described on the basis of ecological theories and concepts. For the river ecosystem the theoretical concepts reviewed are zonation, river continuum, stream hydraulics, resource spiralling, serial discontinuity, flood pulse, riverine productivity and catchment hierarchy. For the shallow lake theories on the eutrophication and rehabilitation of lakes are described.

In the second step the dominant processes and structures of ecosystem functioning are defined. The river ecosystem is steered by abiotic processes, leading to longitudinal and lateral fluxes of matter, which in turn causes a spatial distribution of species. In a shallow lake the predominant effect of increased nutrient loads into lakes is eutrophication, which involves a cascade of direct and indirect effects. This cascade of effects can lead to one of the two equilibrium states of shallow lakes; a turbid, phytoplankton dominated lake in a meso- or eutrophic state . The other equilibrium state is a clear and macrophyte dominated lake, which is in a meso- or oligotrophic state. The balance between these two states depends on a bottom up force determined by producers and a top down force determined by consumers.

In the third step bioindicators are selected, which provide information on ecosystem productivity and structure. The algal biomass is selected as indicator for river and lake productivity. For ecosystem structure, the indicators for river and lake differ. For the river ecosystem the bioindicators describe the diversity and occurrence of life cycles along the longitudinal and lateral dimension. For the lake ecosystem two bioindicators are selected that are characteristic for the switch between the two equilibrium states between the area and biomass of macrophytes, indicating that turbidity has reduced to the extent that macrophytes can grow .

In the fourth step abiotic steering factors and relating human pressures are defined to describe cause–effect relationships. For the river natural dynamics, habitat diversity, connectivity and the water quality are described. For the lake the preconditions for the two forces are selected as indicators: nutrient loading for the bottom-up force productivity and habitat area for predatory.

Finally, reference levels for the bioindicator are discussed in order to assess ecosystem condition. A number of references for ecosystem assessment are presently used. The first types of references are based on an “undisturbed” river or lake having authentic hydrological, geomorphological and ecological characteristics comparable to the ecosystem, which is to be assessed. The second type of references relate to a historical analysis of river characteristics in a pre-”disturbance” phase (natural background water quality, species occurrence, hydro-geomorphological characteristics. The third type are effect reference levels based on the risk of ecological impact. The recognition of the irreversibility of human impact increasingly attracts attention to the return of ecosystem processes as the starting point for ecological assessment (such as flood frequency, sedimentation patterns, succession). Finally, references can be based on policy goals or a reference year for policy evaluation. Which of the abovementioned reference levels is selected for the indicators depends on the goal of the assessment (effectiveness of policy, assessment of ecosystem condition) and the availability of (data on) the reference levels .



**Geostatistic fundamentals of bioindicator**

Like many other spatially differentiated phenomena the components of vegetation and fauna of a study area exhibit such a variability that only a systematic statistical analysis prior to active monitoring activities or following the tentative steps of passive monitoring approaches can ensure the representativeness of data in general and the validity of areal extrapolation procedures based on primarily puctiform measurement data. In this context the term “representative” firstly means reproducing adequately the properties of sets of phenomena in terms of characteristic frequency distributions, and secondly it relates to specific spatial patterns. The latter aspect merits particular attention, when complex entities such as ecosystems are considered which are not discrete independent and ambiguously identifiable objects, as ensues from their epistemological characterization; consequently the habitual statistical procedures must be supplemented by geostatistical analyses. The specific problems relating to areal data like mapping units on thematic maps, e.g. soil associations or ecosystem types, “concern (1 the arbitrariness involved in defining a complex geographical individual, (2) the effects of variation in size and shape of the individual areal units, (3) the nature and measurement of location.

**Geostatistical measures of representativeness**

Difficulties encountered in separating individual areal units from a continuum like soil or vegetation cover are most frequently, and at least partially, overcome by the selection of grid squares as the basic units, geographical characteristics being averaged out for each grid square. Since grid squares are all of the same shape and size their use eliminates variability in these properties and thus solves the second problem. The most commendable solution of the third problem is to make relative location as measured by spatial contiguity the dominant variable of analysis. It can be accomplished by means of geographical diversity analyses or regionalization procedures based on comprehensive data matrices whose elements are derived from the digital evaluation of ecology or ecotope-related topographic, geological, hydrological, geobotanical, etc base maps.

**Prof. Dr. Reyam Naji Ajmi Lec 4**

***In Silico* Docking Techniques:**

*In silico* studies mean a computer aided discovered by using computational methods , most of the natural compounds were discovered by a trial, coincidence and error method. This investigation provides value to the mechanism of action and experimental finding and makes cost-effective decisions before expensive synthesis started that helps compounds discovered and optimized using *in silico* methods . These techniques describe a real method for setting up docking replications, covering analysis and restore of the gotten docking poses possible pitfalls in the docking, discussed and suggestions provided to determine commonly occurring problems. Strategies studies have the highest success rate by the algorithm of a preferred tool for giving a computational prediction of all compound and activity .

Mostly steps are used *In Silico* studies following , Firstly "Target Determination" the known 3D structure of a target initial step in identification target , this usually concluded either by 3dimensional (3D) drawing to detect binding site and analyze the binding energy . Also Docking algorithm Molecular happened when known the target structure, docking molecular consider a preferred method to examine and how natural compound bands with a target . Docking can be considered a most probably heavily to use the tool in computational studies and most correct method to predicate whether a particular compound will indicate a good activity against a target .

**Study of Computational:**

Databases and Programs *In silico* were used for a different purpose:

A- PubChem website "https://pubchem.ncbi.nlm.nih.gov/" for determined the chemical structure of compounds.

B- Swiss Model server "http://swissmodel.expasy.org/" for building the model (Marco *et al.,* 2014).

C- Swiss dock "http://www.swissdock" for purposes molecular docking (Hetal *et al.,* 2013).

D- Chemicalize server "http://www.chemicalize.org/" for dynamic of natural compounds.

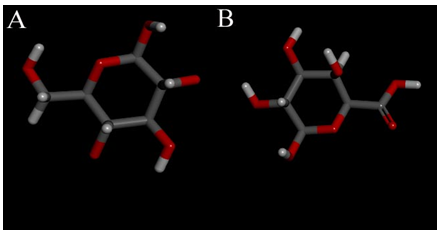
E- Discovery Studio software V.2.5: For bioinformatics purposes (Tsai *et al*., 2009).

It was the first step using waste plants for treating radioactive pollution, so the first step process was done by Discovery Studio 4.1v software; all molecules are prepared for Molecular Docking process in next level of *In silico* studies. Looking for a natural compound target among several characterized radioactive radon (Rn) and Cesium Cs . PubChem website was used to get essential chemistry information about the radioactive elements. The banana and orange peel consist of a complex of many organic compounds particularly pectin, lignin, reducing and non-reducing sugar and other, these organic compounds consist of 43% of the dried peel of banana and orange . All the organic compounds and radioactive Radon (Rn) and Cesium Cs sketched into 2D and created 3D structure and applied the force field to minimize the energy as showed in Figure (3-1a, 3-1b, 3-1c and 3-1d ), all the preparation process was done by Discovery Studio 4.1v software, all molecules are prepared for Molecular Docking process in next level of *In silico* studies. All compounds (lignin, pectin, and sugar) and target radioactive radon (Rn) were subjected to the Docking a logarithm by Swiss Dock Server to estimate the binding affinity, total binding energy and ΔG were used as the main criteria for describing the successful compound. Automatic calculation of molecular docking process was done and the final results of molecular docking for all compounds shown in Table below :

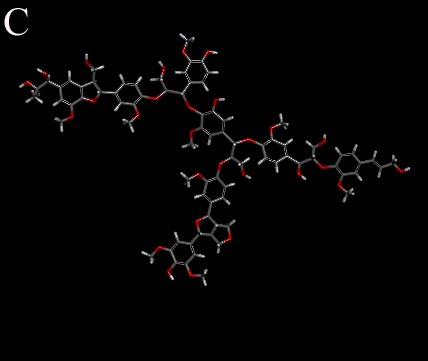
Molecular Docking for All Compounds

|  |  |  |  |
| --- | --- | --- | --- |
| **Molecule** | **Total binding Energy**  **(Kcal/mol)%** | **Estimated ΔG of Rn (kcal/mol)%** | **Estimated ΔG of Cs (kcal/mol)%** |
| Glucose | -12.81 | -7.54 | -6.32 |
| Pectin | -45.26 | -8.05 | -6.9 |
| Lignin | -18.06 | -6.36 | -4.78 |

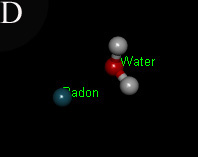
**Fig (3) : 2D A-Glucose Molecule , B- Pectin Molecule Structure to apply the force field to minimize the energy**

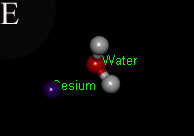
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A-Glucose Molecule , B- Pectin Molecule



C- Lignin Molecule





D- Radioactive Radon & Water Molecule, E- Radioactive Cesium & Water molecule

**Fig (3-1c,d,e): 3D C- Lignin Molecule, D- Radioactive Radon & Water Molecule , E- Radioactive Cesium & Water molecule Structure to apply the force field to minimize the energy**

**Prof. Dr. Reyam Naji Ajmi Lec 5**

**Environmental forensic in ecosystem**

Science is a developing at every moment as a new relatively activity especially in the environmental field , can defined a strong method mostly a new technique driven an aquatic ecosystem within different areas and surrounding it by survey after any incidents occur , that involve any change in morphology , mineralogy, chemistry, geophysics , biology and the waste illegally. Environmental forensics is defined as the systematic and scientific evaluation of physical, chemical and historical information for the purpose of developing defensible scientific and legal conclusions regarding the source or age of a contaminant release into the environment as a good indicator , From 1980 forensics in metals evolved as a result of the potential liability and have enacted similar laws requiring the same environmental tools provided by environmental forensic .Environmental forensics has its roots in metals as the use of sediment, water, soil ,rocks and other geological materials in legal investigations has given rise to a range of forensic geosciences , in which a wide range of scientific data from such fields as geochemistry, mineralogy, stratigraphy, sedimentology, soil science, geomorphology ,hydrology, and geophysics are brought together to address an environmental issue associated with civil and criminal legal investigations.

The most frequently addressed problems in environmental forensics within riverine environments is to define the source and relative contributions of contaminants to a river from human activities in general, and mining, milling, and refining operations in particular. From a legal perspective, source identification is a critical component of many by identifying contaminant source is essential to site mitigation, as well as human and environmental health, because remediation strategies are likely to fail if the source of contaminant influx are not identified and addressed. Environmental forensics is increasingly relying on the use of geochemical mapping and tracing methods to identify the sources of contaminants to these deposits, and to assess the dispersal pathways of contaminants in riverine environments .Geochemical mapping is defined for sediments in river systems as a unique set of physical or chemical properties of the source material that can be used to distinguish it from other sediments within the basin and whose traits are preserved including sediment color ,grain size , particle mineralogy , mineral magnetics , surface morphology and composition , and chemical composition as defined by bulk elemental concentrations, concentration ratios, chemical species, and isotopes .In track the movement and cycling of the material through the drainage system used for several different purposes in riverine environments, including: the identification of contaminant provenance for sediment sampled at a given location, an analysis of the contaminant transport pathways and rates and an assessment of the biogeochemical cycling of a substance within the ecosystem .

**Genotoxicity due to metals stress on plant**

Minerals when entering successfully enters the nucleus and binds to it, they may cause promutagenic damage which includes intra- and inter-molecular cross linkage of DNA and proteins, DNA base modifications, rearrangements, DNA strand breakage and depuration, these damaging effects happen due to oxidative species generated by metal toxicity. It produces promutagenic adduct 8-0xoG (7,8-dihydro8-oxoguanine) that miss pairs with adenine in the absence of DNA repair and leads to trans version of C to T .Forensic sediment and water analysis and extraction of large quality and quantity of DNA often a limiting factor in genetic analysis of bacteria and plant traits important after events to obtain a genomic DNA from thousands of individual lines. Bacteria's DNA in sediment and water analysis provides additional evidence such as plant, and human DNA within unknown forensic water and sediment samples can be compared to that of reference samples Application of DNA as evidence in environmental include Prevention vs. Reaction, Catastrophes and Wars, ID remains of victims (either civilian or soldiers), Holocaust and Mass graves, Military and International Forensics, Illegal waste, Terrorism. The search for weapons of mass destruction , Stored weapons , Genetic profiling of plants when other forms of evidence more commonly non found. More considerations of DNA extraction and achieve reproducible, informative results in plants and bacteria forensic analyses, must be closely monitored contamination to obtain a representative DNA extract of sample under analysis, in addition to lab contamination events at a scene which occur following may also introduce contamination as a result of sample plant ,water and sediment transfer effects and therefore must be taken into consideration several successful tests by researchers to ensure the time lapse between the events and collection of reference samples must be accounted for since temporal and seasonal variation ,the quality of DNA from each line must be consistent from sample to sample to allow equal pooling of DNA . Many high throughput methods to isolate DNA from plant tissues and bacteria are available; however, these methods produce either insufficient amounts or inconsistent quality of DNA. PCR systems are gradually more used in medical and biological studies as they allow immediate amplification of numerous DNA fragments within one reaction. This capacity to decrease the number of reactions wanted to test a sample for diverse targets helps money and saving time and makes multiplex systems useful chiefly when large sample numbers have to be screened. So, multiplex PCR is commonly used to investigate trophic interactions , examining population genetics and parentage assignment , community assessment and for molecular species identification. In case of PCR, the organism is identified depending on a unique gene present in the organism. It takes set in three steps: denaturation, annealing and extension: each step is performed at an optimum temperature ,the denaturation must be temperature higher than the melting point of the primers, but must not be too high as to cause loss of activity to the DNA polymerase. The annealing temperature is dependent on the temperature of the elongation step must be optimum for the polymerase activity and the properties of the primers. The duration of the elongation step must be adjusted to the length of the target sequence. The characterization method chosen was RAPD-PCR, which has been successfully applied to studies with bacteria and plant, and The RAPD technique provides a simple, fast and a comparatively low-cost marker system which has gained wide acceptance, world-wide .

**Environmental Forensic in aquatic plant**

Aquatic plants are accumulated metals ,it absorbs from high metallic materials that are due to the explosive materials as the simplest example or residues of industrial materials that violate the World Health Organization, such as hexachlorine, mercury and lead from factories and factories to places for salons and hair dyes in violation of the law.

The responses of plants to high metals content of soil, sediment, and water vary with species, for instance, some plant species can be injured by the increased metal content in their environment, and some plant species indicators a tolerate metals, reflecting the external metal can be used as bio indicators or bio monitors for quality assessment in aquatic and terrestrial ecosystems Bioavailability and bioaccumulation metals in aquatic ecosystems are gaining tremendous forensic environmental in significance globally. Several of the submerged, emergent and floating aquatic plants are known to accumulate and bio concentrate. Aquatic plant producing an internal concentration several fold greater than their surroundings, there are many cases that helped track minerals inside the water and sediments following the accumulation of events and the disruption of the water and used as vital evidence for element depends on the type of element, and a number of biotic and abiotic factors , the amount of minerals inside the water and sediments with the effect of their accumulations in aquatic plants due to illegal leaks from the plants or the dumping of bodies into rivers or explosions .The adequate monitoring and control of metals in the in soil, sediments, and Aquatic plant requires processing a large amount of samples to characterize the presence and abundance of heavy metals and to reach significant conclusions. There is many application of plants evidence to forensic cases such as leaf and root plant around the event to identification by noting their size, shape, proportions with experience process of collection of samples depending a simple comparison of such physical properties as color, odor, density and hardness is inadequate for the accurate identification of species, it is possible to label date or the time a tree was damaged by a fire, bumping, explosion and accumulation metals.

**Toxic effect of metal on aquatic plant:**

The various environmental factors that influence the bioavailability fate and partitioning of metals include exposure, amounts of ions, metal specificities affect and decomposition capacity of organisms of subject to the mechanisms allows metals to passively enter the tissues, there are relationships between physical and chemical properties of organic chemicals and physiological responses in organisms between the concentration of a chemical in environmental such as water, sediment, plants, soil, and the concentration of a chemical in an organism .Some minerals are similar in structure, but different when analyzed in water , soil or plant, their accumulation and effect on the environment . Lead is counted among the major elements that contaminate the soil resulting from mining ,smelting and natural weather processes ,once entered occurred changes in permeability and reduction in water content give disturbed mineral nutrition. Plants faced with lead toxicity have their photosynthetic pathways adversely affected as it disrupts ultrastructure of chloroplast and blocks synthesis of essential pigments including chlorophyll and carotenoids in addition to plastoquinone. Moreover, it blocks the Calvin cycle and electron transport chain and also produces shortage of carbon dioxide by closing stomata pores .

Mercury acts as a toxic element for the plants and has no beneficiary effect at all . Mercury is unique as it can exist in many forms such as methyl-Hg, HgS and Hg2+ with ionic form prevailing it is absorbed into the clay, sulfides and organic matter and precipitated as carbonate, hydroxide, sulfide and phosphate. It is converted to methylated form by an anaerobic bacterium , toxicity causes visible symptoms of injuries depending on the area it affects. It may enter through water and bind to water channel proteins and obstruct the flow of water to plants. It can also affect the mitochondrial and chloroplast activity by interfering with electron transport chain and inducing oxidative stress along with membrane biomolecules oxidation.

**Health Risk Assessment:**

The United States Environmental Protection Agency (USEPA) (USEPA, 2006) defines the term "risk" as the potential for adverse effects on ecosystems as a result of exposure to an environmental stressor or a combination of environmental factors, and an environmental potential factor is any physical, chemical, environmental stress factors may have negative effects on certain natural resources including plants and animals as well as the environment in which they interact with them. The expression of risk assessment refers to describing the nature, magnitude or amount of health risks to (population, birds, fish and wildlife) resulting from chemical contaminants or any environmental stress agent that may be present in the environment (Chen ,2020 ;Wei *,et al*;2015).Where it is calculated following:

The potential environmental risk index (RI) is calculated by the equation:

**RI = Tin x CRM / Cio**

**Tin = the concentration of the metal in the sample**

**Cio = level of pollution value of the metal**

**CRM= Certificate references materials**

Factors and degrees of the QA and QC of potential ecological risk factor

|  |  |
| --- | --- |
| Eir value | Grades of ecological risk of metals |
| Eir< 10 | Low risk |
| 10 ≤ Ei < 20 R | Moderate risk |
| 20 ≤ Ei < 30 R | Considerable risk |
| 30 ≤ Eir< 40 | High risk |
| 40 ≤ Eir | Very high risk |

To assess concentration the degree of metals contamination, pollution (Cio < 1), low level of pollution (1 Cio< 2), moderate level of pollution (2 ≤ Cio < 3), strong level of pollution (3 Cio< 5) and very strong level of pollution (Cio 5) (Yang *et al.*,2011).The Cio metals for each sample is defined as follows:

Values of toxicity response agent for elements

|  |  |
| --- | --- |
| **Cio (Toxicity response factor ppm)** | **Elements** |
| 10 | As |
| 30 | Cd |
| 2 | Cr |
| 5 | Cu |
| 4 | Hg |
| 5 | Ni |
| 5 | Pb |
| 1 | Zn |

**Elemental isotopes as a tracer:**

Recent studies have demonstrated elements exhibit stable isotopic abundances that fall within a narrow range of values in geological materials. Thus, these isotopes can serve as a highly sensitivity tracer that allow for the determination of sediment and contaminant provenance even where multiple sources exist, or where elemental concentrations are near background values . Many studies have focused extensively on the use of Pb isotopes as an environmental tracer in a wide range of forensic environmental, including air and aerosols , snow and ice , soils , lacustrine and reservoir deposits , plants, mosses, and tree rings , and other biota .Within riverine environments there is little question that the isotopes of Pb have been most extensively utilized as an isotopic tracer in forensic investigations. The pronounced effectiveness of Pb isotopes as a geochemical tracer is related to several factors, first of, the radiogenic isotopes of Pb can be measured with a high degree of precision and accuracy and early on the analysis was primarily carried out by mass spectrometry, a method that provided precise . Lead has a high affinity for particulate matter, and once deposited, is generally immobile . Moreover, this metal combined with the low relative atomic weight differences among its isotopes, limit the degree to which mass-dependent isotope fractionation by physical, chemical, and biological processes occur .The differences in Pb isotopic ratios between the source materials and the alluvial sediments at any given site can therefore be attributed to its mixing with sediments from other Pb sources . Also mercury once released into the environment, its biogeochemical cycling is highly complex, owing in part to the fact that it possesses a stable gaseous form (Hg0), and may undergo a wide range of redox, phase, and biologically mediated transformations. In fact, methyl mercury, the form predominantly accumulated in biota, is primarily produced by sulfate reducing microbes, until recently, understanding the primary sources and biogeochemical cycling of Hg in a particular system relied on an analysis of total Hg concentrations within the media of interest, its chemical speciation in those media, and/or the application of mass balance and geochemical models. It is now clear, however, that numerous inorganic and organic reactions result in Hg isotopic fractionation, producing large variations in the Hg isotopic composition of natural and anthropogenic materials. These variations in Hg isotopic values may be used to assess Hg sources, pollution histories, and transformations during biogeochemical cycling .

**Forensic assessment of metal indicator:**

The tracer of metal source and transport for assessment is complicated because the origin of the geological materials that we are assessing may have been altered from that found at the source. These alterations involve two primarily processes. The first refers to a set of processes through which certain particles are separated from the total material found at the source on the basis of density*,* particle size and shape,the second process is mixing in which sediment weathered from all of the bedrock and alluvial type areas are co-mingled with contaminated sediment from the sources during transport and deposition. The metal used as a tracer allows for the direct assessment of contaminant provenance along the river, its dispersal rates, and the relative contributions from the identified sources. The metals found in sediments and aquatic plant is derived from multiple natural and anthropogenic sources, and the variations in metal concentrations between the sources may be limited. As a result, metal concentration data may prove to be ineffective as a tracer. To address the problem, investigators have combined the analysis of multiple elements with multivariate statistical methods such as principle component analysis, cluster analysis and discriminate analysis . Approach of forensic tracer to utilize stable isotopic has been recognized since at least the 1960s and is based on the ability to characterize a material on subtle differences in the isotopic abundances of a given element , the concentrations of metals pb and Hg isotopes stable tracer in aquatic plant to evaluate their potential to bioaccumulation metals and related influencing factors in river, level in plants, higher than normal level. relatively higher concentrations in aquatic plants , metal in submerged plants, especially in their stems, seemed to be more closely related to metals in water and sediment than those in floating-leaf plants ,the results indicated a high pollution level for the metals, the study also revealed that the contamination levels are very high at the location where a major drain discharges waste water into the canal and the level of contamination was above the limiting value set by the WHO, 2010 . The analyses presume that the analyzed sediment is a mixture of particles from all of the potential Pb sources in the catchment, and that the relative abundance of the radiogenic Pb isotopes in the sediment reflects the contributions from each source. In addition, it is assumed that the isotopes behave conservatively with respect to physical processes hydraulic sorting that may lead to the partitioning of particles on the basis of size and density into specific sediment logic units :

**Prof. Dr. Reyam Naji Ajmi Lec 6**

**Significant sources of polycyclic aromatic hydrocarbon as a biomarkers**

Biomarkers in environmental assessment a concentrate mainly on relationships between the toxic matter in environment and potential risks of pollutants, in past decades, risk assessment levels of pollutants in environment have considered credible because different pollutants can mutually influence their toxic behaviors. Basic main pollutants and chemicals components have a tendency to stick environment such as Cadmium, Lead, Mercury, Copper, Polycyclic aromatic hydrocarbons (PAHs) and others . Primary contaminants cannot degrade more so they can undergo different reversible changes in belonging depending on the chemical environment . Many elements are necessary for life functions , plants and animals possess different mechanisms for accumulating sufficient amounts of elements from their environment also facilitate the treatment of non-essential minerals .

Retention of metals or any other chemical in environmental pollution and the effects of chemicals on the environment and natural resources have become one of the major global problems , the International Agency for Research (IAR) classifies some PAHs as known, Hematology humans among these are benzo pyrene naphthalene, chrysene, benza nthracene, benzo fluoranthene and benzo fluoranthene

Exposure to PAHs at workplace very expected and commonly assessed by measuring biological response such as ranging from molecular to cellular and physiological responses to behavioral changes, that can be associated with exposure to toxic chemicals or their toxic effects , the measurement of 1-hydroxypyrene in blood has been shown to be a reasonably reliable method for monitoring of PAH exposure in the environment that’s indicating a deviation from the natural state can be detected in the organism. Although most of the biomarkers measured at the molecular level. A successful implementation of biomarkers in environmental monitoring programs requires a good understanding of the mechanisms underlying the responses. Biomarker responses are powerful because they integrate a wide array of environmental, toxicological and ecological factors that control and modulate exposure to, as well as effects of, environmental contaminants. However, these same factors may also complicate interpretation of the significance of the biomarker responses in ways that may not always anticipate. Elements pollution related variables may have an additional impact on the various enzyme systems, and may thus interfere with biomarker responses when experimental conditions are not thoroughly analyzed or controlled. Examples of factors are the organisms health condition such as sex, age, nutritional, metabolic activity, migratory behavior, reproductive, developmental status and population density, as well as factors like season, ambient temperature, heterogeneity of the environmental pollution, etc. Unfortunately, most available toxicity data rarely quantify the potency that confounding factors are likely to exhibit in natural environments. Biomarker responses can be applied in integrated monitoring programs in any polluted consists of a particle and a gaseous phase containing a long range of different substances including polycyclic aromatic hydrocarbons (PAH) and activities comprising both chemical and biological measurements in a variety of environmental media or compartments such as analyses of chemical contamination monitoring or assessment of toxicity using bioassays in biological effect monitoring and determination of the in faunal community structure of ecosystem monitoring, to determine the pollution consisting of four different biomonitoring levels using biomarkers, bioassays, bioindicators and ecological indicators .

**PAH as biomarkers**

More studies face difficulties in establishing correlations between these exposures and effects on human health, biomarkers allows detection and measurement PAH concentrations and quantity of substances monitored depends on the sampled and resources of exposure . Environmental assessment of PAH can be based on one that is representative of ecosystem such as benzopyrene, benzo anthracene, chrysene, benzofluoranthene, dibenzoanthracene, benzoperylene and indenopyrene. After the substance enters the body, the unaltered substance or its metabolites searched for in the urine, blood, feces, or other body fluids and tissues. Since PAH generally present a reduced plasma half-life, hydroxyl metabolites are the most frequent option for investigation.PAH compound generally oxidized enzymes through hydroxylation catalyzed by cytochrome P450 monooxygenase enzymes. The metabolites excreted by the blood, urine and feces, the hydroxpyrin can be used as biomarkers based on their ability to indicate the internal dose received . The majority of these reactions result in detoxification but compounds produced that are highly reactive as electrophilic PAH metabolites, which can form covalent interactions with proteins and nucleic acid, resulting in adducts that can compromise normal cell functioning, triggering a series of harmful effects. Hydroxyl PAH metabolites as biomarkers of internal dose metabolites excreted in the blood provide more appropriate estimates of total ingestion as compared to exposure assessments based on environmental data. The choice of the urinary metabolite should consider the constituent of the most common PAH . Hydroxypyrene is the most widely used metabolite in PAH exposure, since pyrene is one of the most abundant hydrocarbons in all PAH mixtures and has as its principal metabolite 1-hydroxypyrene formed in mammals , thus representing a sensitive biomarker of exposure, recommended by various authors as the most relevant parameter in estimates of individual exposure to PAH .

**Human exposure to PAH**

Environmentally relevant sources of PAH are smoking, fossil fuel combustion, vehicle exhaust, and use of lubricant oils. PAH are used as intermediate substances in the production of plasticizers, pigments, drying agents, and pesticides, but the environment probably receives only small amounts as a direct result of these activities; the most significant emissions are from incomplete combustion of organic materials during industrial processes and other anthropogenic activities . Since PAH are lipophilic substances, therefore, it in human body readily dissolved and transported by cell membrane lipoproteins ,blood and urine In general, they are distributed throughout the body and found in any internal organ or tissue, particularly in lipid rich tissues and the gastrointestinal tract. Experimental animal studies have demonstrated that some PAH cause tumors in case of oral or cutaneous exposure. In food exposure, effects have been found in the reproductive system related to fertility, problems during pregnancy and congenital anomalies, major sources of PAHs are formed during the incomplete burning of coal, oil and gas, garbage or other organic substances like tobacco or charbroiled meat . Due to the numerous PAHs exposure sources, humans can be exposed to PAHs through multiple routes, including breathing polluted air, Environmental Tobacco Smoke (ETS), dietary PAHs intake, dermal absorption through soil, air or particulate deposited on skin .

The exposure of PAHs in human has raised public health concerns. The United States Environmental Protection Agency (USEPA, 2012) has designated 16 PAH compounds as priority pollutants, among PAHs, benzo pyrene has been classified as a probable human and animal carcinogen by the International Agency for Research (IAR, 2008) The exposure of PAHs has been linked to the onset of diabetes mellitus , metabolic syndrome and cardiovascular conditions .

Biomarkers can provide an integrated reflection for exposure through inhalation, food and dermal uptake, and takes into account variation in absorption, metabolism, and elimination by the body. Therefore, the urinary metabolites of these compounds are used as preferred biomarkers to estimate the PAHs exposure.

**A protocol for the use of biomarkers in ecological risk assessment:**

Ecological risk assessment consists of two components assessment of current ecosystem status and prediction of future status. With regard to the former, any measurement that is made must clearly indicate how far the ecosystem under investigation has departed from normal conditions and for what reason. With regard to the predictive component, this necessitates knowledge of how populations comprising the ecosystem will respond to a given pollutant load . This can perhaps be estimated to some extent from laboratory biomarker studies and from databases amassed from investigations in other ecosystems with similar characteristics. Such a database is currently being assembled. A protocol for the use of biomarkers in ecological risk . The approach focused on establishing the risk to key components in the ecosystem, the inference being that if these components are adversely affected, the ecosystem structure and/or function ecological integrity will be at risk. Their strategy is developed and reformulated below. Three phases of investigation are envisaged which constitute a sequence moving towards the acquisition of increasingly precise data :

Phase 1. Identification of ecosystems at risk. This involves the identification of potential pollutants, pathways and fate together with recognition of critical populations and communities in the ecosystem under study. Complex interactions among polluting molecules and the ecosystem should be investigated in an interdisciplinary study using a database evaluation of regional features, analysis of diffusion models, chemical analysis and studies of biotic communities. A selected suite of general exposure biomarkers would also be utilized in an initial screening of a broad range of invertebrates to detect pollutant exposures (the justification for focusing on invertebrate species is discussed below). An estimate of how much biomarker values differ among species for a given concentration of the pollutant in the environment may aid the identification of the species at risk. It is important to add here that the biomarker approach is not a replacement for conventional assessment techniques, but is an important supplementary approach of great ecological relevance. Ecological research methods based on the evaluation of the general state of the population (birth rate, mortality, fertility index, relationship between ages) are indispensable for interpreting links between biochemical and cell changes (biomarkers) and adverse effects on populations and communities.

Phase 2. Identification of critical species and target populations Obviously, it is not feasible to perform comprehensive biomarker studies on all the components of an ecosystem. Identification of the most important populations is therefore necessary. Once the general extent of pollutant exposure have been assessed, more specific effects of pollutant toxicity can be examined using extended suites of exposure and effects biomarkers in a limited range of species occupying different trophic levels and ecological niches; in this way in situ verification of the adverse effects.

Phase 3. Predicting the likely impact of chemical pollutants Prediction of the potential of known amounts of specific pollutants to perturb (or further damage) ecosystems can also be aided by the use of biomarkers. It is proposed here that combined laboratory and field biomarker screening tests be evaluated as a means of establishing a firmer scientific basis for extrapolating from laboratory data to real environments. This might comprise of the following:

1. Selection of a range of invertebrate species from diverse phyla that exhibit different feeding strategies and that are present in the ecosystem in question. Sample populations of these organisms should then be exposed to a range of concentrations of the test chemical in the laboratory.
2. Measurement of a suite of biomarkers (biochemical, physiological and behavioral) to assess responses to and toxicity of the test chemical should then be performed. Biochemical biomarkers should reveal the type of detoxification mechanisms induced by the chemical whilst physiological and behavioral biomarkers will signal exposures resulting in adverse effects at the level of the whole organism (such as altered scope for growth, loss of endogenous behavioral rhythmicity, etc.). They will also permit time relationships between chemical exposure and biomarker responses to be established.
3. Residue analysis of the test organisms should be carried out to relate biochemical biomarker responses in specific tissues to tissue concentrations of the test chemical or its derivatives.
4. If the test chemical has been released into other similar ecosystems and biomarker responses have been measured in situ, then results obtained in the laboratory test can be compared with the database compiled from field tests.
5. Once the test chemical has been evaluated and safe concentrations determined, the biomarker approach offers the possibility of genuine validation of the test procedure.

are incomplete combustion of carbonaceous materials and oil spills was defined by (Blumer ,1976) stated that these sources will give mixtures with quite different relative amounts of un substituted PAH compared to other. Although it is difficult to distinguish between the different PAH sources, some authors have presented valuable results using differences in its distribution. Adeniji *et al*.,(2017)utilized a gas chromatograph-mass spectrometer GC-MS method for measuring both total petroleum hydrocarbons and polycyclic aromatic hydrocarbon in river water samples that were collected after oil spill after the samples are extracted with methylene chloride and hexane, the extracts has been fractionated on silica-gel columns and then injected into a GC-MS operating in the selected-ion-monitoring mode**.** Distinguished between inputs of PAH from petroleum and combustion sources are demonstrated on sediment samples. Selected series of aromatics was quantified by computerized GC-MS to combination parameters give a first-order indication of the PAH source. Pyrene and fluoranthene series appear to be especially sensitive as an indicator for detecting PAH from combustion sources.

**1-2-8: Identifying contaminant sources using spatial and geographical patterns in isotopic tracers:**

Geochemical tracing techniques used a contaminant of interest as a tracer and documented the provenance of pollutants by observing the spatial differences that exist throughout the river scheme contaminant / tracer, the method consists of documenting the concentration of an element within the source materials and comparing it with concentrations observed at multiple locations , including areas accepted to be clean .The sampled media may include water, suspended sediment, or sediments from the channel bed or its floodplain, when the focus is on sources that have provided a long-term, semi-continuous supply of contaminants to the river, channel bed sediments are often the media of choice as they tend to show less temporal variations in concentration through time than is found within the water or suspended sediment (Miller and Orbock Miller.,2007;Wang and Pataki.,2009).The factors can complicate the use of spatial patterns in geochemical tracers to identify a contaminant source. The most significant such as sediment logical differences in the sampled sediments, this corresponds to, Moore *,et al*;(1989) and Yi, *et al*;(2020) showed that within many mine contaminated rivers, metals are more uniformly distributed within the sediments. Thus, analyzing the fine-material may lead to reliability indication of contamination results, in studied (Devesa-Rey,*etal*;2009),have also been used to minimize variations associated with sediment logical differences in the samples when the primary goal is to document spatial variations in the contaminant concentrations for the purpose of source damage identification.

**Prof. Dr. Reyam Naji Ajmi Lec 7**

**Stress and nonspecific resistance**

Stress is the state of a biotic or abiotic system under the conditions of a “force applied’ strain is the response to the stress, i.e. its expression before damage occurs while damage is the result of too high a stress that can no longer be compensated for . Focusing on biological systems, it is indicated for reasons of terminological clarity not to apply the term “stress” to fast readjustments of metabolic fluxes, photosynthetic or transpiration rates induced by fluctuations in the photon flux density, slight changes in temperature, or rapid variations in air humidity. Plants are acclimated, i.e. usually respond flexibly to such steadily occurring normal changes of cell metabolism induced by variable environmental conditions. The same applies to the diurnal fluctuations in metabolic activities, growth patterns, and in cell division and differentiation processes. Besides such fast acclimations, plants can also respond to environmental changes by means of somewhat longer-term adaptations such as modifications of size and thickness of leaves, number and density of stomata, ultrastructure and function of the chloroplasts by raising the levels of photo protecting enzymes and of stress metabolites.

When subjected to a stress, an organism is in a state of strain. As long as the strain is completely reversible, it is said to be elastic; beyond this point or threshold, the strain will be only partially reversible, and the irreversible part is called the permanent set or plastic strain. Unlike elastic strains, plastic strains are not constant for specific stresses, since they may eventually lead to disintegration of the system (organ ,organism, population, biocenosis, ecosystem) affected. Since plastic strains may be dependent on the time exposed to the stress, the time factor must be measured whenever the resistance of biological systems to plastic strains is determined. Thus, elastic resistance is a measure of the system’s ability to prevent reversible or elastic strains physical or chemical changes when exposed to a specific environmental stress, while plastic resistance is a measure of its ability to prevent irreversible or plastic strains and, therefore, injurious physical or chemical changes.

Stress resistance has two main components, namely the innate internal properties of an organism which oppose the production of a strain by a specific stress on the one hand, and the repair system which reverses the strain on the other. Both the elastic and plastic resistances of an organism to a specific stress may be subject to changes depending on its adaptive potential. The adaptation may be either stable over a large number of generations, or unstable, depending on the developmental stage of the organism and the environmental factors to which it has been exposed. This adaptation is important for both elastic and plastic strains. Plastic strains are by definition injurious. Therefore the adaptation leading to increased plastic resistance, i.e., resistance adaptation sense will prevent (or at least reduce) injury by stress which would injure (more intensely) the unadapted organism. Although elastic strains are reversible by removal of the stress and therefore per definitionem noninjurious it must be realized that they may also lead to injury and even death if they are maintained for a long enough period. This may, for example, be due to the inability of the organism successfully to compete with others that undergo less elastic strain when subjected to the same stress (e.g., mesophiles versus psychrophiles at low temperatures). An elastic strain may also eventually injure the organism even in the absence of competition, for example, due to a disturbance of the metabolic balance Thus, a low-temperature stress may simply decrease the rates of all metabolic processes reversibly, but to different degrees. As a consequence, if the stress is maintained for a long enough period, the resultant strain may conceivably lead to an accumulation of toxic substances or to a deficiency of essential intermediates. In either case, a sufficiently long exposure to the stress may injure or kill the organism An adapted organism, by way of contrast, may complete its life cycle, and regenerate in the presence of the stress. It displays capacity adaptation , while resistance adaptation may not permit growth and may merely prevent the plastic strain and therefore the injury until the stress is removed or decreased to a level permitting growth and completion of the life cycle ……………

**Bioindication on the basis of biocoenoses and ecosystems**

The susceptibility of ecosystems to disturbances depends on the structure and size of the system and on the nature of the disturbances or stressors affecting it. Thus, the integrative stress reaction of a biocenosis or an ecosystem may be defined as a measurable alteration of the state of the community-forming organisms and their

life-supporting substrates, which renders the individual, the population, or community more vulnerable to further injurious physical or chemical impacts.

**Reactions of aquatic ecosystems to stress**

Like terrestrial ecosystems, aquatic ecosystems under stress undergo changes in both structure and function. Changes in structure are manifested by modifications of the composition of the various biocoenoses and the related physical and chemical characteristics of the ambient water body; changes in function are reflected in differences in the organic matter production of the system and in the rates of utilization and release of different gases and nutrients.

According to Cairns and Niederlehner (1993) indicative stress reactions of aquatic ecosystems are the following:

1. Community respiration increases

2. Productivity/respiration ratio becomes unbalanced

3. Productivity/biomass ratio increases as energy is diverted from growth and reproduction into acclimation and compensation

4. Importance of auxiliary energy increases, i.e., import becomes necessary

5. Export of primary productivity increases

6. Nutrient turnover rates and losses increase

7. One-way transport increases, while internal cycling decreases

8. Lifespan decreases, turnover of organisms increases

9. Trophic dynamics shifts, food chains shorten, functional diversity declines

10. Efficiency of resources use decreases

11. Condition declines.

**Terrestrial ecosystems as integrative stress indicators**

From a very general point of view stress reactions of a terrestrial ecosystem are analogous to those of an aquatic one. Considering various types of strain in the major compartments soil and biocenosis of such a system in greater detail, however, manifests far-reaching differences which are mainly related to functional aspects. Functions can be measured by estimating a “capacity” of the system or, particularly for carrier functions, its potential for use (Hanssen et al., 1991). For most of the functions a putative maximum value exists; depending on the stress-coping potential or resilience of the compartments affected, over-use or over-exploitation may lead to a decrease in capacity and ultimately to complete exhaustion or decline. With regard to the degree of naturalness of an ecosystem two types of functions can be specified. The first are so-called natural or, more precisely, ecological functions; and what is known as evaluation or valuation assessment of conservation values can be considered a measure of these ecological functions, in particular information and regulation functions. The second group of functions, particularly the carrier, storage and most of the production functions, may be called socio-economic functions. Obviously some functions are transitional in character, since they are bound to agricultural environments; consequently and following van der Maarel (1978), they may be designated as rural functions comprising agricultural production, biotic production (e.g. forestry, production of industrial raw materials), rural carrying functions, and recreation. Soil is one of the principal regulatory compartments of all terrestrial (and benthic ecosystems. In a bioindicative context its susceptibility to disturbances should therefore be defined in terms of essential ecological functions such as regulation (comprising filtering, buffering and transformation processes), site (habitat), and productivity functions. Other functions which may attain importance in the framework of planning-related evaluations, e.g. subsoil as raw material for building purposes or as a substrate for waste deposits, soil as a geohistorical archive, are left out of account Soil sensitivity to chemical impact is a highly variable property which can only be determined with a reasonable amount of practical accuracy when related to the ecological soil functions, past and current pedogenic processes, and agricultural or silvicultural use patterns. Chemical element speciation plays no less a role, since different species exhibit different mobilities in soils and sediments, have different plant availability, and different toxicity for organisms.

This and subsequent similar approaches are static and deterministic. Therefore, approaches are needed which are both dynamic (appropriately reflecting the time evolution of perturbation, sensitivity and adaptation) and statistical (defining probability distributions in order to calculate expected values). Among the existing or emerging formal (or formalizable) methodologies there are a number of suitable candidates for vulnerability analysis and assessment. Exemplary options comprise advanced versions of game and criticality theories, re-analysis of historical records, extreme value statistics and non-linear dynamics, semi-quantitative typologies (e.g. degradation syndromes) and complex indicator approaches among the latter the following merit particular attention following :

● fluxes of energy through, and entropy production of, ecosystems

● fluxes of selected macro and micronutrients such as K, Ca, Mg, P, S and Mn, Fe,

Cu, Zn

● duration of biogeochemical cycles

● biomarkers, e.g. stress proteins, phytoalexines

● changes in biodiversity of vegetation stands and faunal assemblages

● dynamics of selected populations

● changes in competitive behaviour of functionally important species

● modifications of food web structure.

Some of the methodological problems involved in the determination of these and

related integrative indicators are referred to in the following example of a novel multidimensional combination of integrative bioindicators.

**Indicators of ecological integrity derived from a concept of biological self-organization**

Indicator construction is based on the following premise: The greater an ecosystem’s capacity for biological self-organization, the more likely is it for the system to be a reliable source of important ecosystem services on a long-term scale and in the face of unspecific ecological risks or stress situations, respectively.

The indicators were tested by comprehensive field data comprising the carbon, water, and energy budgets of adjacent crop field and beech forest systems which are edaphically and climatically similar but considerably different with regard to the intensity of human interference. In terms of the indicative parameters biomass storage, biologically bound nitrogen and phosphorus, species number, total ecosystem respiration per total biomass (qCO2), total ecosystem assimilation per available nutrients, and transpiration per total evapotranspiration, there are clear differences between the systems. By way of contrast, ecosystem surface temperature and the Rn/K\* ratio (with Rn \_ net radiation and K\* \_ short wave radiation balance) were of limited usefulness for characterizing the two systems such as biomass, intrabiotic nitrogen and phosphorus storage, and species

number which all showed clear differences between the systems. Also exergy storage, i.e., the available work of the system (Jorgensen and Nielsen, 1998), is a suitable integrative indicator of ecosystem development since it expresses the distance from thermodynamic equilibrium and covers therefore both the size of the organized structure and its content of thermodynamic information. (Its determination, however, is faced with practical problems.) Species numbers, or biodiversity indicators derived can only be a preliminary indicator of the capacity of ecological systems to self-organize. It is certain that biological information represents a constraint to self-organization processes, but there is no simple correlation between diversity and the actual organizational level attained. Genetic diversity, however, finds its predominant expression in species richness which provides ecological systems with the ability to adapt to changing environmental conditions. For the maintenance of processes under variable conditions, the most important effect of biological diversity is the provision of functionally redundant processors with varying ecological amplitudes .In the second place metabolic quotients reflecting functional or efficiency aspects are used to define system organization. Transpiration reflects the organizational capacity at the ecosystem level because it has a constructive function in addition to its dissipative character. The beech forest, where transpiration accounts for 63% of the total annual evapotranspiration, can be clearly distinguished from the maize field, with only 34%. In addition, the beech forest gained more biomass per available nutrients and proved able to maintain this biomass due to lower biomass-related respiration.