# BIOINDICATORS OF ENVIRONMENTAL HEALTH



**Cooperation beyond borders.**
Interreg-IPA Cross-border Cooperation Romania-Serbia Programme is financed by the European Union under the Instrument for Pre-accession Assistance (IPA II) and co-financed by the partner states in the Programme.
**Project RoRS 337- ROmania Serbia NETwork for assessing and disseminating the impact of copper mining activities on water quality in the cross-border area (RoS-NET2)**

# 1 Introduction

**Biotic stress factors**, such as food supply, intra and interspecific competition, diseases, parasites, predator-prey relationships, as well as **abiotic stress factors**, such as climate change and radiation, are **influencing organisms**, **populations**, **bioceonoses**, and ultimately **ecosystems**. Thus, for all living systems, the ability to react to these stress factors is an important characteristic. No development of the species and ecosystems could be possible without the presence of these stress factors, but in recent centuries a new qualitative and quantitative dimension of these changes has been reached (Markert et al., 2003).

In recent time, the **pollution** of the environment has intensified due to **industrialization** and **urbanization**, new stress factors, such as new substances like **xenobiotics** and **heavy metals**, being added to the natural stress factors due to human activity. The **identification** and **remediation** of the pollution is imperative in aerial, aquatic and terrestrial environments, as all are affected. There are several methods used to identify the degree of pollution, one of these being the use of **bioindicators** (Markert et al., 2003; Parmar et al., 2016).

## 1.1 Bioindicators

The term "**bioindicators**" describes **living organisms** that are used in the assessment of environmental health, in the detection of environmental changes and as biomarkers for assessing the quality of the environment, as well as the possible state of pollution (Parmar et al., 2016). A bioindicator is an organism, a part of an organism or a community of organisms that contain information regarding the quality of the environment (Markert et al., 2003).

The **presence** of bioindicator organisms is **regulated** by certain natural **factors** such as temperature, light, suspended solids, etc., as well as the presence of pollutants and other contaminating factors (Parmar et al., 2016). The organisms that are selected and used as bioindicators are species that **react sensitively to the factor**, natural or anthropic, that is investigated (Markert et al., 2003).

## 1.2 Advantages of bioindicators

The use of bioindicators for the assessment of environmental health and of the degree/level of contamination/pollution has several **advantages**, such as: due to their prevalence, the organisms can be **easily counted**; the synergetic and antagonistic effects of various pollutants can be **monitored** on an organism; the determination of **biological impact** can be done; it enables the monitoring of early stage **diagnosis** and of harmful effects of toxins to different organisms; it is an economically viable alternative compared to different specialized monitoring systems (Parmar et al., 2016).

# 2 Bioindicator types

Bioindicators can be categorized into different types and subtypes, using different criteria:

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- **By detection:**
    - Pollution bioindicators – detect the presence of pollutants;
    - Environmental bioindicators – detect the change in the environment;
    - Biodiversity bioindicators – detect changes in biodiversity;
    - Ecological bioindicators – detect the change in natural surroundings and their impacts (Parmar et al., 2016);
- **By mode of action:**
    - Accumulation bioindicators – accumulate elements and compounds from their environment, by different paths such as:
        - Biomagnification – absorption of substances from nutrients via the epithelia of the intestines;
        - Bioconcentration – direct uptake of the substances from the surrounding media through tissues and organs;
    - Effect / impact bioindicators – in response to exposure to a substance or element, these demonstrate specific or unspecific effects, such as:
        - Changes in morphological structure;
        - Changes in histological structure;
        - Changes in cellular structure;
        - Changes in metabolic and biochemical processes;
        - Changes in behavior;
        - Changes in population structure;
- **By origin:**
    - Active bioindicators – are bred in laboratory and are exposed in the field for a defined period of time; at the end of exposure the organisms are analyzed, and the provoked reactions are observed;
    - Passive bioindicators – are already occurring naturally in the ecosystem and are analyzed for their reaction (Markert et al., 2003).

# 3 Bioindicator cyanobacteria and algae

## 3.1 Cyanobacteria bioindicators

Although many **cyanobacteria** species are regarded as problematic symptoms in eutrophic conditions, some species can be useful as bioindicators. Many species of cyanobacteria have a **wide tolerance** for a great range of **environmental factors and of pollution levels**, however there are **some** cyanobacteria species that are **characteristic for unpolluted waters**, emerging as suitable bioindicators (Mateo et al., 2015).

## 3.2 Algae bioindicators

Micro and macroalgae can be used as bioindicators as these organisms pay an **important role in the removal of pollutants** from their environment. In the aquatic environment, algae play a **key role in the biogeochemical cycling of pollutants** and their **accumulation in sediments**, and these organisms also pass the accumulated pollutants further up in the food chain (Stankovic y Stankovic, 2013).

### 3.2.1 Phytoplankton as bioindicator species

**Phytoplankton** species such as *Pseudokirchneriella subcapitata*, *Chlorella vulgaris* and *Scenedesmus subspicatus*, can be used as bioindicators in **standard tests**, representing the primary producer (Stankovic y Stankovic, 2013). However, the usefulness of some phytoplankton species may be hampered by the wide ecological tolerance of these species (Dokulil, 2003).

**Example**
The pollution of marine ecosystems can be indicated by the changes in biodiversity of phytoplankton species, such as *Euglena clastica*, *Trachelon anas* and *Phacus tortus* (Parmar et al. 2016).

### 3.2.2 Periphyton as bioindicator species

The algal **periphyton** from running waters are **ideal bioindicators** due to their ubiquitous occurrence from clean springs to highly polluted waters, as long as the environmental limits of the species is well understood. The algae category that meets the most of the requirements for being used as bioindicators are **diatoms**, as for these algae the most objective accounts of the tolerance of individual species have been made (Dokulil, 2003).

### 3.2.3 Macroalgae as bioindicator species

**Example**
Algae from the genus *Klebsormidium*, which form algal mats, are bioindicator species for elevated iron concentration in the environment, while the presence of *Fucus vesiculosus* indicate heavy metal pollution in marine ecosystems (Stankovic y Stankovic, 2013).

Marine **macroalgae** are an important part of aquatic communities and can be used as bioindicators to predict the status of marine environment, as these organisms are **immobile**, can **rapidly obtain a state of equilibrium** with their surroundings, have an **important role** in oxygen production, nutrient cycling, sediment stabilization and provide habitat for aquatic life (Parmar et al., 2016; Stankovic y Stankovic, 2013).

### 3.2.4 Examples of cyanobacteria and algae species that could be used as bioindicators

*Table 1. Examples of bioindicator cyanobacteria and algae species*

| Organism category | Species | References |
|---|---|---|
| Cyanobacteria | *Chamaesiphon sp.*<br>*Chroococcus sp.* | (Mateo et al., 2015) |
| Green microalgae | *Chlorella vulgaris*<br>*Scenedesmus subspicatus*<br>*Selenastrum capricornutum* | (Markert et al., 2003) |
| Diatoms | *Skeletonema costatum*<br>*Stephanodiscus neoastrea* | (Dokulil, 2003; Markert et al., 2003) |
| Macroalgae | *Fucus vesiculosus* | (Stankovic y Stankovic, 2013) |
| Terrestrial algae | *Chlamydomonas chlorococcoides*<br>*Xanthonema montanum* | (Markert et al., 2003) |

# 4 Bioindicator lichens and mosses

Due to their bioaccumulative properties, lichens and mosses are particularly used as bioindicators of aerial pollution, being used in international and national monitoring programs in Europe (Stankovic y Stankovic, 2013).

### 4.1.1 Lichens as bioindicators

**Lichens**, as those found in the forest on the trunks of the trees or on rocks, **can react to air pollution** (such as pollutants of sulfur and nitrogen), **climate change** and **air quality change** (such as increased levels of sulfur dioxide). Environmental stress can also lead to the disappearance of lichens in the forest (Parmar et al., 2016).

### 4.1.2 Mosses as bioindicators

Bryophyte **mosses** can be used as bioindicators as these organisms have high surface/volume ratio, making them **able to absorb pollutants**, and these mosses are **able to accumulate the absorbed pollutants**. Factors that make mosses suitable for being used as bioindicators also include their natural characteristics such as: tendency to grow all around the year, able to survive in dry weather conditions, are widespread geographically, have a vegetative section that is usually younger than three years and present a thin cuticle, allowing a more direct interaction with the environment (Aceto et al., 2003).

### 4.1.3 Examples of lichen and moss species that could be used as bioindicators

*Table 2. Examples of lichen and moss species used as bioindicators*

| Organism type | Species | References |
|---|---|---|
| Lichens | *Parmelia sulcate*<br>*Hypnum cupressiforme* | (Stankovic y Stankovic, 2013) |
| Mosses | *Pleurozium schreberi*<br>*Bryum sp.* | (Aceto et al., 2003; Stankovic y Stankovic, 2013) |

# 5 Bioindicator plants

**Plants** are a **very sensitive bioindicator** category that are used as tools for the recognition and prediction of environmental stress (Parmar et al., 2016). Plants are **always exposed to environmental condition** like air, soil, and water pollutants from their sites of growth due to their non-mobile nature (Stankovic y Stankovic, 2013). Thus, the health of the environment can be assessed by the presence or absence of some plant species, providing ample information about the environmental health (Parmar et al., 2016).

**Example**
The Asian watermeal, *Wolffia globosa*, is a duckweed species that is used as an important tool for cadmium contamination indication as it is a cadmium sensitive species (Parmar et al. 2016).

In the absence of mosses, plants can be used as bioindicators of aerial pollution. The most used plant organs for pollutant identification and quantification are leaves and needles or bark from trees such as poplar, oak, olive, spruce, birch, pine, etc. (Stankovic y Stankovic, 2013).

*Table 3. Examples of bioindicator plant species*

| Environment | Species | References |
|---|---|---|
| Aquatic | *Lemna minor*<br>*Lemna gibba* | (Markert et al., 2003) |
| Terrestrial | *Avena sativa*<br>*Brassica rapa*<br>*Pisum sativum* | (Markert et al., 2003) |

# 6 Bioindicator animals

Harmful changes in the environment, caused by pollution, such as with metals, or other factors, can be identified by accumulation in the organism or by variations in the populations of animals, such as the population density which may indicate a negative impact on the assessed ecosystem. The most used animal bioindicators for the assessment of pollution of the environment are zooplankton, invertebrates and vertebrates (Parmar et al., 2016; Stankovic y Stankovic, 2013).

### 6.1.1 Zooplankton as bioindicator species

**Example**
Zooplanktonic species, such as *Cyclops sp.*, *Alona guttata* and *Mesocyclops edax*, are zone-based indicators of pollution (Parmar et al. 2016). *Daphnia magna*, a freshwater cladoceran, is one of the oldest, most widely used zooplankton as a bioindicator in aquatic toxicology due to its advantages such as high sensitivity to chemicals and small size (Stankovic and Stankovic 2013).

**Zooplankton** is a part of plankton which is composed of freely floating organisms in water bodies. For example, **daphnids** are an important link in the food chain as these organisms are both consumers of phytoplankton and represent a food source for predators, both invertebrate and vertebrate (Stankovic y Stankovic, 2013).

### 6.1.2 Macroinvertebrates

**Macroinvertebrates** are particularly **powerful bioindicators** of **water health** as these are easy to distinguish and identify in the laboratory, and these organisms also **occupy key positions at every trophic chain level** (Parmar et al., 2016; Rizo-Patrón V. et al., 2013). The biodiversity of macroinvertebrates, especially of insects, can be affected by exposure to different pollutants, the observed effect being a reduction of species richness and decrease of faunal composition (Rizo-Patrón V. et al., 2013).

There are different assessment approaches based on macroinvertebrates, such as saprobic, diversity, biotic approaches. The saprobic approach provides water quality classification based on the pollution tolerance of the bioindicator species used. In the diversity approach, richness, evenness and abundance are used, these being three components of community structure. The biotic approach incorporates both saprobic and diversity approaches. There are several other approaches that are more complex than the ones above described (Pauw et al., 2006).

**Example**
Several macroinvertebrate complexes are used for monitoring the environmental health, one of these using Ephemeroptera, Plecoptera and Trichoptera species as bioindicators (EPT complex). These organisms are intolerant towards the presence of pollutants in the water, exhibiting effects on their abundance and biodiversity. The presence of EPT complex denotes that parameters in the ecosystem is within the tolerance limit of the species, these organisms being crucial bioindicators of rivers (Ab Hamid y Md Rawi, 2017).

### 6.1.3 Mollusks as bioindicator species

**Example**
The zebra mussel, *Dreissena polymorpha*, a widespread invasive species in Europe and North America, is one of the most frequently used bivalve accumulator species as a bioindicator for reflecting the site specific pollution due to its capacity to accumulate high amounts of pollutants (Stankovic and Stankovic 2013).

An important ecological role in the different aquatic and terrestrial ecosystems is played by **mollusks**, organisms that have an enormous number of species and have an ubiquitous distribution. These organisms have been **successfully used** in the obtaining of information regarding the **quality of both aquatic and terrestrial ecosystems** and in the **quantification of contaminants** present in their environment. This is due to their limited mobility, direct contact with the environment, capability to take up contaminants via diet and from ambient water, sediment or soil, their limited ability to excrete pollutants directly via their kidneys or other excretory organs and tissues, attaining higher bioaccumulation and bioconcentration factors for many pollutants than other organisms. Another important aspect is their widespread distribution and abundance, especially the distribution of gastropods. While bivalves are mainly present in marine ecosystems, as well as in freshwater, gastropods are present in aquatic ecosystems such as oceans, lakes and rivers, as well as terrestrial ones such as forests, alpine mountains, steppes and desserts (Stankovic y Stankovic, 2013).

### 6.1.4 Earthworms and enchytraeids as bioindicator species

Regarding the soil fauna, **earthworms** are usually used as bioindicators. Soil-inhabiting **enchytraeids** are also used as bioindicators as these organisms are an important part of terrestrial biocoenosis in many variate habitats, especially where the occurrence of earthworms is reduced (Stankovic y Stankovic, 2013).

**Example**
*Eisenia foetida*, an earthworm species, is one of the most used terrestrial bioindicators, especially for metal toxicity, being used even in standardized procedures (Stankovic and Stankovic 2013).

### 6.1.5 Fish as bioindicator species

Due to their top position in the trophic chain and their role as food for humans, **fish** are an important category of bioindicators for the aquatic environment. Several fish species are used in **standardized procedures** developed for the **assessment of the ecotoxicological effects of pollutants**. For the investigation of accumulated pollutants fish organs such as gills, liver, muscle and kidney are used (Stankovic y Stankovic, 2013).

### 6.1.6 Herpetofauna as bioindicator species

**Frogs** are an important category of animal bioindicators, as these organisms **are influenced** by the changes that occur in both **aquatic and terrestrial environments** (Parmar et al., 2016). **Lizards** may also represent potentially useful bioindicators for different pollutants, especially pesticides, as lizards are sensitive to these kinds of chemicals. Lizards represent good bioindicator species in **xeric habitats**, where amphibian species are absent (Lambert, 2005).

### 6.1.7 Birds as bioindicator species

**Bird** species are not as used as other animals as bioindicators due to disadvantages like their migration, being difficult to determine where the contamination exposure occurred. The use of birds as bioindicators also present **advantages** such as the fact that they are **easy to identify** and the fact that their **biology and ecology is well known**. The most used bird types as bioindicators are waterfowl, raptors, and seabirds, which accumulate toxic pollutants, affecting their physiology and reproduction, even leading to death. All these effects lead to population declines which are signs of environmental pollution.

### 6.1.8 Mammals as bioindicator species

As for **mammals**, **wildlife** or free ranging animals are the **best bioindicators** because these depend exclusively on the quality of their habitat, including food, water, and air. **Domestic** animals **can also be used** as bioindicators, as long as these animals feed on food that originates from their surrounding environment, an example being grazing sheep. There are different factors that influence the suitability of a species being used as a bioindicator, such as the size of the home range and the better accumulation of metallic pollutants by herbivores and of organohalogen compounds by carnivores (Stankovic y Stankovic, 2013).

> **Example**
> *Lepus europaeus*, the European brown hare, is one of the best bioindicators for agricultural lands in Central Europe, while *Capreolus capreolus*, the roe deer, is one of the most suitable bioindicators for terrestrial ecosystems, being suitable for both agriculture lands and forest areas. For aquatic ecosystems, a suitable bioindicator mammal species is *Mustela vison*, the mink (Stankovic and Stankovic 2013).

### 6.1.9 Examples of animal species that could be used as bioindicators

*Table 4. Examples of bioindicator animal species*

| Environment | Organism category | Species | References |
|---|---|---|---|
| Aquatic | Flagellates | *Entosiphon sulcatum*<br>*Chilomonas paramecium* | (Markert et al., 2003) |
| | Ciliates | *Colpoda maupasi*<br>*Paramecium caudatum* | (Markert et al., 2003) |
| | Snails | *Physa integra*<br>*Physa heterostropha* | (Markert et al., 2003) |

| | | | |
|---|---|---|---|
| | Clams | *Mercenaria mercenaria*<br>*Venerupis sp.* | (Stankovic y Stankovic, 2013) |
| | Mussels | *Dreissena polymorpha*<br>*Mytilus sp.* | (Markert et al., 2003; Stankovic y Stankovic, 2013) |
| | Oysters | *Crassostrea gigas*<br>*Crassostrea virginica* | (Markert et al., 2003; Stankovic y Stankovic, 2013) |
| | Polychaetes | *Capitella capitata* | (Markert et al., 2003) |
| | Daphnids | *Daphnia magna*<br>*Daphnia pulex* | (Markert et al., 2003) |
| | Copepods | *Acartia tonsa*<br>*Acartia clausi* | (Markert et al., 2003) |
| | Amphipods | *Gammarus lacustris*<br>*Gammarus pseudolimnaeus* | (Markert et al., 2003) |
| | Shrimps | *Crangon crangon*<br>*Penaeus duorarum* | (Markert et al., 2003) |
| | Crayfish | *Cambarus sp.*<br>*Oronectes sp.* | (Markert et al., 2003) |
| | Crabs | *Callinectes sapidus*<br>*Pachygrapsus sp.* | (Markert et al., 2003) |
| | Mayflies | *Baetis sp.*<br>*Ephemerella sp.* | (Markert et al., 2003) |
| | Midges | *Chironomus sp.* | (Markert et al., 2003) |
| | Fish | *Brachydanio rerio*<br>*Onchorynchus mykiss* | (Markert et al., 2003) |
| Terrestrial | Oligochaetes | *Eisenia foetida*<br>*Eisenia andrei* | (Markert et al., 2003; Stankovic y Stankovic, 2013) |
| | Birds | *Parus major*<br>*Cyanistes caeruleus* | (Stankovic y Stankovic, 2013) |
| | Mammals | *Vulpes vulpes*<br>*Lepus europaeus* | (Stankovic y Stankovic, 2013) |

# 7 Selection of bioindicators

The selection of **bioindicators species** for assessment of environmental health must be done in order to ensure the quality of the obtained data which depends on:

- The **representativeness** of the observed objects which constitute a random sample;
- The **selection** of object attributes **relevant** for hypothesis testing;
- The **degree of compliance** with the basic criteria of test theory (Markert et al., 2003).

For example, the assessment of the effects of xenobiotics is better done by marine and freshwater species of mollusks, invertebrates, arthropods, and vertebrates than terrestrial species. This is due to the special adaptations that are presented by aquatic animals such as the total or partial hydrophilicity of the epidermis, absence of protective structures against desiccation or the major role of cutaneous respiration (Markert et al., 2003).

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