II- Chapter 02: Biological Analyses of the Environment

In a balanced ecosystem, each organism has specific requirements. Any modification of one of the environmental characteristics can disturb its normal equilibrium. It is also possible to demonstrate the existence of pollution through the study of animal and plant communities. This is known as biological study for determining environmental quality.

1- Fundamental Concepts:

- **Bioindicator**

A bioindicator can be defined as a species or a group of plant or animal species whose presence or absence, abundance, biomass, or other biological characteristics provide information about the ecological state of an environment and its level of degradation.

- **Biomarker**

A biomarker refers to any biochemical, cellular, physiological, or behavioral parameter measured in the tissues or fluids of an organism, used to reveal the effects of exposure to one or more contaminants.

- **Bioaccumulator**

A bioaccumulator refers to the ability of certain organisms (plants, animals, fungi, or microorganisms) to absorb and concentrate within a part of their body—such as plant tissues (bark or wood) or animal structures (shell or horn)—specific chemical substances, which may be rare in the environment (trace elements or toxic elements), without being negatively affected.

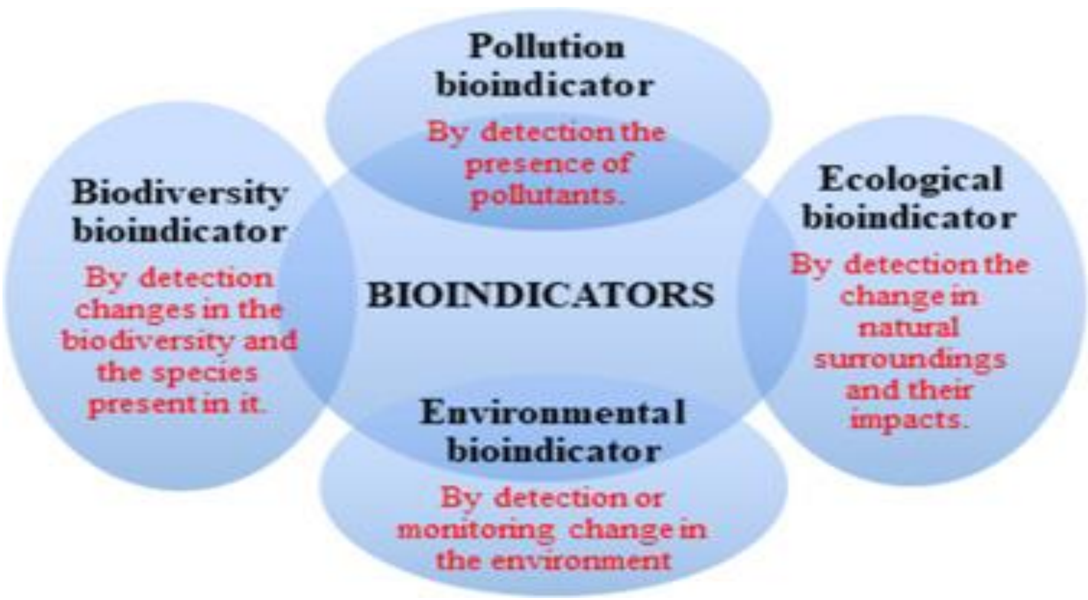

Figure 01. Role of bioindicators in contaminated ecological ecosystems

1-1-Ecological roles of Bioindicators:

Bioindicators should be used to:

- Provide early warning signals of environmental problems.
- Identify cause-and-effect relationships between degrading factors and biological effects.
- Assess the overall stress condition of the environment through the different responses of indicator organisms.
- Improve the effectiveness of restorative measures on the health of biological systems.

1-2- Types of Bioindicators According to Their Reactions:

1-2-1- Accumulation bioindicators:

These are organisms that accumulate one or more substances from their environment, which allows the assessment of their exposure levels.

1-2-2- Effect or impact bioindicators:

These are organisms that reveal biological effects (such as fatigue, disease, or death) when exposed to one or more substances originating from their environment—often affected by agricultural pollution or industrial contamination.

1-2-3- Pollution-sensitive bioindicators:

These are species known to thrive only under specific vital conditions. Their appearance, abundance, or seasonal disappearance indicates the ecological quality of their natural habitat (presence or absence of pollutants).

2- Biological methods for evaluating environmental quality:

2-1- Biochemical methods:

These involve the assessment of biomarkers indicating any observed or measured change at the molecular level (biochemical, cellular, physiological, or behavioral) in an organism, caused by exposure to a toxic chemical substance. Examples include DNA alterations, protein synthesis disturbances, or enzyme activity changes.

2-2- Ecotoxicological Methods:

This involves a set of toxicological tests on a population of organisms exposed to a specific pollutant. These bioassays aim to estimate the concentrations that cause toxic effects (such as reduced physiological and reproductive activities, or increased mortality). The bioaccumulation capacity represents all analyses on bioaccumulators that capture and store these pollutants (toxic molecules, trace elements, pesticides, etc.), which is expressed by a Concentration Factor (CF).

$$\textbf{FBC} = \frac{\text{Concentration of a compound in the living organism}}{\text{Concentration of the same compound in the surrounding environment}}$$

2-3- Biocoenotic methods:

These methods make it possible to monitor the evolution of biocoenoses (animal and plant populations) within a specific space and time. Biocoenotic methods are often carried out through ecological zonation, which divides watercourses according to the slope width or the quality of benthic species (insects).

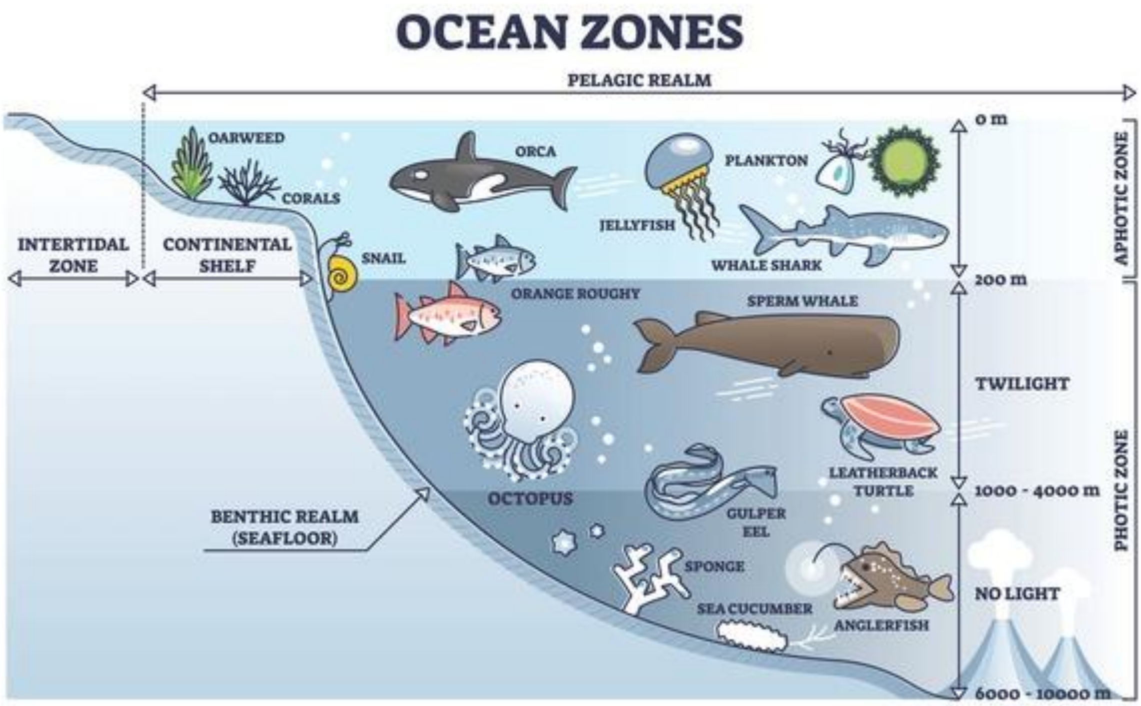

Figure 02. Variable types of Zonation

Biological treatments of biocoenoses also include all quantitative analyses of community structure and biodiversity indices of ecosystems, ranging from species richness to the Shannon-Weaver index.

3- Bioindicators of natural ecosystems:

3-1- In Aquatic environments:

3-1-1- For plant species:

3-1-1-1- Diatomic indices:

Diatoms **(Fig. 03)** are microscopic unicellular algae found in both freshwater and marine environments. They live either suspended in the water or attached to the bottom of lakes and rivers. Diatoms use dissolved phosphorus and nitrogen in water for their growth and are particularly affected by heavy metals and pesticides. The set of diatomic indices allows the evaluation of environmental parameters such as eutrophication, acidification, saprobity, and salinity in aquatic ecosystems. Among these diatomic indices, we can mention:

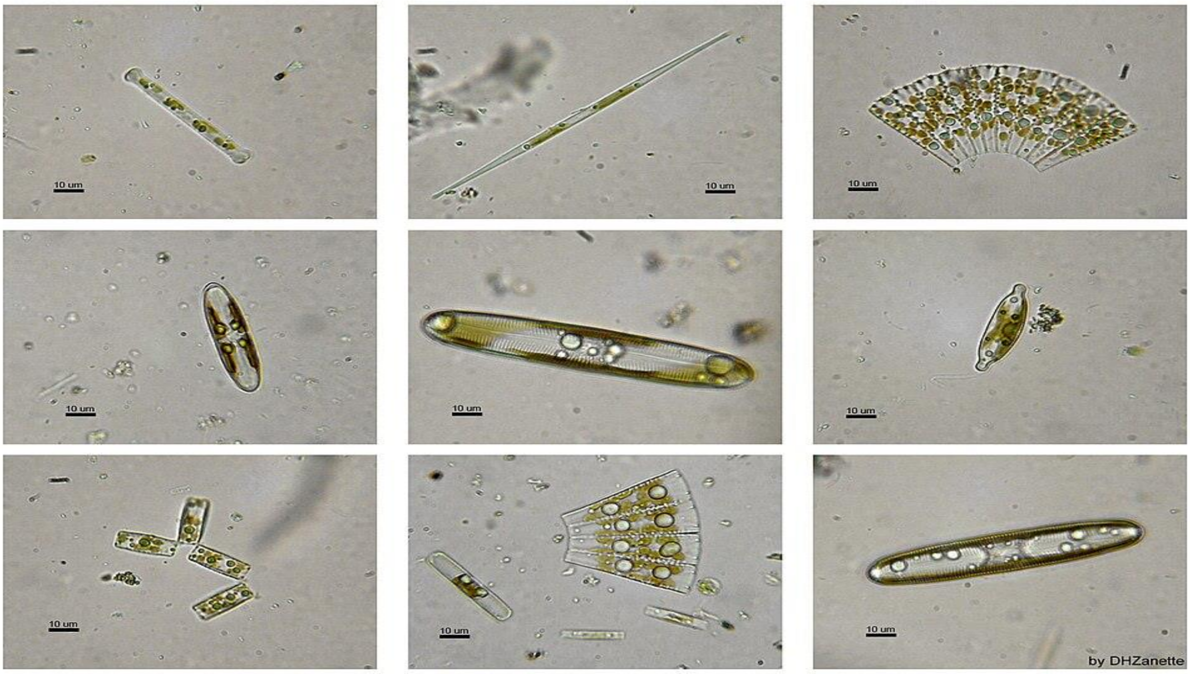

Figure 03. Different Classes of Diatoms in Aquatic Environments

- **Specific Pollution Sensitivity Index of Diatoms:**

This index represents the tolerance level of a diatom species to organic pollution in the aquatic environment (such as the presence of phosphorus or nitrate). It is expressed by the following equation:

$$IPS1 = \frac{\sum(Ai \times Si \times Vi)}{\sum(Ai \times Vi)}$$

Where:

Ai: Relative abundance of species *i*

Si: Sensitivity value of species *i* **(Table 01)**

Vi: Indicator value of species *i* **(Table 01)**

Table 01. Pollution sensitivity (S) and Indicator values (V) of some Diatom species:

Genus	Species	S	V
Achnanthes	*Achnanthes hungarica*	2	3
Amphora	*Amphora pediculus*	4	2
Cratícula	*Cratícula accomoda*	1	3
Cyclotella	*Cyclotella atomus*	2	1
Navícula	*Navícula lanceolata*	3	1
Neidium	*Neidium iridis*	5	2
Melosira	*Melosira nummuloides*	2	3
Sellaphora	*Sellaphora pupula*	2	2

Then, the **IPS** value is estimated from the **IPS₁** value as follows

$$IPS = 4.75 \times IPS1 - 3.75$$

Table 02. IPS and environmental quality

IPS Value	Pollution Classification	Ecological Condition
$17 \leq IPS < 20$	No or low pollution / eutrophication	Very good
$13 \leq IPS < 17$	Moderate eutrophication	Good
$9 \leq IPS < 13$	Moderate pollution or high eutrophication	Fair
$5 \leq IPS < 9$	High pollution	Poor
$1 \leq IPS < 5$	Very high pollution or eutrophication	Very poor

$$\boldsymbol{F(i)} = \frac{\sum_{x=1}^{n} Ax \times Px(i) \times Vx}{\sum_{x=1}^{n} Ax \times Vx}$$

Where:

- **Ax:** Abundance of taxon *x* (expressed in ‰);
- **Px(i):** Probability of occurrence of paired taxon *x* for water quality class *i*;
- **Vx:** Ecological value of paired taxon *x*;
- **n:** Number of paired taxa retained after applying the threshold values of presence.

The seven values of **F(i)** are thus estimated. An index B is then calculated according to the following formula:

$$B=1\times F(1)+2\times F(2)+3\times F(3)+4\times F(4)+5\times F(5)+6\times F(6)+7\times F(7)$$

The **IBD** value is then determined according to the following table:

Table 03. IBD values based on B values

B Value	> 2	**2 to 6**	≥ 6
IBD	1	$4.75 \times B - 8.5$	20

Table 04. IBD and environmental quality

IBD	**≥ 17**	**17 > IBD ≥ 13**	**13 > IBD ≥ 9**	**9 > IBD ≥ 5**	**< 5**
Ecological State	Very good	Good	Moderate	Poor	Bad

3-1-2- For animal species:

3-1-2-1- Saprobic indices:

A saprobe is an aquatic organism that lives in an environment rich in dead or decomposing organic matter, which serves as its food source in oxygen-deficient conditions. The Saprobic Index (SI) therefore reflects the tolerance of organisms to the presence of organic matter and indicates the level of organic pollution in the environment.

3-1-2-2- Invertebrate Indices:

Marine invertebrates are multicellular animals without a vertebral column that live in aquatic environments. They include several taxa such as sponges, cnidarians (jellyfish, corals), marine worms, mollusks (snails, slugs), arthropods (crabs, shrimps, lobsters), and echinoderms (starfish, sea urchins). Macroinvertebrates can indicate the quality of aquatic environments depending on their groups, as follows:

- **Oligochaetes: Oligochaete sediment bioindication Index (IOBS)**

Oligochaetes are a subclass of annelid worms abundant in fine, sandy, or coarse sediments of rivers and lakes. They are known for their varying sensitivity and resistance to organic pollution. Their index, called the Oligochaete Sediment Bioindication Index (IOBS) **(Table 05)**, indicates the ecological impact of organic and metallic micropollutants **(Figure 04)**.

Figure 04. Examples of Marine Oligochaete Species

The value of this index (IOBS) is given by the following equation:

$$IOBS = \frac{10 \times S}{T}$$

With:

S: Total number of taxa identified among the 100 oligochaetes.

T: Percentage of the dominant group.

Table 05. IOBS and sediment quality

IOBS	Biological quality of sediments
≥ 6	Very good
$3 \leq IOBS \leq 6$	Good
$2 \leq IOBS \leq 3$	Moderate
$1 \leq IOBS \leq 2$	Poor
$IOBS < 1$	Bad

❖ **Chironomids: Benthic quality index of Chironomids (IQBC):**

This family belongs to the Nematoceran Diptera and includes small insects related to the Ceratopogonidae, Simuliidae, and Thaumaleidae, bearing a strong resemblance to mosquitoes. In their larval form, these species are highly resistant to pollution and serve as good indicators of aquatic environment quality **(Fig. 05).**

The ecological index of Chironomids, called the Benthic Quality Index of Chironomids (IQBC), takes into account the number of individuals of each indicator species group relative to the total number of individuals of all indicator species.

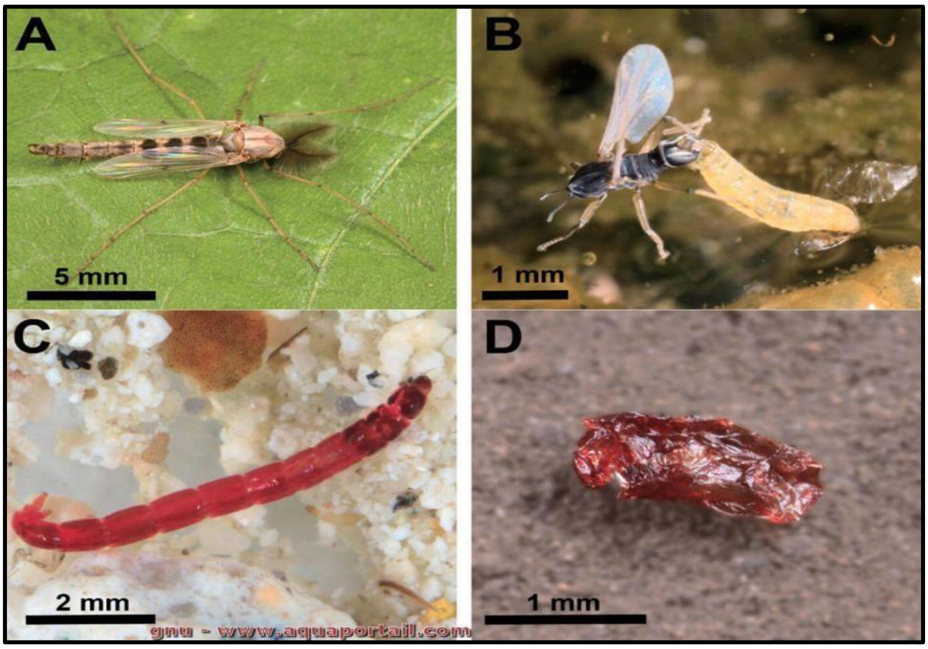

Figure 05. Life cycle of Chironomids (A: Adult; B: Egg laying; C: Larva; D: Pupation)

The value of this index (IQBC) is given by the following equation:

$$IQBC = \frac{\sum_{i=0}^{5} \mathbf{ki} \times \boldsymbol{ni}}{N}$$

With:

N: Total number of individuals of all indicator species.

ni: Number of individuals of the indicator species group *i*.

- ki = 5 for *Macropelopia spp.*, *Paracladopelma nigritula* group, and *Heterotrissocladius spp.*
- ki = 4 for *Micropsectra spp.* and *Paratendipes spp.*
- ki = 3 for *Sergentia coracina* and *Stictochironomus spp.*

- ki = 2 for *Chironomus anthracinus* and *Tanytarsus spp.*
- ki = 1 for *Chironomus plumosus*
- ki = 0 if the indicator species are absent

❖ **Mollusks: Malacological index of lake system quality (IMOL):**

This phylum of lophotrochozoans colonizes the deeper zones of aquatic environments **(Fig. 06)**. They serve as indicators of lake system quality according to the Malacological Index of Lake System Quality (IMOL), which is mainly based on the calculation of mollusk communities inhabiting lakes of maximum depth. To estimate this index, mollusks are collected from three different depths:

- $Z_1 = 9/10$ Zmax,
- $Z_2 = -10$ m,
- $Z_3 = -3$ m.

For each species identified at each specific depth, the IMOL value varies according to the following table **(Table 06).**

Figure 06. Examples of marine mollusk species

Table 06. Standard values of the IMOL Index

Sampling Level	Malacological Indicators	Index
Z_i = 9/10 Z_{max}	Gastropods and bivalves present	8
	Gastropods absent, only bivalves present	7
	Absence of mollusks at Zi	—
Z_2 = –10 m	Two or more genera of gastropods present	6
	Only one genus of gastropod present	5
	Gastropods absent, pisidiids present with more than one individual per grab	4
	Absence of mollusks at Z_2	—
Z_3 = –3 m	Two or more genera of gastropods present	3
	Only one genus of gastropod present	2
	Gastropods absent, pisidiids present with more than one individual per grab	1
	Absence of mollusks	0

3-1-2-3 – Vertebrate indices:

Marine vertebrates are represented by four major classes: mammals, birds, fish, and reptiles. Among these classes, fish **(Fig. 07)** are the species most resistant to pollution and serve as the best indicators of water quality through the estimation of the river fish index **(IPR)**.

• River Fish index (IPR)

The IPR makes it possible to assess the degree of alteration of fish communities based on various characteristics that are sensitive to the intensity of human-induced disturbances. It determines the ecological status of the aquatic environment according to seven parameters:

- Total number of species
- Number of rheophilic species
- Number of lithophilic species
- Density of tolerant individuals
- Density of invertivorous individuals
- Density of omnivorous individuals
- Total density of individuals

The IPR is obtained by summing the seven estimated parameter values. It varie according to the following table **(Table 07).**

Figure 07. River fish species used as indicators of pollution

Table 07. IPR and environmental quality

IPR	**<7**	**7 à 16**	**16 à 25**	**25 à 36**	**>36**
Quality class	Excellent	Good	Fair	Poor	Very Poor

3-2 – In the atmospheric environment:

3-3-1 – For Plant species:

- **Lichens:**

Lichens are formed by a symbiotic association between a fungus and an alga. Their biological characteristics make them highly dependent on-air quality. Therefore, they are very sensitive to gaseous pollutants and may even disappear in polluted or impacted atmospheres **(Fig. 08).**

Figure 08. Variety of lichen species

Among the indices used to indicate lichen quality and their environmental conditions, we can cite:

- **Atmospheric purity index (IPA):**

This is a quantitative approach based on a pollution index related to lichen flora, as well as the number and frequency of each species **(Table 08)**. It is estimated using the following equation:

$$IPA = \frac{1}{10} \times \sum_{i=1}^{n} (Oi \times Fi)$$

With:

- *i*: Lichen species
- *ni*: Number of species at the station
- *Oi*: Ecological index of species *i*
- *Fi*: Cover coefficient of species *i* (from 1, rare, to 5, abundant)

Table 08. IPA and environmental quality

IPA	0 to 15	15 to 30	30 to 45	45 to 60	> 60
Type of Atmospheric Pollution	Very high	high	Moderate	Low	Very low

3-3-2 – For Animal species:

- **Bees:**

Bees pollinate more than 80% of plant species and are also highly sensitive to phytosanitary treatments applied to crops. Therefore, they are considered excellent bioindicators of environmental quality, capable of detecting the presence of pollutant molecules in natural habitats **(Fig. 09).**

Figure 09. Bees as indicators of atmospheric environmental quality.

3-3 – In the terrestrial environment:

3-3-1 – For Plant species:

- **Heavy metal hyperaccumulator Plants:**

In terrestrial environments, analyses are carried out on leaves collected from representative species within the plant community of contaminated soils. These analyses assess the overall phyto-availability of metallic contaminants in pollution-indicator plants. Some accumulator plants have the ability to absorb pollutants from the soil and store them in their tissues, a process used in phytoremediation techniques for polluted soils

Figure 10. *Phyllanthus orbicularis*: Heavy metal hyperaccumulator plant

The index used for plants as indicators of soil quality is:

- **CMT Plant Index:**

This index is based on a comparison between the concentrations of trace metallic elements found in contaminated samples (plant leaves) and those in control samples characteristic of non-contaminated sites **(Table 09).**

Table 09. CMT–Plants and Soil Quality

CMT	**Environmental Quality**
0 – 5	Non-polluted environment
5 – 15	Moderately polluted (Zone monitoring)
> 15	Polluted environment

3-3-2 – For Animal species:

- **Snails:**

Snails **(Fig. 11)** feed on plants, soil, and humus. Their ability to accumulate contaminants such as metals has been used to detect pollution in environments located at the soil–air–plant interface. Snails play an important ecological role as they serve as prey for many consumers, including glowworms, birds, hedgehogs, and humans. Their physiological condition and contamination levels can be assessed using the SET-Snail Index.

Figure 11. Snail; Bioindicator and Bioaccumulator of terrestrial pollution

- **SET-Snail Index:**

This index indicates the zoo-availability of metallic contaminants in soils. It varies according to and influences soil quality as shown in the following table **(Table 10).**

Table 10. SET-Snails and soil quality

SET–Snails	Environmental Quality
0 – 1	Non-polluted environment
1 – 5	Moderately polluted (Zone monitoring)
> 5	Polluted environment