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COURSE HANDOUT

Subject: Evaluation of the quality of ecosystems
Teaching intended for Master's students
Ecology of natural environments

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Master's title: Ecology of natural environments

Semester: S2

Title of Curriculum Unit: Methodology

Subject title: Evaluation of ecosystem quality

Credits: 5

Coefficients: 3

Teaching objectives

Train students to carry out expert assessments of the functioning and malfunctioning of natural environments, based on ecological diagnoses - Ecological diagnosis - Bioindication, biocenotic indicators, sentinel species - Monitoring networks, physico-chemical analysis of water, etc.).

Recommended prior knowledge: Basic notions of ecology.

Contents:

- Physico-chemical analysis of water and soil

- Ecological indicators: background, definition, purpose, typology, selection and minimum requirements

- Hydrobiological analyses: Standardized Global Biological Index (SGBI), Diatom Biological Index

- Biological Index (IBD), Oligochaete Index for Bio-indication of Fine Sediments (IOBS), Macrophytic River Biological Index (IBMR), fish index.

- Soil quality indicators: nematofauna, mesofauna (springtails and mites),

IBQS, bioaccumulation in snails, soil microflora, bacterial and fungal biomass...
- Air quality indicators: lichens, bryophytes, clover, Petunias..

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Objectives

The training program aims to equip students with in-depth expertise in assessing natural ecosystems through the application of various ecological diagnostic tools and methodologies. These methods include bioindication, which involves using indicator species to evaluate environmental quality. The program also covers biocenotic indicators, enabling students to understand the complex interactions within biological communities.

An important part of the training involves the use of sentinel species, organism's sensitive to environmental variations, to detect and assess changes in ecosystems. Students will learn to design and implement ecological monitoring networks to continuously track the health of natural environments.

The course also includes skills in the physic-chemical analysis of water, enabling students to quantify the key parameters of water, soil and air quality. This includes the measurement of chemical composition, temperature, pH and other physical factors that can influence the biodiversity and dynamics of aquatic ecosystems.

By combining these approaches, students will be able to conduct comprehensive surveys of natural ecosystems, covering both biological aspects and the physic-chemical characteristics of the environments studied. This course aims to equip future professionals with the tools necessary to assess, monitor, and propose measures for the preservation and restoration of natural ecosystems.

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Basic notions in ecology

I. Basic notions in ecology.

I. 1. Ecology: What Is It?

Ecology is the study of the relationships that organisms have with their surroundings and with one another. Ecology shares similarities with many other sciences, such as biology, geography, geology, and climatology, despite being a separate scientific field. It is also strongly associated with ethology, the study of animal behavior, and genetics. Furthermore, the fundamental ideas of natural selection and adaptation in evolution provide the basis of contemporary ecological theory.

Ecologists examine a variety of phenomena, such as how creatures interact, how energy flows through them and how they recycle matter, and how diversely distributed organisms are in relation to their surroundings. Ecology has numerous real-world applications. They include, among other things, the preservation of threatened species.

I.2.The Ecological Classification

Researching every living being and its surroundings would be an enormous task. Generally speaking, structuring the biological world into a nested hierarchy makes ecology easier to understand.

The living world above and beyond the level of the individual organism is the usual focus of ecology. These levels are described as follows and are shown in Figure 1:

*A population: all the individual members of the same species that coexist and communicate in the same space make up a population. For instance, the population of angelfish is made up of all the fish that reside in the same region of the ocean.

*A community:refers to all of the various species' populations that coexist and communicate in the same space. The populations of many fish species, corals, and several other organisms make up the aquatic community that is home to angelfish.

*An ecosystem: consists of all the living things and the nonliving surroundings in a certain location. Abiotic elements including water, minerals, and sunshine are part of the nonliving environment.

*A biome:Anywhere in the globe, a biome is a collection of related ecosystems with a common physical environment. The main forms of plant and climate are used to distinguish between different terrestrial biomes. Deserts and tropical rainforests are two examples of terrestrial biomes. In general, the depth of the water and the distance from shore determine aquatic biomes. The shallow water near the coast (littoral zone) and the deepest water at the bottom of a body of water (benthic zone) are two examples of aquatic biomes.

*The biosphere: Every area of the Earth that supports life, such as all of the land, water, and air, is referred to as the biosphere. The largest biological category, the biosphere, is made up of numerous distinct biomes.

I.3.Types of Ecosystems - Definition, Classification and Examples

I.3.1. Definition: A biological system of immense complexity, an ecosystem is essentially a collection of diverse interactions that occur both within and between living things and their surroundings. As a result, ecosystems are made up of a vast array of intraspecific interactions that take place between members of the same species and interspecific interactions that occur between members of different species. These interactions are based on the various resources that the ecosystem offers to living things as well as the various energy flows that take place within it. You may read more about Intraspecific Relationships.

I.3.2. Types of Ecosystems:

Throughout history, many environmentalists have found it challenging to categorize the many ecosystems on Earth. These days, a broad classification of various ecosystem types enables them to be differentiated according to their surroundings. Consequently, the classification

The natural and artificial ecosystems are the two broad categories of ecosystems found in the natural world. There are a few among the first.

*Earthly ecosystems.

*Water-based ecosystems.

*Ecosystems that are mixed (water-land) and aero-terrestrial (air-land).

*Artificial or non-natural ecosystems created by humans that have been altered

II. Assessing the quality of ecosystems

In particular, environmental monitoring needs to be able to:

1. be aware of the ways in which ecosystems' composition, structure, and function are changing; one such way is through the alteration of biodiversity.

2. be aware of the reasons for, the effects, the mechanisms, and the scope of these changes.

3-recognize the effects of land degradation on biodiversity loss and climate change, and vice versa

4. Determining the relationships between the local population and its biological environment.

5. Recognize and comprehend changes in the quantity and quality of natural resources as well as socioeconomic characteristics:

• Over time: short, medium, and long term

• In space: from the local to the global, via the regional

6. providing relevant information to enable clear decision-making

• broad management of biodiversity

• putting into practice sustainable land use practices in line with changing socioeconomic parameters, such as adjusting pastoral management to ecological capacity

II.1. Function of assessing the quality of ecosystems

1. Monitor and assess the condition of ecosystems and resources natural and ecological processes: deterioration of soil, water, and air; loss of biodiversity; and change (variability) of the climate.

2. Provide practical assistance in the sustainable management of natural resources and land use by suggesting measures like restoration, rehabilitation, and/or re-affectation.

Part I

Physicochemical analysis of water and soil

Chapter1: Physicochemical parameters of water

1. Introduction

The measurement of physicochemical characteristics and the existence or lack of aquatic creatures and microorganisms—indicators of a generally high quality of the water—are the foundations for assessing the quality of surface water. Sediments (sludge), which are a "memory" of the river's past and can include episodes of heavy metal, polychlorinated biphenyl (PCB), polycyclic aromatic hydrocarbon (PAH), and other non-biodegradable organic material pollution, can be analyzed to add to these data. Waterways' ability to self-purify and the extent of their pollution can both be evaluated thanks to these components.

Figure 1: Some instruments for physical-chemical water analysis

2. Physical-chemical water parameters

2.1 The temperature

Temperature of the water is very important, especially when it comes to the solubility of gases and minerals, such oxygen, which is necessary for the aquatic life's equilibrium. The rates of chemical and biological reactions are also significantly impacted by temperature; these rates usually increase two to three times for every 10 degrees Celsius (°C) increase. Aquatic creatures' metabolic activity increases in tandem with this temperature rise.

It should be mentioned that potential hot waste water discharges have an impact on the water's temperature in addition to the outside air temperature. Abrupt temperature fluctuations, frequently over 3°C, can be detrimental to aquatic environments. Therefore, the aquatic environment's thermal stability is essential to preserving the conditions that support life and biodiversity.

2.2. The hydrogen potential pH

The hydrogen potential, or pH, is a unit of measurement used to determine how basic or acidic an aqueous solution—including water—is. The concentration of hydrogen ions (H+) determines it. At 25°C, a pH of 0 indicates a very acidic solution, a pH of 14 indicates a very alkaline solution, and a pH of 7 is neutral.

Water's natural pH range can change based on the geological features of the area it flows through. The pH range of natural waters is 4 (acidic) to 10 (basic). A low pH, which denotes acidic waters, raises the possibility that metals exist in an even more hazardous ionic form. The danger of harm could rise if certain metals become more soluble and thus more accessible to aquatic species.

Conversely, elevated pH levels may encourage higher ammonia concentrations. Ammonia can be harmful to aquatic habitats and is toxic to fish. For aquatic ecosystems to remain healthy, it is crucial to monitor and keep pH within a certain range. The specific needs of the aquatic ecosystem in issue may require interventions to modify the pH as needed.

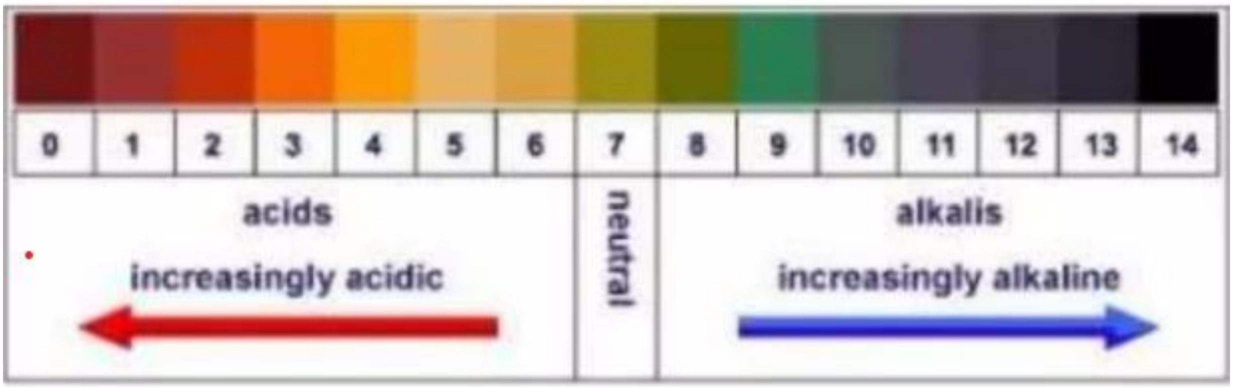


Figure 2: PH values

2.3. Electrical conductivity (EC)

A metric called electrical conductivity (EC) assesses a solution's ability to carry electrical current. This feature is especially significant in the water domain, where the chemical composition of the solution can vary significantly. In general, electrical conductivity is expressed in millisiemen per meter (mS/m) at a standard temperature of 20 °C.

The majority of minerals in solution, including the ions calcium, magnesium, sodium, and potassium, are good electrical conductors. On the other hand, organic compounds are poor conductors. Thus, the measurement of electrical conductivity can yield information on the concentration of dispersible minerals in a solution.

Although natural water's conductivity can vary, it typically falls between 50 and 1500 microsiemens per centimeter (µS/cm). This variance is dependent on the particular chemical composition of the water, which can be influenced by factors such as the local geology, human activity, and the presence of dispersants.

One can multiply the conductivity value by an empirical factor that depends on the type of dissous particles and water temperature to estimate the total amount of dissoute materials in the water. This estimate is significant since it provides information on the water's quality in terms of the substances that are present.

Understanding the content of Dissolved salts is essential since each aquatic organism has unique requirements regarding Dissolved salts. Significant fluctuations in the dissolution concentration, such as those resulting from dilution of used water, may negatively impact aquatic organisms by upsetting their osmotic balance and altering their physiology. For this reason, monitoring electrical conductivity is a crucial tool for maintaining and managing aquatic ecosystems.

2.4. The redox potential

The redox potential, often known as Eh, is a measurement that indicates a system's propensity to either accept or reject electrons. It is vital to aquatic systems because it affects the oxygenation states of elements like Hydrogen (H), carbon (C), nitrogen (N), oxygen (O), sulfur (S), iron (Fe), and other elements. Conditions of oxidation predominate in an aquatic environment with adequate oxygenation, meaning that chemical reactions involve electron loss. Un potentiel redox plus élevé pourrait servir à symboliser cela. As oxygen concentrations drop, the environment becomes more reactive, which is manifested as an increase in electron availability and a decrease in redox potential.

Monitoring the evolution of redox potential in natural waters can yield invaluable insights about changes in the aquatic system. For instance, a decrease in redox potential may indicate more restrictive conditions, which may be linked to a reduction in oxygen or other redox processes.

The redox potential is measured in millivolts (mV). Certain equipment, such as redox electrodes, are used to measure these fluctuations in water potential. The obtained data can be used to monitor biogeochemical processes, assess water quality, and identify changes in the redox balance of the aquatic system.

2.5. Suspended matter

All mineral or organic particles that do not dissolve in water are referred to as "matter en suspension" (MES) in water. This includes a variety of items such as plankton and other microorganisms found in water, as well as clay, sand, silt, and small organic and mineral matter. The concentration of these suspended materials may vary depending on the season and the water's evaporation rate. The presence of suspended materials has multiple effects on the water's quality. First of all, they may impair water transparency by reducing light penetration, which may have an impact on photosynthetic efficiency. Additionally, these suspended particles may impede fish breathing by obstructing their branchers.

The fact that compounds in suspension may act as vectors for the buildup of toxic substances in water is another significant factor. Certain compounds, such as heavy metals, pesticides, mineral oils, and polycyclic aromatic hydrocarbons, can stick to these particles and increase the risk of water contamination.

The measurement of suspended materials is typically expressed in milligrams per liter (mg/l), allowing one to determine the concentration of these particles in water. This measurement is a significant indicator of water quality and is used to assess the health of aquatic ecosystems as well as pollution.

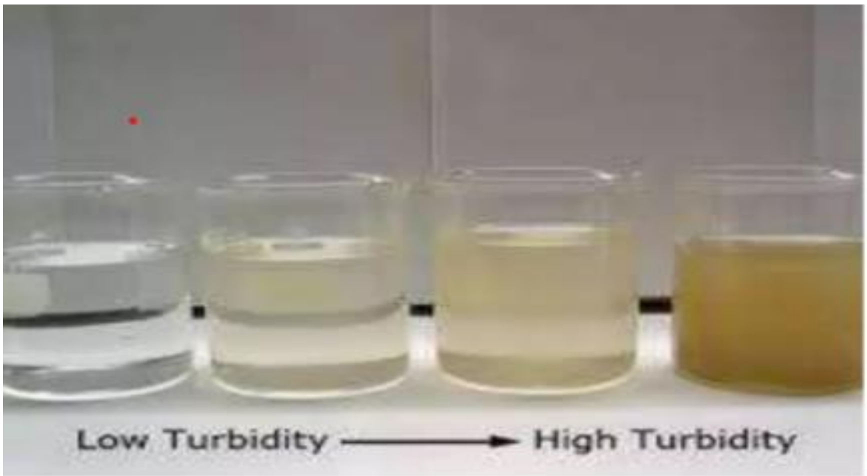


Figure3: Turbidity

2.6. The hydrocarbons.

Hydrocarbons are organic compounds composed solely of hydrogen and carbon atoms. In the context of pollution, mineral oils, including various types of hydrocarbons such as alkanes and alkenes, are frequently mentioned. These compounds pose significant environmental and health risks."

The amount of oxygen in surface waters may decrease as a result of hydrocarbons, especially at high concentrations. This could seriously affect aquatic life by interfering with the natural oxygenation processes that are essential to aquatic organisms' survival.

The complex organic compounds known as HAP result from the assembly of several benzene rings. They tend to strongly adsorb to soils and suspended matter, are relatively stable, and are barely soluble in water. Their solubility in fats promotes their bioaccumulation in human and animal tissues, increasing health risks

1.7. The elements in solution

The elements in solution, such as chlorides (Cl-) and sulfates (SO4--), are frequently closely monitored within the context of water quality analysis due to their significance in terms of pollution and potential effects on the environment and human health.

*__Chlorides (Cl-)__:

Sources of pollution: A high quantity of chlorine can indicate domestic water pollution, particularly due to the use of regenerated sulfur in laundry detergents. The industrial wastewaters, especially those from businesses that use salt-containing chemical products, may also be contributing factors to rising chloride levels.

***Sulfates (SO4--):**

Pollution sources: Sulfates may be present in the water used by several businesses. Certain industrial processes, such the refining of petroleum and the making of paper, can release sulfates into the water.

Potential effects: Sharp changes in sulfate concentrations have the potential to harm the environment, affecting drinking water quality and having undesirable effects on aquatic life, among other things.

Other ions such as calcium (Ca++), magnesium (Mg++), potassium (K+), fluorine (F-) can also be measured. The elements in solution are expressed in mg/l.

Monitoring of the elements in solution, such as chlorides and sulfates, is crucial to determining the water quality, locating potential pollution sources, and implementing the necessary precautions to safeguard the public's health and the environment. Significant variations in these concentrations may act as markers for possible disturbances to the aquatic ecosystem.

2.8. Water's hardness (or hydrometric pressure)

Water hardness is determined by the highest concentration of metal ions, excluding alkalinity metals (Na^+, K^+, and H^+). It is commonly caused by Mg^{2+} and Ca^{2+} ions. These two cations often reduce metal toxicity in water. Hardness is expressed in milligrams of $CaCO_3$ per liter.

2.9. Dissolved oxygen (DO) and oxygen saturation percentage

Along with pH values, the quantities of dissolvable oxygen make up one of the most crucial aspects of water quality for aquatic life. The oxygen released into surface waters depends on many variables, including the partial pressure of oxygen in the atmosphere, water temperature, salinity, light penetration, water agitation, and nutrient availability. As a result, the concentration of oxygen fluctuates daily and seasonally. This concentration is also influenced by the rate at which oxygen is absorbed by the surrounding environment due to the activity of aquatic organisms and the oxidation and breakdown of organic matter in the water

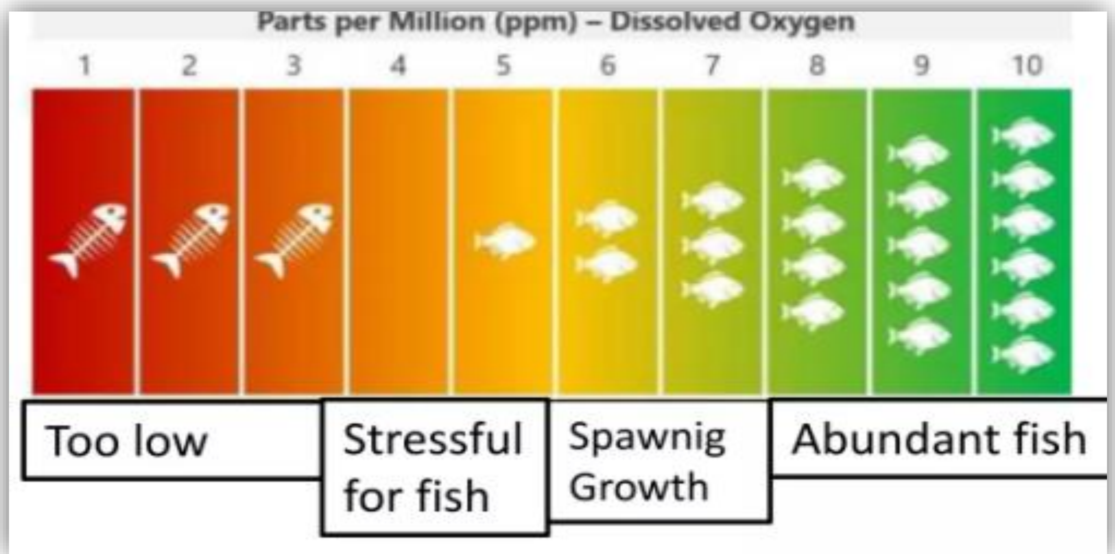


Figure 4: Value of dissolved oxygen

Globally, a river's ability to absorb pollutants increases with its content of oxygen dioxide (OD) near saturation: A value of less than 1 mg of O2 per liter suggests an almost anaerobic state. This condition results from the oxidation processes of mineral waste, organic matter, and nutrients using up more oxygen than is available. A low partial pressure of oxygen leads to an increase in the solubility of toxic elements that liberate sediments.

A value between one and two milligrams of O2 per liter indicates a heavily contaminated but reversible river;

Good-quality water has an O_2 concentration of 4 to 6 mg/liter. Higher concentrations than the natural saturation point of oxygen indicate eutrophication of the environment, which is reflected in high photosynthetic activity.

One way to express the concentration of dissolved oxygen is in mg of O_2 per liter or as a percentage of oxygen saturation. The relationship between these two values depends on temperature, as the table below illustrates:

Table 1: Solubility of oxygen in water as a function of temperature

Temperature (c)	Solubility of oxygen O2 mg/l
0	14.16
5	12.37
10	10.92
15	9.76
20	8.84
25	8.81

2.10. Charge in organic matter: DBO and DCO demands for biochemical and oxygen-containing materials

.The two methods commonly used to assess the amount of organic matter present in water are the Chemical Oxygen Demand (COD) and the Biochemical Oxygen Demand (BOD). These two approaches are based on the distinction between the initial oxygen demand and the final oxygen demand that occurs after the organic matter in a water sample has been oxidized."

*The quantity of oxygen required by bacteria to partially or completely decompose biochemically oxidizable substances (such as fats, carbohydrates, surfactants, etc.) present in water is known as Biochemical Oxygen Demand (BOD). This oxygen consumption occurs at the expense of other aquatic organisms. In the case of domestic wastewater, over 70% of organic compounds typically begin to degrade after five days, and the process is essentially complete after twenty.

* The term 'Chemical Oxygen Demand' (COD) refers to the amount of oxygen required for the chemical degradation of organic compounds in water, which is accomplished with the aid of a strong oxidant. It allows for the measurement of the total content of organic matter (except for a few non-degradable compounds), including those that are not broken down by bacteria. Thus, it is a crucial parameter for describing the overall contamination of a body of water by organic compounds

There is a distinction between COD and BOD due to the compounds that cannot be broken down biologically. An indicative measure of the 'biodegradability' of the compounds present in water is given by the ratio of BOD to COD. After complete breakdown, the COD/BOD ratio changes from about 2.5 (in recently reversed residual water) to 10–20. In this latter case, we are discussing well-mineralized water. However, the presence of toxic compounds slows down biological activity, resulting in less oxygen being consumed after five days. This also leads to a high COD/BOD ratio. Both BOD and COD are measured in milligrams of O_2 per liter

2.11. Eutrophic substances: various forms of phosphorus and nitrogen (nutrients)

Nitrogen (N) and phosphorus (P) are two examples of elements that are essential nutrients for plants. The compounds that contain them, such as phosphates and nitrates, are the preferred source of nutrients for plants from the start.
Excessive concentrations of nitrates and phosphates lead to the phenomenon known as eutrophication, which causes the overgrowth of aquatic life. Normally, the mineralization of organic matter produces these compounds. However, when they are present in excessive amounts due to runoff from precipitation, they encourage the growth of algae and small photosynthetic organisms, which reduce light penetration in deeper water layers.

If these algae and microorganisms that are photosynthetic produce oxygen during the day, they will consume it at night, and these changes in oxygen concentration could be harmful to fish.

Additionally, the breakdown of dead algae results in oxygen consumption. Anaerobic conditions may also lead to the accumulation of ammoniacal and nitrite compounds, which have the potential to poison plants and wildlife when oxygen levels in water are too low.
Key parameters for monitoring the quality of surface waters include the concentrations of nitrites (NO_2^-), nitrates (NO_3^-), ammonia (NH_3), ammonium (NH_4^+), phosphates (PO_4^{3-}), nitrogen (N), and phosphorus (P). 'Kjeldahl' nitrogen refers to both ammoniacal and organic nitrogen (such as amino acids and urea). When referring to 'total' nitrogen, it encompasses the sum of organic nitrogen, ammoniacal nitrogen, nitrites, and nitrates.

2.12. Chlorophyll

Chlorophyll content is a sensitive indicator of the abundance of photosynthetic and algae-forming organisms, and, consequently, the degree of eutrophication in the water.

2.13. Heavy metals

It is especially crucial to monitor concentrations of heavy metals (densities more than 5 g/cm3) due to their potential for bioaccumulation along food chains and their toxicity. En contraste avec les polluants organiques, les métaux ne peuvent pas subir des dégradations chimiques or biologiques. Regular measurements are made of the concentrations of copper, nickel, chromium, plumb, zinc, cadmium, and arsenic. Heavy metals are a characteristic of some types of pollution, like as:

-The presence of copper and nickel indicates waste from surface metal treatment companies;

-The presence of a tannery is indicated by the chromium;

- Lead is associated with diffuse pollution, resulting from the presence of industrial sites impacted by pollution and the use of motor vehicles.
Zinc is released by industries involved in galvanization or the production of alloys like bronze, as well as by rust-corrosion water coming into contact with galvanized materials (e.g., metal furniture, gutters). Cadmium is released, for example, by electroplating plants and chemical textile and dye industries.

It dissolves heavy metals very well in acidic (low pH) water. They precipitate and concentrate primarily in the solid phase (sludge) in neutral or alkaline waters. This allows for an overview of all heavy metal discharges that have occurred, both in terms of quantity and type, through the analysis of these sludges.
Zinc's toxicity, which is influenced by temperature, oxygen content, and water hardness, primarily affects plants and algae. Copper's toxicity to the aquatic environment is mostly dependent on its alkalinity, pH, and organic matter content. Salmonids (salmon, trout) are generally quite sensitive to copper and zinc.

3-Monitoring the health of aquatic ecosystems

A vital component in ensuring the preservation of aquatic ecosystems is the ongoing monitoring of their overall health, including the quality of their waters, the diversity of organisms that inhabit them, and the condition of their habitats.
Pollution may be evident (e.g., floating corpses, foam, etc.). However, this is not always the case; heavily contaminated clear water can still occur. Measuring a watercourse's pollution requires the use of specific measurements

Figure 5: On-site analysis of physicochemical parameters

Numerous physical-chemical analysis techniques make it possible to determine the amounts of various compounds, including pollutants, in water. Certain of these actions can be carried out continuously on-site. Additional tests must be conducted in a laboratory using water samples taken from the natural environment. However, pollutants that have established an aquatic ecosystem are not content to stay in the water. They are distributed among the various ecosystem compartments which include water, the physical environment, and living things. Therefore, physical-chemical measurements of water are insufficient to accurately assess the ecological quality of aquatic ecosystems. Additionally, they are ill-suited to the detection of intermittent or sporadic pollutions that do not always arise during the collection of sample data for analysis and do not account for physical disturbances in the environment.

Chapter 2: Physicochemical parameters of soil

1. Introduction

Derived from the Latin word *solum*, the term 'soil' refers to the fine layer of earthly materials that covers the Earth's surface, formed by the weathering and fragmentation of rocks. Composed of organic matter, minerals, and living organisms, soil is essential for providing nutrients to plants, which, in turn, sustain most living things. In addition to its nutritional role, soil can also filter impurities, neutralize pathogens, and break down pollutants.

Soil serves as a physical support for elements such as water, air, nutrients, temperature regulation, and protection against harmful toxins. Its composition, influenced by various constituents, determines its physical characteristics, such as structure and porosity, which in turn affect the movement of water and air within it. Soil plays a vital role in the quantification of physical, chemical, and biological parameters that impact agricultural productivity and sustainability.

Over time, soil forms distinct layers, or horizons, that make up its profile. These layers include the uppermost horizon, O, and the arable layer. Occasionally, the uppermost layer consists of accumulated plant material. Some soils have specific horizons, such as horizon B, which is rich in clay, and horizon C, which consists of the underlying bedrock or parent material.

As a vital natural resource, soil supports a diverse ecosystem that sustains the surrounding environment. It influences meteorological conditions, aids in plant growth, facilitates nutrient cycling, and contributes to water purification. Proper soil preservation is crucial to maintaining this invaluable resource, which is essential for both environmental health and food security

2. Physicochemical analyses

Soil physicochemical analyses are techniques used to evaluate the physical and chemical characteristics of a soil. This information is crucial to understanding soil fertility, water retention capacity and other properties important for plant growth. Here are some of the analyses commonly carried out:

2.1. Granulometric analysis: This measures the distribution of particle sizes in the soil, classifying them into sand, silt ad clay. This gives an indication of soil texture.

Figure 6:particle size analysis

2.2. Organic matter analysis: This evaluates the amount of organic matter present in the soil, which is essential for soil fertility.

2.3. pH analysis: pH measures the acidity or basicity of the soil. Plants have specific pH preferences for optimal growth.

2.4. Analysis of cation exchange capacity (CEC): CEC measures the soil's ability to retain and exchange ions, which is crucial for the availability of nutrients to plants.

2.5. Nutrient analysis: This measures the concentration of nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, etc., which are essential for plant growth. These elements are essential for plant growth.

2.6. Trace element analysis: Certain elements in minute quantities, such as iron, zinc and copper, are also analyzed, as they are required by plants in limited quantities.

2.7. Electrical conductivity analysis: This measures the soil's ability to conduct electricity, which can provide information on soil salinity.

2.8. Soil moisture analysis: Evaluates the amount of water retained in the soil at different moisture levels.

3. Soil pollution

3.1. Definition: The abnormal, exogenous accumulation in soil of mineral or organic elements or compounds, or pathogens, thereby affecting its quality.

3.2. Different soil pollutants

3.2.1 Soil contamination by heavy metals

*Metals (cadmium, lead, etc.) and metalloids (boron, arsenic, etc.) are naturally present in soils. Discharges from industry, households, transport and agriculture all contribute to the diffuse contamination of soil by metals.

*Toxic in varying doses to humans, flora and fauna, they can contaminate ecosystems via the food chain (livestock farming) and water resources. Due to the high affinity between lead and soil organic matter, this metal is not readily available to plants and migrates very little at depth.

High levels at the surface can be attributed to human activities (transport, industry, mining, urban sludge, phytosanitary treatments), while at depth, they are more the result of rock alteration.

*Like lead, copper is not very mobile in soils, except in highly acidic environments or under poor drainage conditions. It is also an essential trace element in very small quantities.

3.2.2 Hydrocarbon contamination

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants, produced mainly by the combustion of organic matter (forest fires, fossil fuel combustion). Toxic to human health and the environment, they are generally not very biodegradable.

In addition to their ability to be transported over long distances, they accumulate in living tissue due to their high fat solubility (bioaccumulation). Lastly, they readily attach themselves to organic matter, suspended solids and river sediments.

3.2.3 Soil contamination by pesticides

The prolonged use of persistent organic pollutants is the source of diffuse pollution that can affect a large part of the country. This is the case for organochlorine pesticides such as lindane, which has been used for over 50 years and is considered toxic for humans and dangerous for the environment. Lindane is not very mobile in soils, but can be evaporated and transported into the air under the influence of the nature and humidity of the soil, as well as the way it is applied. The nature of the soil, the climate and the depth to which lindane is buried also influence how long it takes to degrade in the soil (up to 40 years).

Part II

Ecological indicators

1. Definition of ecological indicators

Ecological indicators are specific measurements, either quantitative or qualitative, that are used to assess the health, performance, and state of ecosystems. These indicators allow one to monitor environmental changes and quantify the effects of human activity on natural resources, biodiversity, air and water quality, and other environmental components.

To put it another way, ecological indicators are tools that help to simplify complex information on the state of the environment so that it is easier to understand, communicate, and make decisions. They play a crucial role in maintaining biodiversity, managing natural resources sustainably, and assessing the efficacy of environmental policies. These indicators may differ depending on specific goals, but they are all intended to provide clear and meaningful information on the ecological state of a particular area.

2. Objective of ecological indicators:

The main goal of ecological indicators is to provide information that enables clear decision-making regarding environmental management. They make it easier to monitor environmental health, help spot long-term trends, assess the efficacy of environmental policies, and play a significant role in raising public awareness of environmental issues.

3. Typology of ecological indicators:

*Pressure indicators: Track human activities that have an impact on the environment, such as industrial waste discharges, deforestation, etc.

*Indicators of state: Assess the current state of the environment, such as the variety of species, the quality of the air, etc.

*Response indicators: Track how ecosystems respond to pressures, such as recovering from disturbances.

*Durability indicators: Assess the ability of ecological systems to continue operating over an extended period of time.

4. Minimum requirements for ecological indicators:

Representativeness: The indicators used must be indicative of the ecosystem or phenomenon they are measuring.

*Fiability: Precise and dependable data must be used to calculate the indicators.

*Sensitivity: The indicators must be responsive to relevant changes.

*Reliability: The measurement techniques should provide consistent evaluations throughout time.

Chapter I: Hydrobiological indicators

1.Introduction

Prior to 1971, a variety of researchers and scientists from throughout the world contributed to the development of concepts related to the use of biological indicators to assess the health of aquatic ecosystems. These forerunners established the theoretical and methodological foundations that were later refined and developed.

The first biological index based on benthic macro-invertebrates is introduced in 1971. This approach, developed by Verneaux and Tuffery, becomes a valuable tool in assessing the quality of aquatic environments on a global scale.

International recognition of the significance of biological indices is demonstrated by the 1992 enactment of the Water Law in France, which advocates the use of normalized biological indicators to assess the condition of aquatic environments.

Over the course of the 2000s, the international scientific community has come to understand the importance of conducting an integrated assessment of the ecological state of aquatic environments. This leads to the creation of new multimetric indices that aim to provide a more comprehensive assessment of aquatic ecosystems.

An attempt is being made in 2003 to bring the methods of water plan evaluation up to date on a global scale. These updates aim to increase the accuracy and relevance of evaluation tools used in various nations. 2007 saw the start of an international effort aimed at creating comparable indicators that would enable a coherent assessment of the ecological state of aquatic environments worldwide. This partnership reflects the growing awareness of the need for coordinated approaches to monitor and safeguard aquatic resources on a global scale.

1. Biomonitoring of aquatic ecosystems

The physical-chemical methods used to monitor the quality of environments consist of measuring the concentrations of various components present in the environment and comparing them to regulatory values. However, despite technological advancements in microbiological systems, these methods do not directly provide information on the effects of pollutants on organisms. According to Van Haluwyn et al. (2011), biosurveillance—which is based on fish or plant models—remains the only method capable of assessing the biological effects of environmental alteration.

Garrec and Van Haluwyn (2002) formalized the concept of biosurveillance and its various aspects. According to their definition, biosurveillance involves the use of an organism's or a group of organisms' responses at all biological levels of organization (molecular, biochemical, cellular, physiological, tissue, morphological, and ecological) to predict and/or reveal environmental changes and track their evolution. These approaches include observing and measuring the overall response of organisms to pollutants, taking into account the effects of compound mixtures present in the environment, as well as the ecological and climatic characteristics of the area (Van Haluwyn, 1998). Biosurveillance includes four key principles: the use of biomarkers, bioindication, biointegration, and bioaccumulation.

According to Lagadic et al. (1997), the use of biomarkers is defined as "a change observable and/or mesurable at the molecular, biochimical, cellular or physiological level that reveals an individual's exposure, either past or present, to at least one polluting chemical substance." These biomarkers indicate early, non-visible, and specific effects of stress at the infra-individual level. This is a relatively new idea in the field of plant biosurveillance that is now undergoing significant development. It depends, for instance, on monitoring chlorophyll fluorescence and photosynthetic activity (Catalyud&Barreno, 2004; Crous et al., 2006),The parameters that can be affected are enzyme doses (Rai& Agrawal, 2008), membrane integrity, or genotoxicity (Rzepka&Cuny, 2011; Misik et al., 2011).

The bioindicationis situated at the individual level. It is based on the observation of the visual and clinical effects of pollution on an organism. These effects might be seen at the morphological, tissue, or physiological levels. It involves, for instance, the development of foliar necrosis (Silva et al., 2012) or alteration of radial growth (Manier et al., 2009).

With the use **of biointegration**, long-term effects on communities can be accessed through the study of population density and specific diversity within ecosystems. The sentinel organisms that are typically used are lichens, bryophytes, and higher plants (Thimonier et al., 1994; Takahashi &Miyajima, 2010).

The amount of pollutants accumulated in resistant tissues is what determines the **bioaccumulation**, not an organism's immediate response. In this case, the organism being used primarily serves as a matrix for quantifying various pollutants resulting from environmental transfer mechanisms and their distribution among various tissues. This approach is widely used nowadays to assess the effects of pollutants' pollution of the environment and track changes in it over time and space (Larsen et al., 2007; Cloquet et al., 2009; Gerdol et al., 2014). In this case, the tissue concentrations reflect what is present in the environment and correlate with the biodisposable percentage that ultimately stays within the organism. Therefore, these amounts do not directly reflect those of the surrounding environment.

I. Standardized global biological index (IBGN)

I.1. Definition

Standardized global biological index or IBGN, is a tool used to assess the hydrobiologic quality of a watercourse by analyzing the species composition of the populations of benthos that inhabit various habitats (Support torque/velocity). In the waterways that allow the visibility and accessibility to the various prospecting supports, one proceeds by avoiding the source zones, the lower courses of the larger waterways, and the atypical environments (canaux and estuaries).

Figure 7:Watercourse.

I.2. Objective

*Situate the biological quality of a site's running water within a general typological range
*Monitor the evolution of the biological quality of a site
-Over time
- In space (upstream/downstream)
*Evaluate the effect of a disturbance (example: a release) on the environment.

I.3. Applicability domains and boundaries of the IBGN

I.3.1. Principal categories of disturbances identified by the IBGN

It is especially recommended to use the IBGN for disturbances that result in changes to the organic quality and substrate type of the water:

* Eutrophication due to sediment denaturation and urban-type discharges with a dominant organic component.

* Pollution caused by suspended materials

*and secondary effects of some types of waste (metals, organic).

Moreover, the IBGN, which represents the long-term structure of a biocenosis made up of integrating organisms, is primarily sensitive to chronic disturbances or, at the very least, intermittent perturbations that are severe enough to cause immediate death.

I.3.2.Areas concerned

*Every watercourse for which: the depth does not exceed one meter

*The current speed is not too high, allowing for the entire habitat mosaic to be examined.

*The water's turbidity does not make it impossible to see the supports.

I.3.3. Environments eliminate

*Sources,
* **Streams and estuaries**

*The major waterways

I.4.How the index is calculated

*A list of particular organizations

Based on the examination of populations of benthic macro-invertebrates (depending on the substrate), the quality of the environment is evaluated. There are 138 taxa in the directory of organisms used to calculate the IBGN. With the exception of a few faunal groups, where it is the phylum or class, the family remains the taxonomic unit. Of the 138 taxa, 38 make up the nine faunal indicator groups (GI), which are arranged in increasing order of pollution sensitivity in the determination table, from 1 to 9.

I.4.1. Advantages:

- ✓ several species of bioindicators.
- ✓ dispersion throughout aquatic environments.
- ✓ relative population stability over time and space.

Invertebrates found in the ecosystem at various trophic levels, such as primary, secondary, and decomposers…

I.5. Simpling

I.5.1. Choice of sampling station

A station is characterized as a segment of a watercourse that, at the time of sampling, is roughly ten times longer than it is wide.

The purpose of the study guides the choice of site. For the section of the watercourse under study, a "representative" station is selected in order to evaluate the overall condition of the ecosystem. The samples being collected in similar ecosystems must be comparable across two stations monitoring a polluted discharge whose impacts we wish to assess.

I.5.2.Sampling practice

Benthic fauna sampling for a station consists of 8 1/20 m2 samples taken independently in 8 different habitats. Prior to sampling, each of the eight habitats must be identified. For example, marking each one with a flag can be helpful. By using this strategy, you can select the most representative habitats and get a good overview of the station. With caution, the location is made so as not to disrupt the bed's underneath**.**

A. Sampling of wildlife

Utilize a "Surber" fig 8.-style sampler with a surface area of 1/20 m2 for lotic facies (flowing water). The Surber's base is set on the bed's bottom to frame the habitat that will be sampled, and the net's entrance faces the stream. By hand, the support is "cleaned". Samples of loose substrates will be taken down to a few centimeters in thickness.

When sampling lentic facies, such as stagnant water or slow flowing, use a "Haveneau" style sampler. Pulling over 50 cm is the recommended method for prospecting; if not, moving back and forth over a surface of similar size will do. A sieve with 500 mesh can also be used.

Figure 8: The surber.

B. Plant sampling

The entire tuft, or 1/20 m2 of surface area at the base, is used for the prospecting of submerged plants. Place the plant inside the net, then trim and tidy it.

C. Sample conservation

After gathering the samples, put them in a 10% formalin solution. For shelled invertebrates, use calcium carbonate to neutralize formalin.

Condition each of the eight samples independently; their individual analyses frequently yield insightful information for interpretation.

I.6. Physicochemical measurements

I.6.1. Physical attributes

***Watercourse width**: a rough estimate will do. Put something conspicuously noticeable along the bank. Follow the water away from the object. Use a one-meter-long cord to measure the distance between the object and the opposite bank when they appear to be at the same distance.

*** Depth:** using a weighted wire (ranging from 4 to 500 g) that has marks spaced 10 and 50 cm apart.

***Current speed**: The two pupils in charge of collecting a sample are spaced two meters apart with respect to the current. The third student stays on the bank and multiplies the time it takes for a piece of wood to get from one of the other two students to the other. Repeat the experiment multiple times, then average the outcomes.

***Temperature:** Take a sample of water from the bank or a bridge, and note its temperature right away.

*When using a Secch disk**, turbidity**

I.6.2.Measurable physical-chemical parameters on the ground

Certain physical and chemical parameters can be measured on-site using portable measurement instruments (e.g., conductimeter, pH meter). Additional measurements on the field measurements need to be made in the lab.

I.7.Calculating the index

The determination table, which includes the 14 taxonomic variety classes on the abscissa and the 9 indicator faunal groups on the ordinate, is used to establish the IBGN.

We progressively ascertain:Even if each taxonomic variation is only represented by one person, **the sample's taxonomic variety (St)** is equal to the total number of taxa gathered. The classes shown on the table's abscissa are comparable to this figure….

The indicator faunal group (GI), which only considers the indicator taxa that have three or ten individuals, depending on the taxon, represented in the samples.

The process of determining the GI involves prospecting the table's ordinate from top to bottom (GI 9 to GI 1), with the inspection ceasing at the first instance of a taxon's considerable presence (n > 3 individuals or n > 10 individuals) on the ordinate of the table.

From the table's ordinate (GI) and abscissa (St), we can infer its IBGN. As an illustration:

IBGN = 17 if GI = 8 and St = 33.

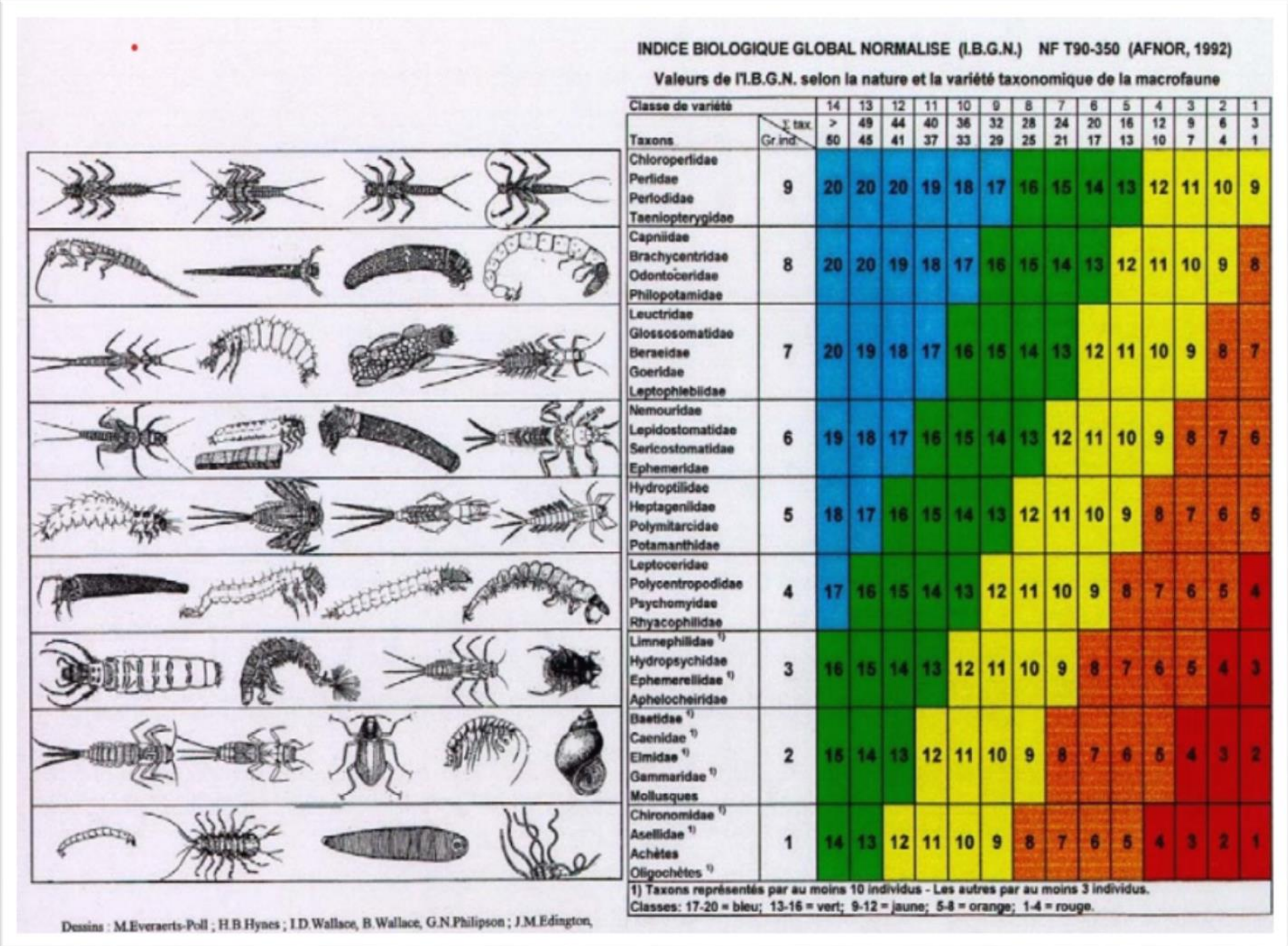
IBGN = 13 if GI = 5 and St = 30.

IBGN = 7 if GI = 3 and St = 14.

For a cartographic representation of the results, each section of watercourse is assigned a color according to the value of the IBGN.

Table 2:The values and classes of IBGN.

IBGN	>ou = à17	16-13	12-19	8-5	<ou = à 4
Color	Bleu	Green	Yellow	Orange	Red



INDICE BIOLOGIQUE GLOBAL NORMALISE (I.B.G.N.) NF T90-350 (AFNOR, 1992)

Valeurs de l'I.B.G.N. selon la nature et la variété taxonomique de la macrofaune

Classe de variété		14	13	12	11	10	9	8	7	6	5	4	3	2	1
Taxons	Σ tax / Gr ind	> 50	49 45	44 41	40 37	36 33	32 29	28 25	24 21	20 17	16 13	12 10	9 7	6 4	3 1
Chloroperlidae, Perlidae, Perlodidae, Taeniopterygidae	9	20	20	20	19	18	17	16	15	14	13	12	11	10	9
Capniidae, Brachycentridae, Odontoceridae, Philopotamidae	8	20	20	19	18	17	16	15	14	13	12	11	10	9	8
Leuctridae, Glossosomatidae, Beraeidae, Goeridae, Leptophlebiidae	7	20	19	18	17	16	15	14	13	12	11	10	9	8	7
Nemouridae, Lepidostomatidae, Sericostomatidae, Ephemeridae	6	19	18	17	16	15	14	13	12	11	10	9	8	7	6
Hydroptilidae, Heptageniidae, Polymitarcidae, Potamanthidae	5	18	17	16	15	14	13	12	11	10	9	8	7	6	5
Leptoceridae, Polycentropodidae, Psychomyidae, Rhyacophilidae	4	17	16	15	14	13	12	11	10	9	8	7	6	5	4
Limnephilidae [1], Hydropsychidae, Ephemerellidae [1], Aphelocheiridae	3	16	15	14	13	12	11	10	9	8	7	6	5	4	3
Baetidae [1], Caenidae [1], Elmidae [1], Gammaridae [1], Mollusques	2	15	14	13	12	11	10	9	8	7	6	5	4	3	2
Chironomidae [1], Asellidae [1], Achètes, Oligochètes [1]	1	14	13	12	11	10	9	8	7	6	5	4	3	2	1

1) Taxons représentés par au moins 10 individus - Les autres par au moins 3 individus.
Classes: 17-20 = bleu; 13-16 = vert; 9-12 = jaune; 5-8 = orange; 1-4 = rouge.

Dessins : M.Everaerts-Poll ; H.B Hynes ; I.D.Wallace, B.Wallace, G.N.Philipson ; J.M.Edington,

Figure 9:Standardized biological index calculation table.

I.8. Terms and duration of collecting

Only once the flow rate has steadied for a minimum of ten days can sampling be carried out. Wait at least fifteen days following a river's inundation or drying out. The summer-autumn low water period is the suggested time to sample since it has the highest pollution concentration, the highest temperatures, the fewest hydraulic disturbances, and ideal sampling circumstances. But the primary factor considered when selecting the sampling period is the study's goal. It might be challenging to distinguish between species that are in their larval stage and those that are in their aerial phase between June and October. It takes at least two occasions a year to accurately assess an environment. It is feasible to calculate the difference between the most unfavorable scenario and a second sample period.

II.Diatom Biological Index

II.1.Introduction

Diatoms' unique sensitivity to a range of ecological variables has made them a popular tool for monitoring changes in the water quality of streams and rivers. Diatoms have also been used in paleolimnological studies. Their tolerances and preferences for various environmental factors, including pH, conductivity, salinity, humidity, organic matter, saprobity, trophic state, oxygen requirements, nutrients, and current velocity in freshwater streams, rivers, lakes, wetlands, and estuaries, have been defined. Although indices of biotic integrity based on periphyton, diatoms, non-diatom "soft" algae, such as cyanobacteria, macroalgae, and macrophytes assemblages, have also been developed for biological monitoring, biotic indices using diatoms based on the relative abundance of the species weighted by their autoecological values have been developed globally.

II.2.Definition of Diatoms

Diatoms, a common form of unicellular phytoplankton, are shown in the photo below. They most likely first appeared in the Jurassic Period. Diatoms have the ability to create colonies that have certain shapes, such as ribbons, fans, or stars. They are protected by a special silica cell wall called a frustule. Different diatom species have quite different frustules that are bilaterally symmetrical and can fit inside one other. Diatoms are employed in ecology to check the water quality of huge bodies of water.

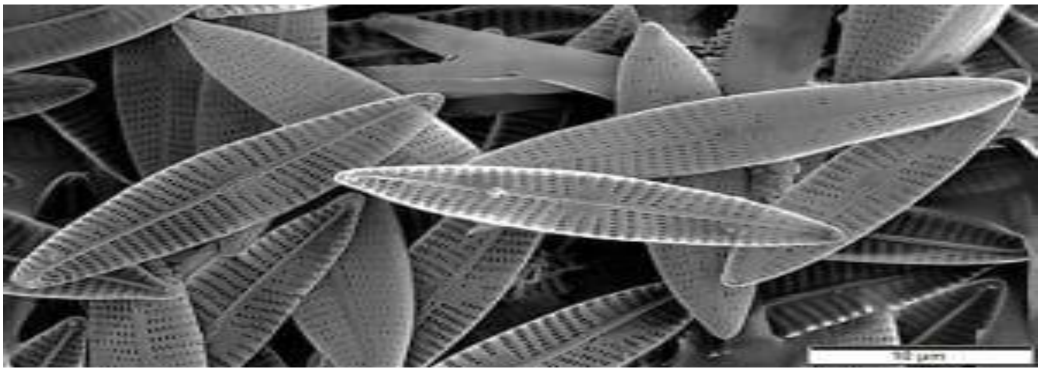


Figure 10: Diatoms.

II.3.Factors that affect Diatom Growth

Diverse environmental conditions have a substantial impact on the growth and dispersion of diatoms. Among these elements are the following:

*pH: The growth and spread of diatoms can be influenced by the water's acidity or alkalinity, as shown by the pH scale. Certain species might be better suited to environments that are more alkaline or acidic.

*Diatoms are especially sensitive to the amounts of nitrogen and phosphorus in water, among other nutrients. These are necessary for their growth, and fluctuations in their availability can affect the makeup of communities of diatoms.

*The existence of organic materials: Diatom formation may also be influenced by the amount of organic matter in the water. Certain kinds of diatoms may develop more readily in environments with high organic matter concentrations.

*Low oxygenation of waters: The amount of dissolved oxygen in the water can have an impact on diatoms. Certain diatom species may be more prevalent than others depending on how well they have adapted to low oxygen levels.

In conclusion, environmental parameters including pH, nutrients, the presence of organic matter, and the degree of oxygenation in water substantially affect the existence, distribution, and abundance of diatom species, which are identified based on the features of their frustule.

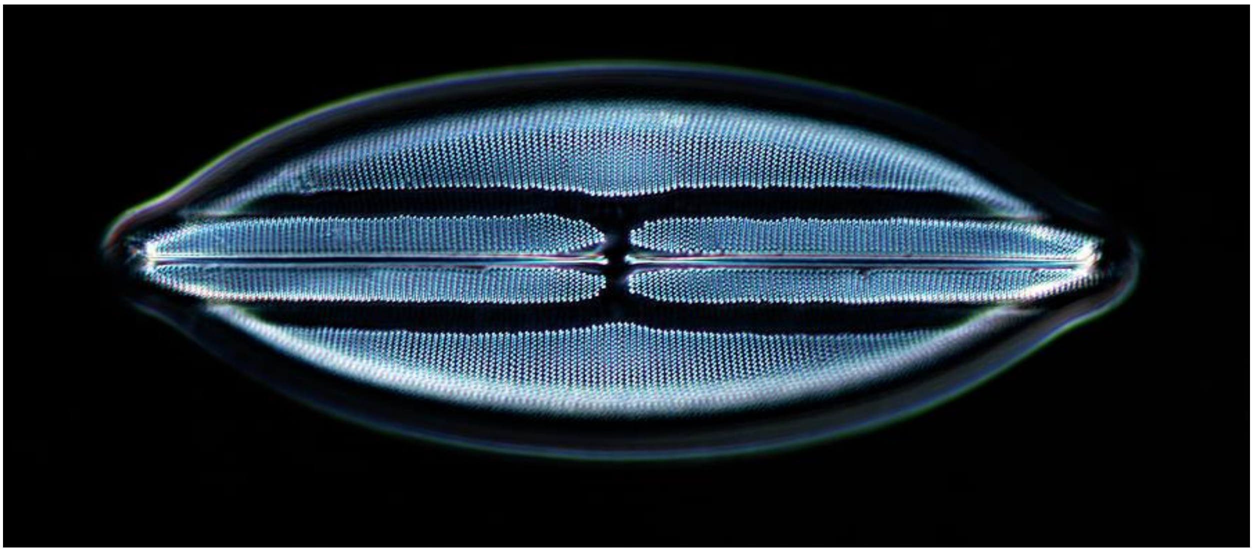


Figure 11: photo Diatom Furstule.

II.4.benefits of using diatoms

There are numerous significant benefits to using diatoms as bioindicators, such as:

*__Fast life cycle__: Diatoms can adapt quickly to changes in their surroundings because of their relatively fast life cycle. Because of this, they are indicators that are sensitive to short-term influences, making it possible to detect environmental changes early on.

*__Aquatic ecosystems mostly rely on diatoms, which are photosynthetic algae__ with a single cell. As a result, changes in the physical and chemical characteristics of water, such as temperature, light, the concentration of nutrients, and pollutants, have an immediate impact on them. They are useful in determining the quality of water because of their sensitivity to changes in the environment.

***Ease of Sampling**: Gathering diatom samples is a reasonably easy task that costs little money and needs a small staff. Diatom sampling is a more efficient way to monitor the environment than other technologies available. Because sampling is so simple, large-scale, frequent monitoring is made possible, which is essential for evaluating how the environment is changing.

***Minimizing Impacts on Wildlife:** Gathering diatoms usually has little effect on the local wildlife, in contrast to certain animal species that might be vulnerable to invasive sampling techniques. This guarantees that the ecosystem under study won't be considerably impacted by the employment of diatoms as bioindicators, protecting the environment's integrity while data is being collected.

Les diatoms are excellent bioindicators because of their great sensitivity to changes in the environment, ease of sampling, and capacity to reduce effects on nearby wildlife. These qualities make them useful instruments for maintaining aquatic ecosystems and keeping an eye on the quality of the water.

II.5.Sampling method

Diatoms frequently form biofilms in rivers, therefore gathering samples there is essential to determining the health of the aquatic ecosystem and the water quality. Below are further specifics regarding the mentioned sample method:

***Finding sample Sites**: Considering the study's objectives, carefully select the sample sites before starting the sampling procedure. Sites may be chosen on the basis of possible pollution sources, geographic features, or diversity of habitat.

***Diatoms often grow through the production of biofilm**s. These biofilms are intricate communities made up of different microorganisms that are heterotrophic (bacteria, hyphomycetes, and protozoa) and autotrophic (algae and cyanobacteria). A representative sample of the aquatic ecosystem's biodiversity is offered by these communities.

Brush Sampling Technique: Using a toothbrush or a brush made especially for this purpose is a frequent way to collect diatoms. To gather connected diatoms, hard surfaces like pebbles, boulders, or artificial substrates are brushed. It is feasible to gather representative samples of the biofilm that is present in the river using this technique.

Use of artificial substrates: To promote the growth of biofilms, artificial substrates can be added to the river beforehand in addition to natural pebbles and rocks. These substrates, which provide diatoms more surfaces to colonize, can be plastic plates or other inert materials.

Figure 12: Diatom sampling method.

***Storage**: Because diatoms are photosensitive, the samples must be kept in suitable containers that are shielded from light to prevent any alterations. Samples should ideally be kept at room temperature until they are examined in a lab.

This approach makes it feasible to get insightful information on the makeup of diatoms in rivers, which is crucial for understanding the condition of the aquatic ecosystem and the quality of the water.

II.6. The calculation of the Biological Diatom Index (BDI)

Based on a particular methodology, the Biological Diatom Index (BDI) is computed using a sample of 209 diatom taxa, of which 57 are matched. Here are some more specifics regarding the IBD calculating procedure:

II.7. Sampling and Identification: The previously outlined procedure is followed for sampling diatoms. After that, diatoms are classified using specialist microscopes and identification aids at the taxonomic level. Usually, the count is based on a 400-person sample.

II.8. Taxonomic Selection: Of the 209 taxa, only 57 are matched. This indicates that the IBD computation makes use of these 57 distinct taxa. The computation does not include other taxa, allowing one to concentrate on the species that are most important for evaluating the quality of the water.

II.9. Assigning indicator Values: Based on its resistance to oxidizable compounds and salinity, each taxon included in the IBD computation is given an indicator value. These indicator values are used to evaluate the quality of water by showing how diatoms react to their surroundings.

II.10. IBD calculation: A formula that considers the relative abundances of the 57 paired taxa is used to compute IBD. The numerical number produced by this formula is equivalent to the water quality index. The water quality improves with a higher value.

II.11. Water Quality classifications: Next, according on already established water quality classifications, the IBD results are interpreted. Every quality class has a designated color, which ranges from red (poor quality) to blue (great quality). Results can be communicated to interested parties more easily thanks to this visual classification.

II.12.Interpretation and Reporting: By contrasting the computed value with water quality classes, it is possible to understand IBD results. Decision-makers can utilize this data to evaluate the overall health of the aquatic ecosystem, identify potential changes to the environment, and learn more about the quality of the water in a particular area.

IBD offers a comprehensive evaluation of water quality by taking into account factors like salinity and oxidizable elements, and it is based on specific taxa. Its usage of colored quality classes makes it possible to communicate results to non-specialists in an efficient manner.

Table 3: The values of IBD with water quality classes.

Value IBD	**17 à 20**	**13 à 17**	**9 à 13**	**5 à 9**	**1 à 5**
Class	A	B	C	D	E
Ecologicalstatus	Very good state	Good state	Average state	Bade state	Verypoor state

Here are some diatoms grouped by plate indicating the quality class of the waters

Very poor-quality

Poor quality

medium quality

Good quality

Excellent quality

Table 4: Ecological interpretation of 5 IBD classes.

Class IBD	Ecological interpretation
A	The diatom community corresponds to reference conditions (undisturbed). Little or no alteration of human origin and low organic pollution during the previous weeks. Oligotrophic watercourse
B	Slight modifications compared to the reference communities. Low level of distortion linked to human activities. Low nutrient concentration and organic pollution in previous weeks
C	Moderate changes compared to reference communities. Moderate level of distortion linked to human activities. During the previous weeks, episodes where the concentration of nutrients and/or organic pollution were high. Mesotrophic watercourse
D	The diatom community is seriously altered by human activity, compared to reference conditions. Species sensitive to pollution are absent. During the previous weeks, there were episodes where the concentration of nutrients and/or organic pollution was high.
E	The diatom community is the most degraded compared to reference conditions. The population is exclusively composed of species that are very tolerant to pollution. During the previous weeks, the concentration of nutrients and/or organic pollution was constantly high. Eutrophic rivers

III. The Oligochaete Bio-indication Index for fine sediments (IOBS)

III.1. Introduction

In ecology, the Index Oligochètes de Bio-indication des Sédiments Fins (IOBS) is a tool used to assess the quality of fine sediments, such as those found at the bottom of watercourses, lakes, or other aquatic environments. This index is based on the study of oligochètes, which are aquatic vertebrates that belong to the Oligochaeta class. These organisms are sensitive to environmental conditions, and their abundance, diversity, and presence can provide important information about the health of the aquatic ecosystem.

III.2. General characteristics of oligochaetes

Oligochaetes are a group of annelids, more specifically segmented worms, belonging to the class Clitellata Figure 13. Oligochaetes have an elongated and segmented body, divided into rings. They are characterized by the presence of a clitellum, a thickened region located near the head or in the middle of the body, involved in reproduction.

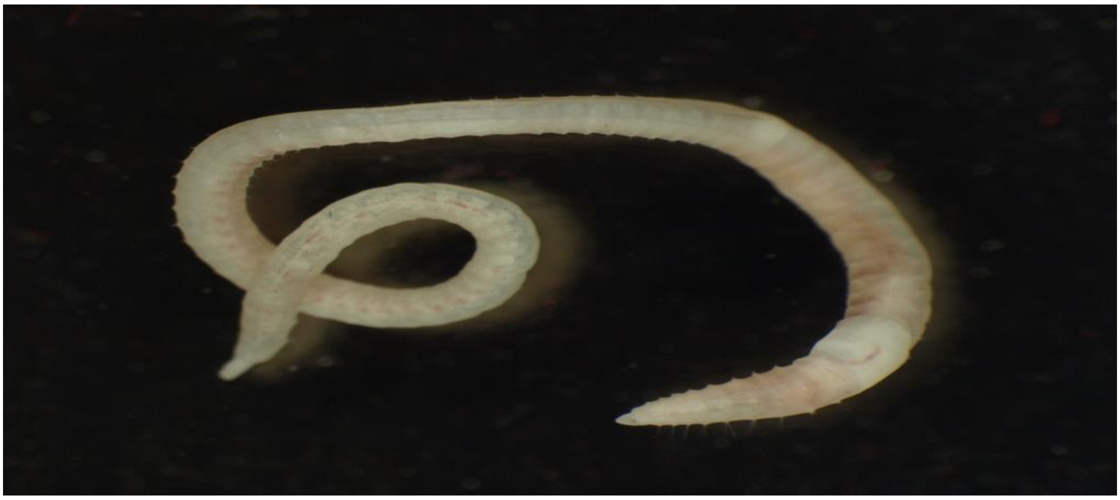


Figure 13: Oligochaetes.

Ecological role: Oligochaetes play an essential role in aquatic ecosystems by participating in the recycling of organic debris. They are often involved in the decomposition of organic matter and contribute to water quality.

These sedentary and burrowing organisms are characterized by their capacity to adapt and colonize very diverse habitats. Their sensitivity to pollution varies depending on the species, which makes their study interesting for assessing the quality of sediments. This index is not used for monitoring the quality of water bodies

The Oligochaete Sediment Bioindication Index (IOBS - AFNOR NFT 90-390, April 2002) makes it possible to evaluate the biological quality of fine and sandy sediments present in natural or artificial watercourses. It provides information on the influence of organic and mineral micropollutants on aquatic environments.

III.3. Sampling

The sampling campaign must be carried out under permanent low water conditions or at least 10 days after a high-water event. After a stressful hydrological event (flood), it is advisable to wait two months before taking samples, to allow time for the environment to stabilize.

Samples are taken as a priority in the dominant sediment at a given station. In principle, only one sample is taken per station. However, if two different types of sediment are significantly present at a given station, it is possible to take two samples per station (one of mud, one of muddy sand.

Sampling is carried out differently depending on the environment, in particular depending on the nature of the sediment and the water height. We use in particular those which are used in the IBGN standard (sampler net/haven, NF T 90-350), which can be suitable for collecting oligochaetes.

However, if IBGN equipment is used, it is imperative to equip Surber and Haveneau with a net with a maximum mesh gap of 0.315 mm (Lafont2002).

Figure 14.Oligochaete sampling method.

In all cases, sampling the first 10 centimeters of sediment, where the majority of oligochaetesare found, is preferred.

- A sample will be made up of at least three samples, for a surface area of at least 100 cm². A station measures between 100 and 150 steps and the interval between sampling points must be 30 steps.
- In the laboratory, the oligochaetesare extracted from the sample by sieving at 0.5 mm. To enable their identification under a microscope.
- The biological quality of the sediments is assessed by the IOBS:

$$IOBS = 10 \times S.T - 1$$

with

S: total number of taxa identified among the 100 oligochaetes;

T: percentage of the dominant group of Tubificidae, with or without capillary bristles, adults and immatures combined

The taxonomic richness (number of different species) of the sample and the percentage of individuals most tolerant to pollution (family Tubificidae) makes it possible to calculate the IOBS which is expressed according to 5 quality classes. When the index value is greater than or equal to 6, it means that the sediment quality level is “Very good”. Conversely, when the index is strictly less than 1, this indicates that the sediment quality level is “Poor”.

Table 5: Biological quality classes of sediments established using the IOBS index

Colors	Index values IOBS	Biological quality level
Bleu	**>or = 6**	Very good
Green	**≤ IOBS < 6**	Good
Yellow	**2 ≤ IOBS < 3**	Average
Orange	**1 ≤ IOBS < 2**	Poor
Red	IOBS < 1	Bad

IV. The macrophytic biological index in rivers (IBMR)

IV.1. Introduction

The Biological Macrophytic Index in Rivers (IBMR) is an indicator used in aquatic ecology to assess the biological quality of waterways based on the composition and structure of macrophyte communities. Macrophytes are aquatic plants visible to the naked eye. IBMR is an important tool for assessing the health of aquatic ecosystems and monitoring the impact of human activities on waterways.

IV.2. Definition of macrophytes

Macrophytesare aquatic plants visible to the naked eye that live in aquatic environments such as lakes, rivers, ponds, marshes, and other freshwater ecosystems. These plants grow in water or at the boundary between water and land, and they play an important role in aquatic ecosystems by influencing water quality, habitat structure and biodiversity. The presence of macrophytescan be used as an indicator of the health of an aquatic ecosystem.

➢ The different types of macrophytic

- multicellular algae,
- bryophytes,
- pteridophytes,
- Spermaphytes

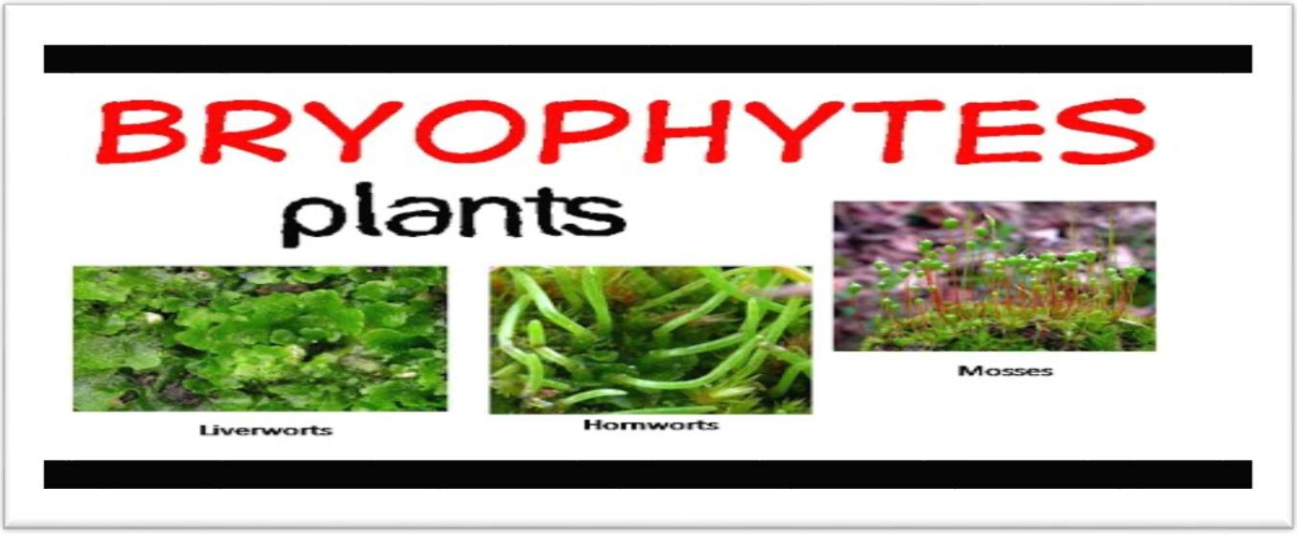


Figure 15:Macrophytics.

IV.3.General characteristics of macrophytes:

- **Habitat**: Macrophytesare found in a variety of aquatic environments, from stagnant pond water to fast-flowing streams. Some can grow completely submerged, while others partially or completely emerge from the water's surface.

- **Types:** There are different types of macrophytes, including rooted plants that grow in the stream bottom, buoyant plants that float on the surface of the water, and amphibious plants that can survive in flooded or emerged areas.

- **Ecological role**: Macrophytes play an essential role in aquatic ecosystems. They contribute to the oxygenation of water through photosynthesis, provide habitats and feeding areas for many aquatic organisms, help stabilize sediments, and can play a role in water purification by absorbing certain nutrients.

- **Adaptations:**Macrophytes have evolved various adaptations to survive in aquatic environments, such as special structures to aid flotation, roots that anchor plants in the soil, and leaves often adapted to underwater life.

- **Sensitivity to environmental conditions:** The presence, diversity, and health of macrophytescan be influenced by factors such as water quality, depth, light, temperature, turbidity, and nutrient availability.

- **Use in Ecology:** In addition to being indicators of the health of aquatic ecosystems, macrophytesare also used in ecological studies, environmental monitoring programs and water quality assessments.

IV.4.Description of the indicator

The IBMR is based on the examination of aquatic macro-plants to assess the trophic status of rivers. This index reflects the degree of trophy of rivers linked to their content of ammonium (reduced form of nitrates) and orthophosphates, as well as to major organic pollution. The score obtained may also vary depending on certain physical characteristics of the environment such as the intensity of lighting and flow.

IV.4.1. Methodology

- **Survey of aquatic plants:**

IBMR involves carrying out an exhaustive survey of all aquatic plants present in the sampling area. This includes rooted plants, floating plants and emergent plants.

- **Species identification:**

Each plant is identified to species level when possible. Precise knowledge of species is crucial for assigning indicative values and estimating their tolerance to environmental conditions.

Figure 16:species identification in the laboratory.

- **Estimated recovery rate:**

For each species identified, we estimate the percentage of the surface area of the sampling station that it occupies. The coverage rate is often assessed visually, by estimating the proportion of the area covered by each species.

- **Collection period:**

Samples for the IBMR must be taken at low flow, that is to say during the period of minimum flow. This provides a more stable image of environmental conditions and minimizes the impact of hydrological variations.

Figure 17:Macrophytic sampling method.

- **Note from the IBMR:**

The IBMR score ranges from 0 to 20. Each species of aquatic plant is assigned an indicative value based on its tolerance to environmental conditions, and the total score is calculated by aggregating these values for all species present.

- **Highlighting the trophic level:**

The IBMR highlights the trophic level of the watercourse. It indicates the trophic conditions, that is to say the level of nutrients available in the environment. This information can be used to understand the dynamics of biological productivity.

- **Non-expression of the “quality” of water:**

It is important to note that IBMR does not directly express the "quality" of the impaired water. Instead, it focuses on the composition of macrophyte communities to provide information on trophic conditions and stream productivity.

IV.5. Calculate index

$$IBMR = \frac{\sum_i^n CS_i \, . \, E_i \, . \, K_i}{\sum_i^n E_i \, . \, K_i}$$

Ei= steneocy coefficientDegree of bioindication (1-3)

i= contributing taxon

n= total number of contributing taxa

CSi= specific rating (0-20) (Affinity for conditionsenvironmental trophic

Ki= abundance coefficient (1-5) (Specific recovery classe).

The IBMR is established by carrying out a survey of all aquatic plants, identifying them, and estimating their percentage recovery rate at the sampling station. Samples are to be taken at low water. The score varies from 0 to 20. It highlights **the trophic level** of the watercourse and does not strictly speaking express an altered “quality” of water.

Table 6: Biological quality classes of macrophytic

Index IBMR	IBMR > 14	12 < IBMR ≤ 14	10 < IBMR ≤ 12	8 < IBMR ≤ 10	IBMR < 8
Trophiclevel some water	Veryweak	High	Average	Strong	Very high

V. River Fish index IPR

V.1. Introduction

At the core of river ecology and aquatic resource management is the River Fish Index (RPI), a specialized tool. Its primary responsibility is to carefully assess how well the fish population in a waterway is doing. This complex system depends on a rigorous scientific and methodological approach to deliver vital information for comprehending the ecological well-being of river systems. In fact, by using a comprehensive approach, the IPR takes into account species diversity, relative density, and other pertinent factors in addition to the straightforward estimation of fish populations.

Using IPR is a part of the effort to place fish population health in the perspective of ideal circumstances, which are frequently ecosystems that have not seen significant alterations from human activity. With the use of specialized sampling methods like electrofishing, IPR offers precise and representative data. A detailed evaluation of the possible effects of human activity on aquatic ecosystems is then provided by comparing these results to those predicted under reference conditions.

V.2. Basic principal

The IPR compares the fish population composition seen in a river from particular sampling (usually by electrofishing) with the composition predicted under reference conditions, which are thought to be low or unaffected by human activity.

V.3. Data collection

Fish samples from the electrofishing technique must be gathered in order to deploy IPR. This approach was selected due to its ability to effectively capture a large range of fish with the least amount of harm to the individual fish that is captured.

V.3.1. Data necessary for calculating the index

Results of sampling of the station by electric fishing: area sampled and number of individuals captured for each species or group of species.

Environmental data: catchment area, source distance, average width, slope, average depth, altitude, interannual average air temperature in July and January, hydrographic unit.

Figure 18: Electric fishing.

V.4. The seven metrics

The seven metrics you mentioned are specific criteria taken into account to calculate this index. Here is a brief explanation of each of them:

- **Total number of species**: This represents the overall diversity of fish species present in the river. Higher numbers of species generally indicate better ecological health of the river.
- **Number of species of rheophiles**: These are fish species adapted to areas with strong currents. The presence of rheophilic fish can indicate good habitat quality and a certain water dynamic.
- **Number of lithophile species**: Lithophile fish are those that prefer rocky substrates. The presence of these species can provide information on the quality of the substrate and habitat available in the river.
- **Total density**: This is the total number of fish individuals in the river, which gives an indication of the overall abundance of the fish population.
- **Total Tolerant Individual Density**: This metric focuses on the density of individuals belonging to fish species considered tolerant to certain specific environmental conditions.

- **Density of total invertivore individuals**: Invertivores are fish that feed primarily on aquatic invertebrates. The density of these fish can provide information on the availability of prey in the river.
- **Total Omnivorous Individual Density**: This metric relates to the density of individuals belonging to omnivorous fish species, which feed on both plant and animal matter. The presence of omnivores may reflect the availability of diverse food resources.

The River Fish Index combines these metrics to provide an overall assessment of the ecological health of the river, taking into account both species diversity and the abundance of fish populations adapted to different habitat types.

Figure 19: Sensitive species TruiteFario.

*Some tolerant species

Figure 20: fish Loach.

Figure 21:fish Chub.

The score of these “metrics” describes the importance of the gap (called “deviation”) between the observation resulting from sampling and the value of the “expected” metric in the reference situation. The assessment is probabilistic;

An IPR of 0 means that there is no gap between the measured situation and the situation deemed "ideal"; A high IPR signals a significant gap. The IPR is also able to assign quality classes to different parts of watercourses, according to the following scale:

Table 7: Ecological interpretation of IPR.

Score TPR	Estimated quality
less than or equal to 7	Excellent quality
Score between 7 and 16	Good quality
Score between 16 and 25	Mediocre quality
Score between 25 and 36	Bad quality
better than 36	Really bad quality

Example

A station having obtained a poor IPR score, with a probability of the presence of trout close to 1 (therefore very high), can be taken as an example. If no trout was caught, this supports the diagnosis that this river is seriously degraded.

The IPR was subject to an adjustment based on the establishment of a new set of reference sites with little or no disturbance, the taking into account of new metrics and their sensitivity to pressure (quality of water). water, hydrological, morphological pressures, connectivity.

Chapter II. Soil quality indicators

1. Introduction

One of the five main components that contribute to the creation of soil is organisms (Coleman, 2008). Thus, the variety and number of soil organisms influence how well the soil functions (Loranger et al. 1998). According to current estimations, soil animals compriseroughly 23% of the diversity of all living things that have been described up to this point (Lavelle et al. 2006). The writers concur that the fauna and flora of the soil are essential to the operation of terrestrial ecosystems. They are therefore regarded as the structure that keeps life on Earth going. Due to all of this, interest in soil biodiversity and its functions has increased.operational (Barrios 2007).

The term "planned" biodiversity in an agroecosystem refers to the products of crops and/or livestock that farmers wish to create; "unforeseen" biodiversity, on the other hand, refers to all existing fauna and plants that are introduced into the system. These plants and animals can be

beneficial—like pollinating insects—or detrimental—like infections, parasites, or weeds. All of this "unplanned" biodiversity has the potential to become "planned" in the sense of being effectively controlled. Such management can focus on improving mechanisms that control populations to eradicate plagues, for example, or ecological processes like nitrogen fixation, which are linked to species diversity and functional group diversity, respectively (Brussaard et al. 2007).

The largest invertebrates (diameter > 2 mm) among the species that comprise the soil fauna are found in the edaphic macrofauna, which includes termites, ants, beetles, spiders, worms, myriapods, and other similar groups. Conversely, the mesofauna consists of thetiny invertebrates, including mites and springtails, with a diameter of less than 2 mm.

1.1. Soil quality indicators

Until recently, physicochemical markers were the primary means of determining the condition of soils and the effects of specific contamination on their ecosystems. Most importantly, these allow for the detection of contaminants and their concentrations; they do not, however, offer comprehensive details on the behavior of these contaminants or the response of terrestrial ecosystems.

For this reason, studies on biological soil indicators—which enable the identification of an ecosystem's biological reactions to contamination—are now being conducted. Assessing the quality of a soil involves more than just counting the number of degradations (such as reduced fertility, erosion, compaction, etc.); it also necessitates considering the purposes and mechanisms behind these degradations.

The majority of the biodiversity on Earth is found in the soil, not above it. It is full of abundant and incredibly diverse macrofauna (earthworms, termites, ants, etc.), mesofauna (insects, mites, microarthropods, etc.), microfauna (protozoa, nematodes, etc.), and microflora (bacteria, viruses, fungi, etc.), in addition to plants, whose root activity and litter input (dead leaves, pieces of wood, etc.) are crucial to its functioning. To ensure that the primary tasks of the soil are realized, a wide variety of species coexist, interact, complement, or replicate each other (a process known as functional redundancy).

Since the smallest organisms are the most numerous and diverse, it is estimated that there are between 10,000 and 100,000 different types of bacteria per gramme of soil. This diversity has recently and still imperfectly been quantified. For instance, there are around 5*1030 bacteria on Earth Figure 20.

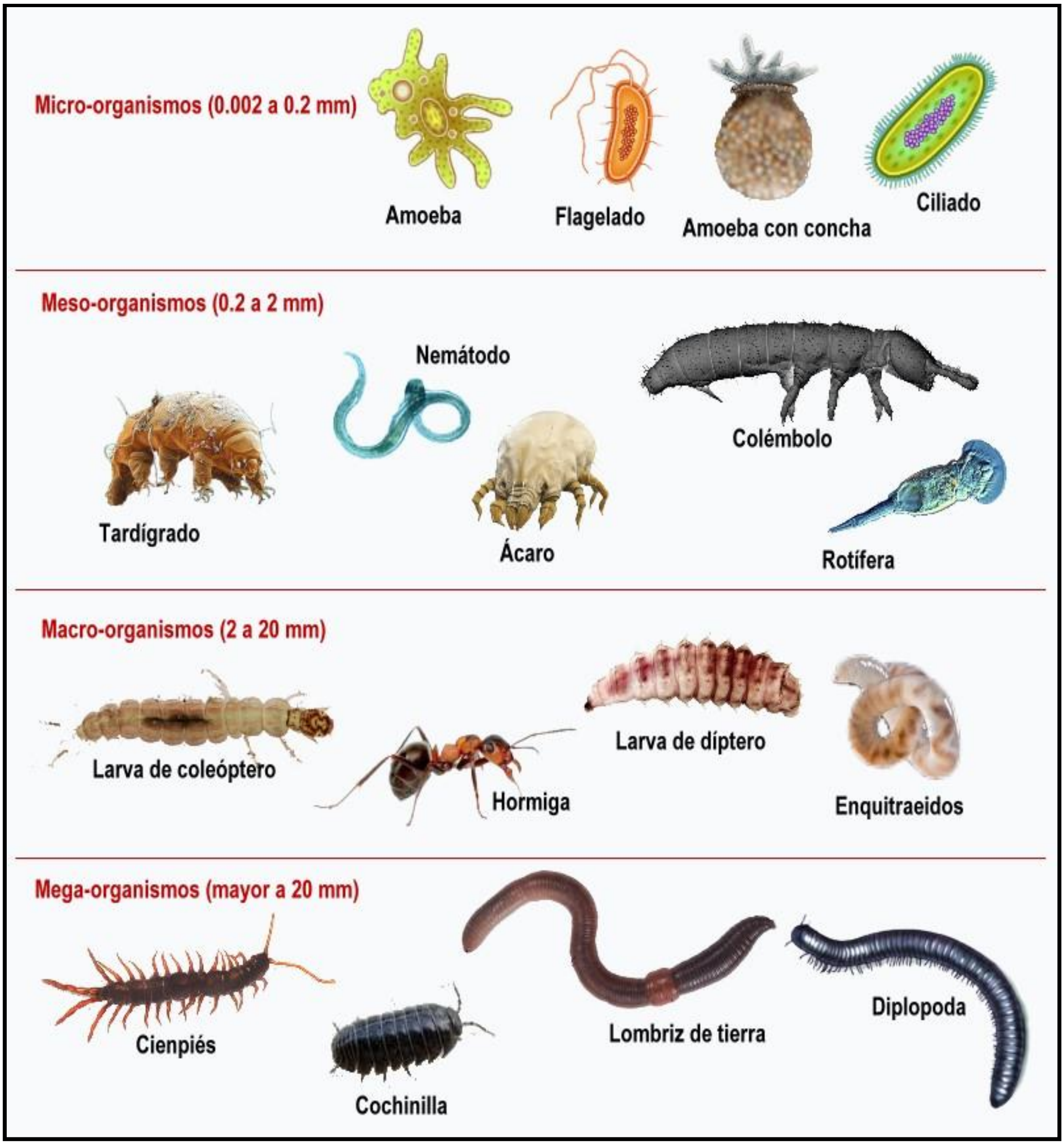


Figure 22: Classification by size of organisms present in the soil.

1.2. The living organisms as a diagnostic tool for soil quality

Numerous biological parameters can be employed as diagnostic tools for the soil quality (Stone et al., 2016), turning them into bio-indicators. "Organisations qui répondent à un stress par leur présence ou leur absence, par les modifications de certaines caractéristiques ou activités particulières, ou par une bioaccumulation de certains contaminants" (Eijsackers, 1983) is one definition for bio-indicators, alsoknown as biological indicators. As a result, they may be able to detect changes in the ecosystem. According to Poge (2012), a perturbation is defined as "a modification of a system that is placed outside of its normal state." Among biological indicators, microflora, and micro-, meso-, and macrofauna are frequently used.

S/Chapter I: Bioindicator of soil quality based on the study of macroinvertebrate populations.

1. Definition

Biological Soil Quality Index (BSQI) is based on the analysis of all macro-invertebrate population populations on the soil as bioindicators of its physical, chemical, and ecological states.

These organisms actively contribute to the functioning of the soil by enabling it to provide a range of ecosystemically important services to the planet.

The soil's macro-invertebrates are constantly in contact with the environment in which they live, feed, and reproduce. They live in a wide range of environments and have incredibly diverse lifestyles. Because of this, they are able to adapt to changes in their environment

throughout time and place and respond to them to varying degrees of intensity depending on their way of life.

2. Sampling protocol

Three planting zones are established on the ground according to the level of the study site. Within each of these zones, ten sampling points are selected. At each collection point (Figures A and B, Figure 2), macroinvertebrates are sampled using the TSBF method (Anderson & Ingram, 1993; ISO 23611-51), which combines chemical extraction (formalin solution) with the manual soil method. It uses simple and reasonably priced equipment. The collected invertebrates are preserved in 4% formalin solution, which fixes the colors and tissues.

Figure 23: Illustrations of the TSBF (Tropical Soil Biology and Fertility) in four steps.

A-Cleaning the extraction surface

B-Chemical extraction with formalinated water (0.2%)

C-Taking a block of soil(25cm*25cm*25cm)

D-Manual soil sorting

After the samples are cleaned in the lab, the primary macroinvertebrate orders are separated and de-nombrated. The individuals are identified by following the taxonomy of species up to the 70% alcohol content..

3. Determine the IBQS, or Index Biologique de Qualité des Sols.

The index is determined by calculating the average abundance of the indicator taxa found in the échantillons (Di) and the indicator taxons' power (Si). There are 22 indicator taxa, and their selection was based on how sensitive they were to disturbances. Depending on that, a power to indicate has been assigned to them. This one is much smaller than the taxon and more susceptible to disturbances.

The IBQS is calculated according to the following formula:

IBQS = Σ Ln (Di+1) x Si

Table 8: Group of families to calculate the index.

Taxonomic group	Family	Indicator value
Gasteropoda	Arionidae	52
Gasteropodes	Cochlicopidae	67
Gastéropodes	Endodontidae	60
Isopodes	Trichoniscidae	68
Isopodes	Oniscidae	62
Pseudoscorpions	Neobisiidae	80
Chilopodes	Lithobiidae	58
Chilopodes	Cryptopidae	63
Chilopodes	Henicopidae	73
Diplopodes	Polydesmidae	58
Diplopodes	Glomeridae	60
Coléoptères	Lathridiidae	73
Coléoptères	Hydophilidae	75

Coléoptères	Coccinelidae	75
Oligochètes	lumbricidae épigés	54
Oligochètes	lumbricidae anéciques	45
Aranea	Araneidae	56
Aranea	Tetragnatidae	90
Aranea	Thomisidae	52
Aranea	Agelenidae	80
Aranea	Hahniidae	80
Lépitoptères (larves)		57

Using the following calibration table, one can assign a note to 20 of the sampled sols using the IBQS calculation:

Table 9: Ecological interpretation of index.

IBQS	Note	Soil quality	Qualification
<282-685	1-4	I	Bad
686-1089	5-8	II	Average
1090-1492	9-12	III	Good
1492-1997	13-17	IV	Very Good
1998-2300 or more	18-20	V	Optimal

S/Chapter II: Nematode indices

1. Definition

Soil nematodes can be used as bio-indicators by analyzing the nematofauna to generate various indicators (also known as parameters) that can be interpreted to understand the state of the soil.

Nematodes are microscopic worms that are extremely abundant (> 1 million per m2) and diverse (over 70 species on a site) in soils, and are commonly used as soil bioindicators.

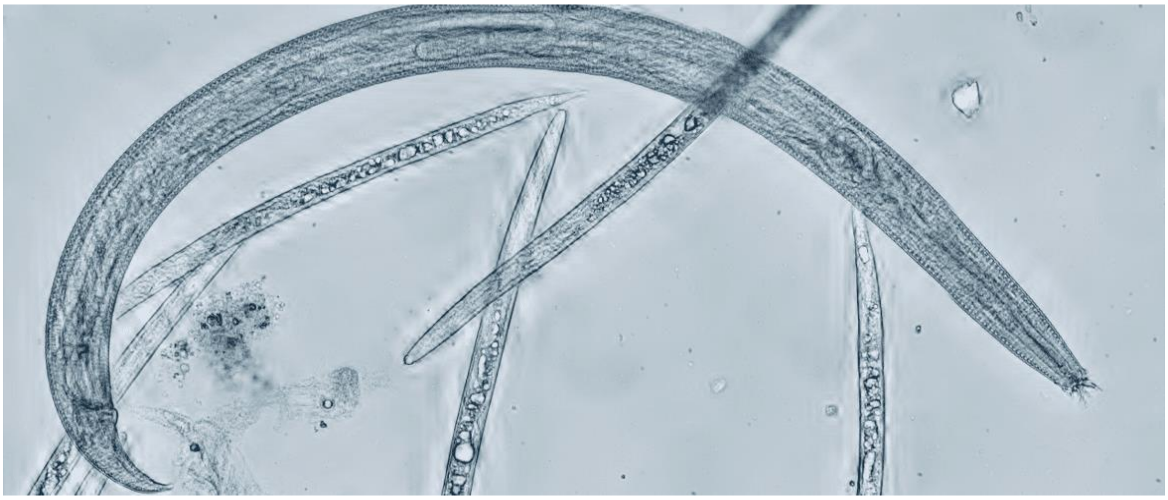


Figure 24: Nematodes.

Analysis of the nematode community, also known as the nematofauna, is carried out in the laboratory in accordance with ISO 23611-4 (2008). This analysis yields 8 major parameters. It enables us to assess the level of biological activity, nutrient flows, environmental stability and diversity of organisms in the soil.

2. The 8 major parameters derived from a nematofauna analysis are as follows:

***Total nematode abundance:** abundance of free-living nematodes (beneficial to soil functioning) and phytophagous nematodes (feeding on plant roots). This is an indicator of the level of biological activity in the soil**.**

***Abundance of beneficial nematodes**: Abundance of nematodes beneficial to soil functioning (i.e. non-parasitic): bacterivores + fungivores + omnivores + carnivores + facultative phytophages. Their abundance is an indicator of the level of biological activity in the soil.

***Abundance of plant-parasitic nematodes**: Abundance of nematodes feeding on plant roots and likely to limit their growth. Their abundance can be used to calculate an index of parasitic pressure linked to these organisms.

***Enrichment Index**: gives an indication of the resources readily available in the soil and the activity of primary decomposers. It increases with the availability of nutrients (particularly nitrogen**).**

***Structure Index**: provides information on the structure of the soil's food web, and therefore on the stability of the environment. The higher the index, the less disturbed the environment, and the more complex the soil's biological network.

***Organic matter decomposition pathways index**: provides information on the decomposition pathways of soil organic matter. The lower the index, the more important the bacterial pathway.

***Taxonomic richness**: Number of nematode families present in the sample. It provides information on the level of heritage diversity. The higher it is, the greater the diversity.

***Shannon Diversity Index**: Provides information on the level of heritage diversity, and also takes into account the relative abundance of the different families present. The higher the index, the greater the diversity.

Depending on the context, other minor parameters derived from the analysis are used, such as the abundance of omnivorous and carnivorous nematodes, or the abundance of opportunistic bacterivorous nematodes, or the abundance of major phytoparasites(Pratylenchus or Meloidogyne, for example).

3. Samples and measurements:

300–500 g composite soil samples are used for nematofaunal analysis. Simple soil sample involves coring the top 0–20 cm of soil. While sampling can be done any time of year, the best times to do it are in the spring and fall. A table of the abundances of the various nematode species is created by removing organisms from the soil, counting them, and identifying them. This table is then used to make diagnoses

Figure 25: species identification in the laboratory.

3.1. Application

3.1.1. Patrimoine biodiversityis indicated.

A soil's quality as an environment for its organisms is indicated by its non-matofaunal biodiversity index, which is based on three non-matofaunal parameters: total abundance of nematodes, structural index, and taxonomic richness.

The non-faunal biodiversity index is sensitive to soil management practices and usage. The higher the values of this indicator, the more stable and viable (or structurally suitable) the soil is for soil organisms that have access to high-quality nutritional resources (organic matter). Conversely, the deterioration of this indicator points to an unstable and altered environment (pollution, deteriorated structural state, insufficient nutritional resources) that prevents the establishment of the soil's microfauna.

3.1.2. Functioning biological is indicated

The term "nematofaunique operation index" refers to the ability of the soil to perform specific functions, such as recycling nutrients, transforming carbon, or regulating biological processes. It is derived from five non-nematofaunal parameters: the abundance of beneficial algae, the Shannon diversity index, the Enrichment Index (EI), the Structure Index (SI), the Index of Voies de Décomposition of Organic Matter (IVD), and the Index of Diversity.

The higher the nematode function index, the more capable the soil is of providing an efficient set of functions (nutrient recycling, carbon transformation, and biological regulation). This indicator is sensitive to soil usage, management techniques, and the availability of the soil's nutritional resources (amount and quality of organic matter). A low value for this index indicates an unstable environment that does not guarantee all soil functions and is conducive to the growth of plant parasitic nematodes.

S/ Chapter III: Bio-indicators of accumulation in Snails

1. Indicator principle

Snails feed on plants, soil and humus. Their ability to accumulate contaminants such as metals has been used to reveal environmental contamination at the soil-air-plant interface.

Here's how it works:

Snail selection: Snails of known origin and age are placed in cages in the field. Knowing this information is crucial to interpreting the results correctly.

Exposure time: Snails are exposed to the environment (soil) for a period of 28 days. This allows the snails to accumulate the metal contaminants present in the soil over this period.

Snail feeding: Snails feed on plants, soil and humus during the exposure period. This simulates their natural behavior and allows them to accumulate contaminants present in these food sources.

Contaminant accumulation: During the 28-day exposure period, snails accumulate metal contaminants present in the soil. Metals can come from a variety of sources, such as industrial activities, agriculture, or other sources of environmental pollution.

Concentration analysis: At the end of the exposure period, snails are collected and their tissues analyzed to determine accumulated metal concentrations. These analyses provide information on environmental contamination, as snails act as bioindicators, reflecting the presence of metals in their environment.

Interpretation of results: Metal concentrations measured in snails can be interpreted as an indication of soil metal contamination. Higher concentrations suggest greater metal availability in the environment, which may have implications for ecosystem health and food safety, given that snails are consumed by a variety of predators.

2. Sampling and measurements:

- Sub-adult snails from the farm are placed in stainless steel cages, with 15 snails per cage. The cages are cylinders 25 cm in diameter and 25 cm high.
- Before being placed in the microcosms (simulated environments), the snails are moistened to wake them up.
- The snails are exposed to the soil, plants growing on the site and ambient air for a period of 28 days.
- Pieces of tile are added to the cage to provide shelter for the snails.
- The cage is closed with a stainless-steel grid and held in place with stainless steel stakes.
- After the 28-day exposure period, the snails are collected and returned to special storage boxes.
- The samples (snails) are then sent to the laboratory for analysis.

3. Laboratory analysis

Contaminant concentrations are measured in the snails' soft tissues (viscera).
For metal contaminants, an analysis is carried out on 6 snails sampled after 28 days of exposure. This analysis was carried out by ICP-MS (inductively coupled plasma mass spectrometry) after digestion in an acid medium.

For other contaminants such as pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins, etc., larger masses of tissue may be required. In this case, all 15 snails in the cage are analyzed..

Figure 26: Expiration of snails.

4. Results

Metallic contaminants: Concentrations of 14 metallic contaminants (As, Cd, Co, Cu, Cr, Hg, Mo, Ni, Pb, Sb, Sn, Sr, Tl, Zn) are measured.

Indicator: Results are evaluated by comparison with internal reference concentrations. The index reflects the difference between the concentrations measured in snails exposed at the contaminated site and the values measured in snails exposed at an uncontaminated site.

In summary, this study aims to assess the impact of site contamination on snails by measuring the concentrations of various contaminants in their soft tissues. The index created will make it possible to compare these concentrations with those of snails exposed to an uncontaminated site, thus providing an indication of the extent of contamination.

Table 10: Ecological interpretation.

Transfer status	No abnormal transfers	Low to moderate transfer	High transfer
Index	0 à 1	1 à 5	> 5

S/ Chapter IV. MICROFLORE DU SOL

1. Definition

Soil microflora refers to all microorganisms living in the soil. This includes bacteria, fungi, protozoa, algae and other microscopic organisms.

Role: Soil microflora play a crucial role in the decomposition of organic matter, nutrient cycling, humus formation and the regulation of soil health.

2. Bacteria in Soil

2.1. Abundance and activity: Bacteria are the most abundant microbial component in soil. Their density can reach several billion per gram of soil. Because of their abundance, bacteria are key players in soil biogeochemical processes.

2.2. Physiological diversity

- **Autotrophic and heterotrophic**: Bacteria can be autotrophic, capable of producing their own food from inorganic compounds, or heterotrophic, dependent on external organic matter.
- **Mesophilic, thermophilic and psychrophilic**: Mesophilic bacteria thrive at moderate temperatures, thermophilic at high temperatures, and psychrophilic at low temperatures.
- **Aerobic and Anaerobic**: Some bacteria require oxygen (aerobic) for their metabolism, while others thrive in the absence of oxygen (anaerobic).

2.3. Genetic diversity

Due to the diversity of soil properties, all bacterial physiological types can be represented. The genetic diversity of soil bacteria is immense, and new species are regularly discovered appropriate microbiological techniques. Selective culture media can promote the growth of specific groups of bacteria, enabling them to be identified.

2.4.Ecological role:

- Decomposition of Organic Matter: Bacteria are key decomposers, playing an essential role in breaking down dead organic matter and recycling nutrients.
- Nitrogen fixation: Certain types of bacteria are able to fix atmospheric nitrogen, making it available to plants.
- Nutrient cycle: Bacteria actively participate in the cycling of nutrients such as carbon, nitrogen, phosphorus and sulfur.
- Responses to soil conditions: Bacteria can adapt to a variety of soil conditions, such as pH, moisture content, nutrient availability and temperature. Their physiological diversity enables them to adapt to a variety of environments.
- Natural environment: Although soil is a rich reservoir of bacteria, this does not mean that all bacteria are naturally present in the soil. Some bacteria may be introduced by external inputs, or may be influenced by specific soil conditions.

Figure 27: Bacteria photos.

2.5. Fungi

Soil fungi play an essential role in the dynamics of terrestrial ecosystems, making a significant contribution to soil biomass, often equivalent to that of bacteria. Their impact on the ecological balance of soils is manifested through a diversity of fundamental metabolic activities. Their ability to establish symbiotic interactions with plant root systems, notably through the formation of mycorrhizae, is particularly crucial. These beneficial associations promote an exchange of nutrients between fungi and plants, enhancing the plants' ability to absorb essential elements from the soil.

At the same time, fungi demonstrate a remarkable ability to colonize different substrates. Through their hyphae, they can explore areas of the soil inaccessible to plant roots, thus extending their influence on soil biodiversity. This ability to colonize also promotes the availability of nutrients in a variety of ecological niches, contributing to the resilience and stability of ecosystems.

A crucial aspect of the metabolic activities of soil fungi lies in their role as decomposers. They are skilled at breaking down large-scale organic debris, such as dead leaves and branches. This decomposition process releases essential nutrients in a form accessible to soil organisms, thus contributing to soil fertility and element cycling. Just as significantly, fungi are capable of degrading complex organic compounds, including the lignins present in plant matter, helping to decompose more resistant organic structures.

In conclusion, the importance of soil fungi in terrestrial ecosystems goes beyond their simple contribution to soil biomass. Their interactions with plants, their colonization capacity and their role in the degradation of organic matter are crucial elements influencing soil health and sustainability, as well as the overall balance of terrestrial ecosystems.

2.6. Algae and protozoa

Algae and protozoa play specific roles in soil ecosystems, although algae are often considered relatively scarce. Their presence, however, is common and diverse. Soil algae include a variety of species, both coccoid and filamentous, with the Chlorophyceae constituting the most common groups. Among photosynthetic soil microorganisms, Cyanobacteria are often dominant in neutral and alkaline soils, while algae predominate in more acidic soils. Algae contribute to the production of organic matter through photosynthesis, enriching the soil with organic compounds. As microbial predators, protozoa play a key role in controlling bacterial populations and recycling nutrients by degrading organic matter. So, although often less abundant than other soil microbial components, algae and protozoa contribute significantly to the dynamics and biodiversity of soil ecosystems.

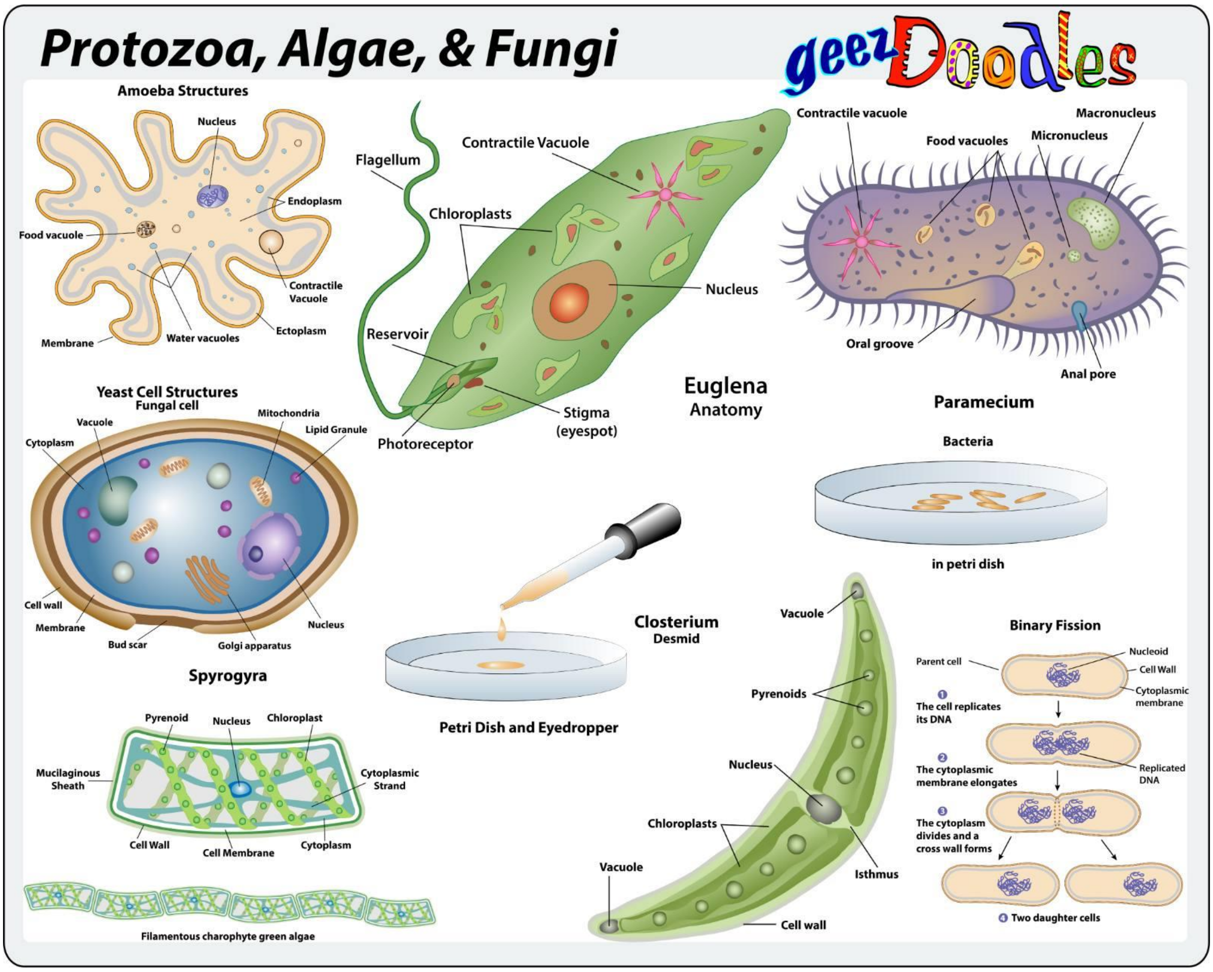


Figure28: Protozoa-Algae and Fungi.

Microbial biomass, often used as an indicator of soil quality and evolution, refers to the quantity of microorganisms present in the soil, measured by weight. Soil microorganisms play a crucial role in biogeochemical cycles, such as the cycling of nitrogen, carbon and other essential elements. To assess microbial biomass and understand microbial population dynamics, several methods are employed:

3.Direct enumeration of microorganisms:

Petri plate method: Microorganisms are grown on specific media, and the resulting colonies are counted.

Microscopy: Using microscopes, microorganisms can be observed and counted directly in the soil.

Molecular methods: DNA or RNA are extracted from the soil, amplified by PCR, and quantified to estimate the diversity and quantity of microorganisms.

3.1. Measurement of overall microorganism activity:

Measuring soil respiration: The quantity of carbon dioxide emitted by the soil is measured to assess overall microbial activity.

Measurement of enzyme activity: Certain enzymes produced by microorganisms can be measured to estimate their metabolic activity.

3.2. The specific activity of a group of microorganisms:

Use of specific markers: Labeling specific groups of microorganisms with stable isotopes enables us to measure their activity in the soil.

Measuringthe fixation of specific substrates: Specific substrates are added to the soil to measure the activity of particular microbial groups.

4. The principle of calculating microbial biomass in soil

Given that organic carbon is a significant component of soil microorganisms, this method aims to estimate the amount of soluble organic carbon in the soil, which may serve as an indirect indicator of microbial biomass. This method may yield information about soil fertility and microbial dynamics related to soluble organic matter.

The assessment of microbiological biomass by measuring the amount of soluble organic carbon in a soil sample. Here is a more thorough explanation of the described protocol:

4.1. Setting up the earth simple

A freshly harvested, non-gelled sample of soil is sieved and ground into 5 mm particles. This makes it possible to achieve particle homogeneity and makes subsequent analyses easier.

The tamed simpleis kept at 4°C to preserve its freshness and prevent any alteration of its microbiological properties.

4.2. Establishment of two equal lots from the simple

Two equal lots, each weighing 25 g of dry land, are created from the original simple. This makes it possible to have duplicates in order to guarantee the reproducibility of the results.

4.3.Dosage of soluble organic carbon

Measuring the amount of soluble organic carbon involves adding a K2SO4 solution (0.05 N) to the fresh soil mixture. This solution is used as an extractant to dissolve the organic carbon that is present in the soil.

The fresh soil mixture with K2SO4 0.05 N solution is allowed to come into contact for 45 minutes at room temperature. This contact time allows for the efficient extraction of soluble organic carbon.

4.4. Analysis of soluble organic carbon

Measuring the amount of soluble organic carbon in the solution occurs after the contact time. This measurement can be carried out using a variety of analytical techniques, such as spectrophotometry or chromatography, depending on the equipment that is available and the necessary sensitivity.

5-Calcul

First, the amount of extractable carbon of microbial origin is calculated:

ExtractableCarbon (E.C.) = Fumigated extracted C - Non-fumigated extracted C

This quantity is directlyproportional to microbial biomass.

It can be converted into microbial biomassbiomass (expressed in mgC/kg soil) using a using a proportionality coefficient (CarbonBiomass (in mgC/kg soil) = Microbial Biomass (MB)

Microbial Biomass (MB) = C.E. / KECwith KEC = 0.45.

5.1. Significance

Microbial biomass represents the quantity of "living carbon" contained in soil microbes, mainly bacteria and fungi. It is an early indicator of organic matter dynamics organic matter, which reacts quickly to favorable or unfavorable the environment. It is both a transformative

compartment (soil mineralization potential) and a storage compartment (storage) capable of trapping elements such as nitrogen (100 mg of BM per kg of soil represents a stock of 45 kg nitrogen/ha).

The analysis can also be used to determine the BM/Soil Organic Carbon ratio. This is an important qualitative parameter for soil functioning.

This parameter assesses the nutritional quality of soil organic matter, as well as the quality

Chemicaland physical environment of the microbial biomass.

5.2. Elements of interpretation

Microbial biomass varies between 0 and 700 to 800 mg C/kg soil in agricultural soils. The factors in the variation of microbial biomass are temperature, humidity, energy status of the soil
of the soil (OM reserves, particularly those that are easily degradable OM), the physical environment (structure and porosity) and chemical environment (CEC, pH, calcium). Microbial biomass content is linked to soil type, crop type and cultivation techniques.
The BM/C ratio, in %, varies between 0 and 5%, usually. The lowest values indicate, for a given soil type: a physical environment unfavorable to life (compaction, compaction, hydromorphy),a chemical environment unfavorable to life (acid pH, calcium deficiency, copper toxicity, etc.).

Chapter III: Air quality indicator

1. Definition

The term "air pollution" refers to the existence of harmful elements in the atmosphere in amounts that are higher than those found in nature or that may have an adverse effect on human health, wildlife, plants, materials, and/or the overall quality of the air. These substances, also referred to as atmospheric pollutants, can take on a variety of forms, including gases, liquid or solid particulates, volatile organic compounds, heavy metals, and other chemical agents. The main causes of air pollution are industrial emissions, transportation-related activities, the burning of fossil fuels, agriculture, and other human activities.

Air pollution can have serious health effects as well as ecological effects, affecting the quality of water and soil as well as contributing to climate change. Health issues include respiratory and cardiovascular diseases. The global fight against air pollution entails initiatives to cut pollution emissions and encourage sustainable practices across a range of industries.

2. The pollutants and their resources

The main human activities that have intensified over the past few centuries are the cause of air pollution, with transportation and industry being the biggest contributors. The combustion of fossil fuels, industrial processes, vehicle emissions, and other human activities all contribute to emissions. Numerous atmospheric pollutants are released as a result of these activities, including carbon monoxide (CO), dioxyde of sulfur (SO2), fine particulates, oxydes of azote (NOx), and volatile organic compounds (VOCs).

It becomes possible to quantify, monitor, and control air pollution while developing strategies aimed at containing these harmful emissions by understanding the sources of the pollutants and using bioindicators. This approach helps to minimize the influence of human activity on the atmospheric environment by promoting sustainable practices and safeguarding air quality.

3.Biological marker lichens

Definition

Lichens are compound organisms resulting from a symbiosis between a fungus and a chlorophyll alga (cyanobacteria). The fungus provides the algae with water and mineral salts, and the algae provides the fungus with certain foods that it synthesizes through photosynthesis.

- are perennial organisms
- are widely distributed organisms
- have no means of protection (stomata, cuticle, etc.)
- have no roots
- absorb water and essential elements from the atmosphere
- develop very slowly

As indicator organisms of environmental quality. As pioneers and colonizers, lichens are exposed to a variety of threats, including environmental changes resulting from human activity, such as land-use planning, agriculture, forestry, pollution and tourism.

Rootless and respiratory, lichens depend on atmospheric inputs and run-off water for their survival. Their fragility is reinforced by their symbiotic constitution, where the aggression of just one of the two partners can upset the balance and lead to the loss of vitality or even the complete destruction of the thallus.

As indicators of environmental quality, lichens are widely used to monitor terrestrial ecosystems. Their observation can detect the diffusion of various pollutants such as heavy metals, radionuclides and chemical substances, helping to assess ecosystem health

Figure 29: Lichens

Lichens are also used as a tool for assessing the quality of management of natural areas. Concrete examples, such as the Pays de Bitche Nature Reserve of rocks and peatlands in Moselle, illustrate how the lichen inventory made it possible to evaluate management choices and put in place practical recommendations for conservation.

The study of lichens can also raise awareness of the consequences of human development on biological diversity, particularly for rare or endangered plant species. Where lichens disappear, other threatened species may be at risk.

4. Bryophytes,

Bryophytes, which include mosses, liverworts and anthocerota, are also used as bioindicators of environmental quality. Like lichens, bryophytes are sensitive to changes in their environment and can provide important information on ecosystem health. Here are some points to consider regarding the use of bryophytes as bioindicators:

- **Sensitivity to pollutants:** Bryophytes react to atmospheric pollutants and the deposition of toxic substances. Their presence, abundance and diversity can indicate the level of air and soil pollution.

- Responses to climate change: Bryophytes are also sensitive to climatic variations. Changes in their geographical distribution, abundance or diversity can be linked to climate fluctuations and provide indications of local climatic trends.

- **Habitat and ecosystem management**: Bryophytes are highly dependent on humidity and water availability. Their presence or absence can reflect the state of aquatic or wetland ecosystems, and their study can help assess the effectiveness of habitat conservation and management measures.

Figure 30: Bryophytes.

5. Trefoils

1. Definition

Clovers are herbaceous leguminous plants that are sensitive to ozone, an atmospheric pollutant harmful to human health, flora and fauna. These plants mainly exchange information with the atmosphere at leaf level, through stomata, tiny orifices on the surface of the epidermis.

Stomata play a crucial role in regulating gas exchange between the plant and the environment. However, they are also a potential entry point for atmospheric pollutants such as ozone. The greater the quantity absorbed by the leaf, the greater the impact of pollutants. Thus, the quantity of pollutant absorbed depends on both the concentration of pollutant in the air and the degree of stomatal opening.

The concentration of pollutants in the air can vary according to various factors such as industrial activities, automobile traffic and other sources of air pollution. Consequently, as ozone-sensitive plants, clovers can be affected by the quality of the surrounding air. Air pollution management is therefore essential to minimize adverse effects on plant, animal and human health.

Figure 31: Different plant Trefoils

The impact of this pollutant on plants is manifested by:

- **Foliar damage**: the appearance of spots or necrosis on leaf surfaces after a few hours' exposure to ozone, even at relatively modest concentrations.
- **Damage**: the appearance of metabolic disturbances without apparent damage, but which lead to a reduction in crop growth or productivity.
- **Senescence** is the aging phenomenon whereby leaves turn yellow, dry out and eventually die, under the effect of ozone.

6. Petunias

It seems that petunias (Petunia hybrida) are used as bio-indicators to detect the presence of traces of hydrocarbons, probably in the surrounding soil or water. Bioindicators are organisms that respond in observable ways to changes in their environment, providing information about its quality.

Figure 32: Petunias.

Effects observed on petunias in the presence of hydrocarbons include leaf necrosis and chlorosis. Necrosis manifests itself as areas of dead tissue, while chlorosis results in yellowing of the leaves due to a lack of chlorophyll. These symptoms generally indicate physiological stress or a disturbance in the plant's metabolism.

In addition, the overall development of the plant seems to be affected, with a reduction in the number of flowers and leaves, a smaller stem and less branching. These changes may be the result of a disruption in the plant's normal growth process, affecting its ability to produce organs and develop in a healthy way.

These observations suggest that petunias react specifically to hydrocarbons, making them useful indicators for the detection of these substances in the environment. The presence of hydrocarbons in soil or water can have important environmental implications, as these compounds are often associated with pollution and can be toxic to many organisms, including plants.

Figure 33: Petunias exhibition at hydrocarbons on the first day.

Figure 34: Petunias after 4 weeks of exposure.

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