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Chapter 3

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Biomonitoring in the Assessment of Chemical Threats to the Environment

The notion of biomonitoring as an assessment of chemical exposure of humans to xenobiotics emerged about 130 years ago, when doctors monitored the content of salicyluric acid in the urine of patients treated with large doses of salicylic acid. Many years later, this term was used in the monitoring of ecosystems, when it typically meant long-term observations concerning the condition of the environment based on the presence and the number of indicator organisms. The first publications concerning contamination and accumulation of persistent organic compounds and their effects on the environment are 50 years old, while intensive application and development of biological methods in ecotoxicology date back to 30 years ago. The term “bioindication” has a slightly different, narrower meaning. Its aim is to establish the quality condition of the environment on the basis of reactions of biocenose organisms (including disorders of reproduction, behaviour, growth and development). Taking into account the generally non-specific reactions of biocenose organisms to stress, the main aim of bioindication is to assess physiological effects, instead of directly determining the concentration of harmful factors in the environment. In practice, misunderstandings associated with appropriate terminology, still are quite often.

Chemical contamination of the environment and threats resulting from this fact are of crucial importance for the further existence of the mankind. It is estimated that the number of new chemical substances emitted to the environment is increasing every year by about 1,000. It is necessary to systematically conduct their analyses in order to quickly and reliably assess the condition of the environment before these unfavourable changes occur to a larger extent and became obvious. At the same time, it is also necessary to take into account factors which have a modifying effect on the behaviour of xenobiotics and on their interactions on biocenose organisms. It can be demonstrated by the example of human health, where the need for a complex assessment is emphasized in a diagnosis of effects of professional or environmental exposure. In 1997 Nelson listed the following factors as examples of interactions affecting the results of chemical exposure: 1) ontogenetic (age, sex, general health conditions, life style, inclination to addictions, 2) family and domestic (type of diet, passive addition to tobacco smoke, various stress situations), 3) chemical (recurring exposure to identical or structurally similar chemical compounds can cause induction or inhibition of the activity of specific metabolic enzymes), 4) physical (noise, temperature, vibrations, electromagnetic radiation, 5) environmental (seasonal changes in temperature, humidity, noise volume).

Traditional chemical analyses, in the context of acceptable norms specifying the content of hazardous substances, do not provide full information in this regard. They mainly indicate dependencies between the content of pollutants in soil, water and air and their uptake and movement in the biological matter. Such research does not take into account unavoidable interactions with the huge amount of existing chemical compounds and antagonistic and synergic effects related to them. This may result in disorders in the operation of entire ecosystems, which are difficult to predict. A high degree of complexity of modern analytic procedures, which are becoming increasingly more time- and labour-consuming, is equally important. The main obstacle in interpreting the results of this type of research is the difficulty in determining the dependencies between the concentration of a xenobiotic in the environment and intensification of changes occurring in the population and ecosystems. Unlike field conditions, laboratory research involves only one species (of the same age and health conditions) exposed to one or several chemical or physical factors. Ecosystems feature additional species of organisms of various functional significance, and each of them is represented by subjects of various ages and a corresponding variability of physical and biological interactions (*e.g.* competition, predation). Anthropogenic stress factors occurring under these conditions are characterized by a large variability, starting with small fluctuations in concentration, up to the condition of chronic influence. Sometimes, as is the case with water organisms, this can take place throughout their lifespan. The presence of large amounts of chemical factors result in the common occurrence of addition and synergism effects, with a simultaneous increase in the concentration of some compounds in the environment.

One of the main factors determining the efficiency of chemical interactions on organisms in ecosystems and under laboratory conditions is the bioavailability of potentially toxic substances. Accordingly, contaminants can be classified as bioavailable, *i.e.* such that within a specific time can freely transfer from the matrix (*e.g.* soil, bottom deposits) through cell membranes into organisms where they undergo processes of assimilation, accumulation, transformation and degradation. The second group which, at the present moment, have no physical contact with biological material is called potentially bioavailable. The main reason for the unavailability of chemical contaminations is soil sorption, which can be significant even at 0.1% content of organic substances. Additionally, strongly absorbed compounds are susceptible to abiotic and biotic decomposition in the soil to a limited extent. This means that a chemically determined level of contamination is not always related to real environmental threats.

In this context, biological methods are increasingly gaining in importance. Their basic advantage, besides relatively low costs, is the possibility of carrying out a summary assessment of biotic disorders occurring under the influence of xenobiotics throughout the entire period of exposure (including those that occur below the limit of detection of a conventional chemical analysis). Biological methods, when properly used, can became perfect early warning indicators against threats related to contamination of the environment, based on physiological and behavioural reactions. The disadvantages of biological methods include the large variability of the sensitivity of test organisms, which – besides the choice of appropriate species, requires standardization of research conditions and methods of their evaluation. A biological assessment of risks caused by xenobiotics should be based on knowledge concerning the phenomenon of stress, *i.e.* the conditions of the organism threatened with the loss of balance under the influence of biological, chemical and physical factors (Fig. 1).

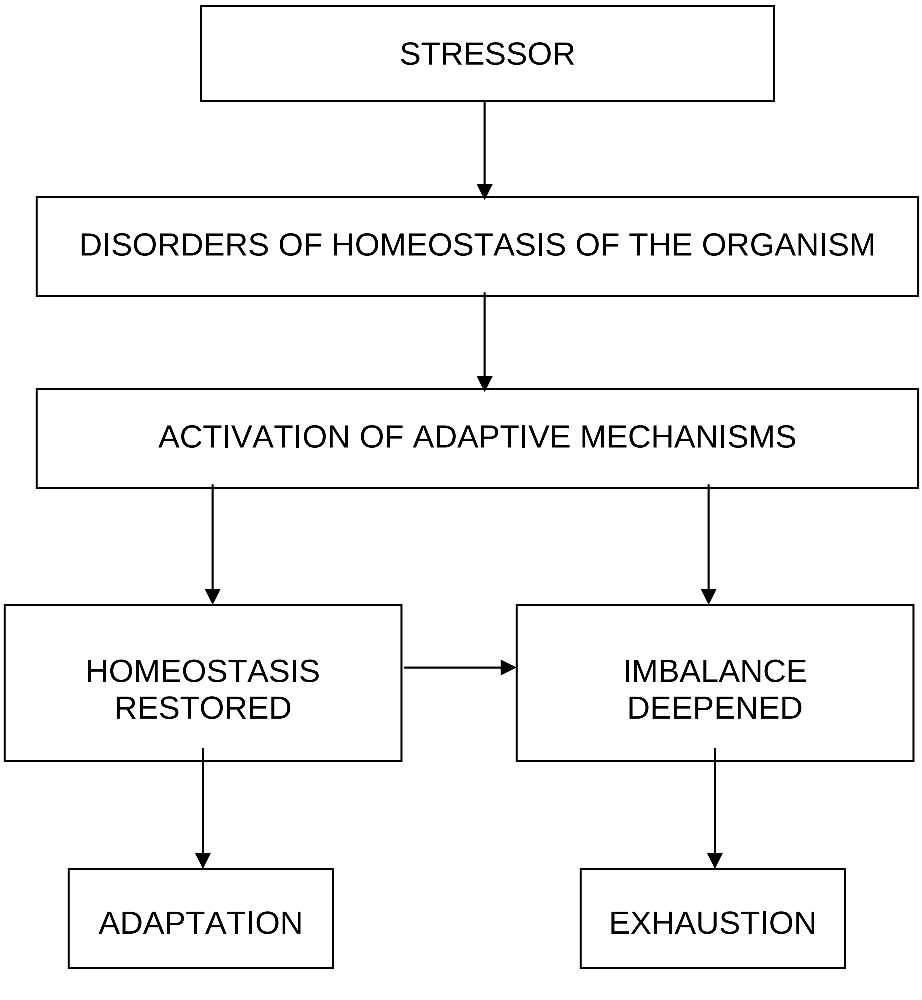

Figure 1. Scheme of environmental stress

Before the occurrence of a stress, the organism is in a standard physiological situation, which indicates a relative optimum of environmental factors. In the case of plants, those factors are related to the availability of light, water and nutrients. Individual stress factors or complex stress evoke a series of reactions in an organism, which consists of three phases. In the first phase, referred to as alarm, certain physiological functions of plants are disturbed, which can lead to inhibition of their growth. The effects of stress occurring at this stage are direct (structural changes) and indirect (metabolic, or functional changes). These changes can occur at various levels of biological matter organization. The need to distinguish between phenomena related to function disorders (which are usually reversible) from damage to the structure (which involves degradation of cell membranes and irreversible inactivation of enzymes) is considered to be the basic criterion for assessing damage to plants caused by environmental stress. The first group includes such disorders as inhibition of photosynthesis, anomalies concerning the respiratory process, energy production and growth inhibition. Structural disorders are manifested, among others, in changes in plants colouring, which leads to tissue drying up symptoms (necrosis), leaks of intercellular electrolytes and water loss by plants, reduced activity of some enzymes that are crucial for the organism, e.g. dehydrogenase. Structural and functional disorders caused by stress factors occur not only on the level of single organisms, but also on the level of entire ecosystems (Fig. 2). Changes in the structure are demonstrated here by modification of biocenose composition (*i.e.* changes in the choice of phytoplankton species), which is related to the new chemical and physical parameters of the surrounding environment. Functional changes consist, among others, in diversification in the organic substance production, rate of using up resources, emission of gases and circulation of nutrients. Accordingly, the following stress indicators in water ecosystems can be mentioned:

- increase in respiration level,
- lack of balance in the productivity/respiration ratio,
- increase in the productivity/biomass ratio resulting from energy expenditure for acclimatisation and compensation instead of using it for growth and reproduction of organisms,
- increase in the significance of additional energy (it becomes necessary to supply it externally),
- increase in the export of primary production,
- increase in nutrient losses,
- reduced lifetime of water organisms and deterioration of their condition,
- shortening of food chains with reduction of their functional diversity, and lower efficiency of resources.

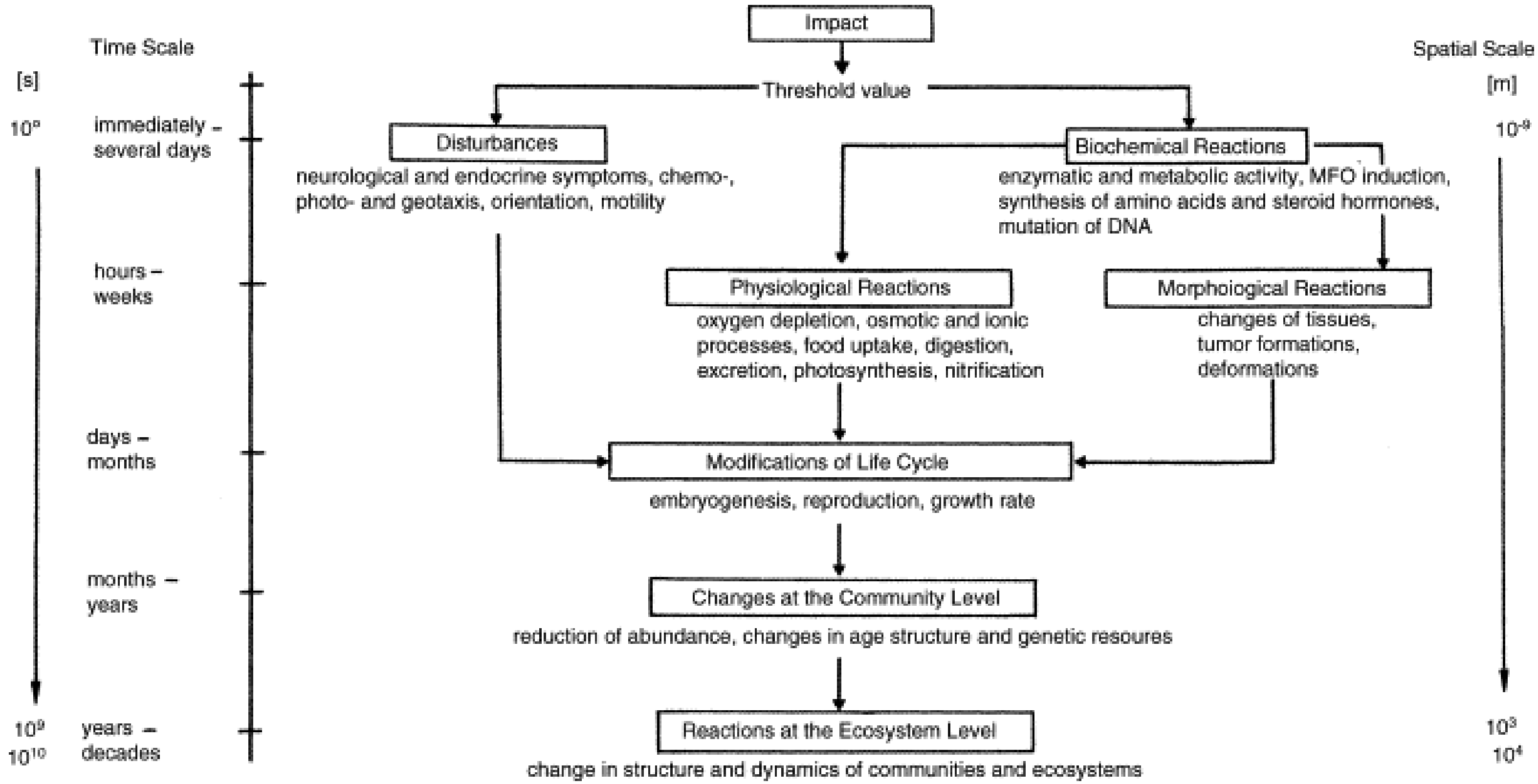

Figure 2. Average stress response times of biotic systems as related to size and structural complexity (Fränzle, 2006, p. 4)

In general, stress reactions occurring in land ecosystems are of a similar nature as in water ecosystems. A complex approach to the problem of integrity or disorders in these ecosystems is related to the assessment of activity of soil micro-organisms, which in turn reflects ATP content (excluding the participation of multi-cellular organisms) and soil respiration.

Organisms of low stress resistance or low stress tolerance reveal symptoms of serious damage or aging. The end of this phase involves the activation of defence mechanisms, such as acclimatization of metabolic fluids, activation of repair processes and long-term metabolic and morphologic adaptation. During the next phase – return to the initial state – new physiological standards are established, which means preservation of a high level of plant resistance. This type of reaction of an organism to the stress factor, known as eustress, can sometimes be positive. An example is the phenomenon of overcompensation, which consists in the stimulation of plant yielding resulting from certain pests feeding on them.

Under the conditions of long-term, prolonged stress which exceeds defence possibilities of an organism, a phase of exhaustion, also referred to as distress, takes place. It leads to gradual reduction of physiological activity and vitality, resulting in serious damage and death of the organism. In this phase, regeneration and development of new physiological standards of the organism are still possible, provided that the stress factor is removed in time. Sometimes it is a long process, which can last for years. E.g. Fränzle (2006) studied water eutrophication, where the balance is restored after 10-12 years following the removal of the source of chemical stress, while in case of rivers with a heavy water flow, this can happen within two years.

The effects of environmental stress concern a wide range of organisms, starting from cell organelles, bacteria and unicellular animal organisms, through plants, water organisms (e.g. algae, plankton, squids, bivalves, fish), soil nematodes, insects, birds, mammals, to humans. A basic criterion here should be the choice of indicative organisms that are representative for a given ecosystem. Examples include edaphic algae (particularly useful in the assessment of land ecosystems) and lichens (considered to be very good indicators of air quality).

Biotest research, to a broad extent, assesses the results of all environmental effects on a biological system. Reactions of an organism to stress factors are determined on behavioural, genetic, enzymatic, physiological and morphological levels, with the application of biological parameters known as biomarkers. It is obvious that biotest material subject to evaluation (bioindication) should be representative for a given research hypothesis, selected on the basis of explicitly specified taxonomic, ecologic, and toxicologic criteria and the type of exposure. Good bioindicators should be characterized by a relatively narrow range of ecological tolerance (specialised species are ideal), long life cycle, large population size, broad range of occurrence and easy determination.

Two types of bioindication can be distinguished – active and passive. In case of active bioindication, test material is subject to temporary exposure under controlled conditions of temperature, humidity and lighting. The aim of passive bioindication is to assess the effect of environmental stress factors on selected components of biocenose and ecosystem under natural conditions. Bearing in mind the non-specific reactions of an organism to stress factors, the primary aim of bioindication is to assess physiological effects, instead of performing direct measurements of the content of the stress factor in the environment. Taking into consideration modes of operation, bioindicators can be divided into two groups of organisms: indicators of effect (reaction) and indicators of bioaccumulation. In the first case, measurable effects of chemical stress are achieved very quickly. This means that as well as a narrow range of tolerance in relation to specific environmental factors, they should be characterized by a low adaptation potential and a low degree of stress resistance. In contrast, indicators of accumulation should reveal a quite high level of resistance, which allows them to assimilate potentially toxic substances within a specific time, without any symptoms of damage. Numerous examples of bioindicators of accumulation include moss and lichens, as well as higher fungi. This results from their fast growth rate, combined with a large assimilation area. Lichen communities are often used as complex bioindicators of air purity, which is particularly important in urbanized and industrial areas. The limitations of this method include an infrequent occurrence of these organisms over the examined area, large differences in xenobiotic uptake from the specific ground, and occurrences of synergy or antagonist effects. Soil organisms, earthworms, nematodes and snails, are also used as bioindicators of accumulation, as they consume large amounts of low energy food, which involves assimilation (also in large amounts) of potentially toxic chemical compounds. Research based on biomonitoring requires using various species of test organisms, since there is no universal biomonitor for different chemical substances. The good example is the biotic index, which was introduced to assess the ecological condition of rivers in the European Union. It is based on sets of organisms (phytoplankton, macrophytes, macrozoobenthos, fish), as biological elements of the environmental quality.

For many years, biomonitoring (bioindication) was based on acute, sub-acute and chronic toxicity tests, as well as on reproduction tests. The simplest of them, acute toxicity tests on animals, determine the results of exposing an organism to chemical contamination of the environment in the form of a lethal effect or a stress reaction to a specific chemical compounds penetrated into organisms through the alimentary tract, respiratory tract or skin and conjunctivas. A basis for acute toxicity assessment is the LD_{50} index in mg/kg body weight, which means a lethal effect for 50% individuals of a given population within 14 days from the exposure. Sub-acute toxicity tests determine the long-term effects of a reduced dose of LD_{50} (generally to 20, 10 and 5%) within 14-90 days. Similarly, on the base of dose-effect relationship, NOEL index (No Observed Effect Level/Concentration) is calculated.

Chronic toxicity, the most difficult to assess, is typically determined based on the exposure of a large number of test organisms over their lifespans. Reproduction tests, including teratological examinations, provide information concerning embryotoxic effects, even for three generations of organisms. A disputable issue is the assessment of the condition of the entire ecosystem based on the reaction of a single bioindicator. Toxicological tests conducted on various levels of biocenose organizations are a response to this problem. The most popular representatives of test organisms for land include adaphic algae (e.g. *Chlamydomonas* *chlorococcoides*), some higher plants (e.g. *Avena sativa, Brassica rapa, Chenopodium album, Pisum sativum*), earthworms and birds. It is estimated that over 500 species are currently used in test examinations, of which, according to the BUA report, 25 species are prevailing. The first species on this list are daphnia *Daphnia magna* – 137 tested substances, ide *Leuciscus idus* (fish of the family Cyprinidae) – 94, and *Pseudomonas putida* bacteria – 82.

Another issue is also the large diversification of the biological material which can be used for bioindication and biomonitoring. This can be exemplified by heavy metals, which are detected *e.g.* in microorganisms, peryphiton, conidia and spores of fungi, duckweed, plant pollen, plankton, fish scales, bones, hair and blood of mammals, tree rings and excrements. However, bioassays have often been deceptive in environmental monitoring due to difficulties in communication, taxonomy, performance indicators, time scale, consistency of habitat, standardization of techniques and expression of results. Many species of higher plants, including cultivated species and weeds, demonstrate an ability to uptake xenobiotics from the soil and from the atmosphere, including heavy metals, sewage, petroleum-originated substances and pesticides. Since the 1980s, much attention has been paid to the response of plant communities to various stress factors, and to air pollution in particular. This has resulted in distinguishing three levels of phytocenose sensitivity – high, medium and low, from clear symptoms of acute and chronic toxicity, to periodical disturbances of its operations. The third degree indicates e.g. a slight reduction in the number of organisms, with no transfer of hazardous chemical compounds to the neighbouring ecosystem.

Unlike plants, animals have developed more defence mechanisms against stress factors. This applies in particular to such species that can escape the polluted area. It should be noted that some changes of insect behaviour, such as disruptions of bees' orientation under the influence of sub-lethal doses of pesticides, can also have a toxicological aspect. Due to the important role of bees as pollinators and their high susceptibility to chemicals, they are particularly well suited as indicators of environmental pollution. Other species of insects can be also used as bioindicators. Generally, water organisms are more sensitive than land organisms, although the effects of chemical stress in the soil environment can last much longer than in the water environment. Some noteworthy soil bioindicators also include numerous species of fungi, enchytraeidae, springtails, saprophytes and insects. Apart from simple biotests or their sets, assessments of the susceptibility of the soil environment to chemical contamination involve experiments, which refer, in their assumptions, to natural conditions. They can be exemplified by research in microcosms, in which sets of organisms are introduced to large, several-hundred-litre vessels filled with soil. This helps to simulate conditions in order to assess the effects of toxic substances on specific species, trophic levels and entire populations of organisms. The research also involves controlled exposure in field conditions with a differentiated level of biodiversity.

Another form of research, which is quite rarely applied because of its costs, is the use of mesocosm systems, taking the form of isolated fragments of lakes or artificial streams, into which chemical compounds are introduced in a controlled manner.

Nowadays, apart from traditional floristic, faunistic and biocenotic research, in which the main element of assessment is the absence or the presence of specified species, much more popular are second-generation biological tests, known as “microbiotests”. They are a result of the development of bioindication, which has occurred within the last 20 years, including new analytic techniques and advances in molecular biology. A good example is immunoanalysis, a method that has been known since the 1950s. Currently, it is used to determine the presence of pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, benzene, toluene and xylene, dioxins and heavy metals. Microbiotests have an advantage over traditional acute toxicity tests resulting from their lower costs, fast performance and no ethical controversy related to inflicting pain on test organisms. This means that they can be very useful for a complex assessment of the condition of the environment. “Toxkit”-type research, instead of acute toxicity tests conducted on higher organisms, assumes a criterion of shock and death of test organisms and disorders in physiological processes, such as changes in growth, respiration and reproduction. On the ground of large surface area and direct contact of cell membranes with investigated medium, microorganisms show higher susceptibility to toxic substances than invertebrates or fish. The range of specifically-prepared tests include both single test organisms and their various species representing specific trophic levels, on the basis of which extrapolation can be made to real conditions found in ecosystems. These sets of quick tests, ready for immediate use, are available under various names and contain unicellular organisms, early developmental stages of multicelullar organisms or small invertebrates. The first biotest of this type, created in 1979, was the Microtox^{TM}, system, which used bioluminescent bacteria of *Vibrio fischeri*. Lyophilized bacteria, kept at -20 ^{o} C, can be used at any moment. During the test, they emit constant radiation, visible for 1-1.5 h. The intensity of radiation diminishes with the growth in toxicity of the examined sample, in which the bacterial suspension is placed. This easy-to-use test is applied to assess the toxicity of such various substances as pesticides, mycotoxins, liquid industrial waste, medical materials and cosmetics. Phytotests, on the other hand, use algae (e.g. green algae and diatoms), duckweed and rooted water and land plants.

A good illustration of the usefulness of this method in monitoring environmental pollution could be the results of research conducted in 2006-2008 on soil contamination in the vicinity of an inactive pesticide dump in Warlity near Ostróda (north-eastern part of Poland). This object, located at the border of forest, on a small, gravely hill was liquidated in 2004. However, the risk of the emission of toxic substances, which had accumulated in the soil for many years of its usage and later penetrated to ground water, still exists. Soil samples for research were collected at the depth of 20-30 cm in various locations from the centre of the dump. The experiment was conducted as a test of seed germination and early growth of plants, PHYTOTOXKIT^{TM}, with the use of white mustard (*Sinapis alba*), shallot cress (*Lepidum sativum*) and sorghum (*Sorghum saccharatum*), and a chronic toxicity test, OSTRACODTOXKIT F, with the use of *Heterocypris incogruens* crustacean. The analysis of results obtained in 2006 revealed inhibition of seed germination and inhibition of the growth of test plant roots after 3-day exposure in all examined objects (see photo below with white mustard as tested plant)..

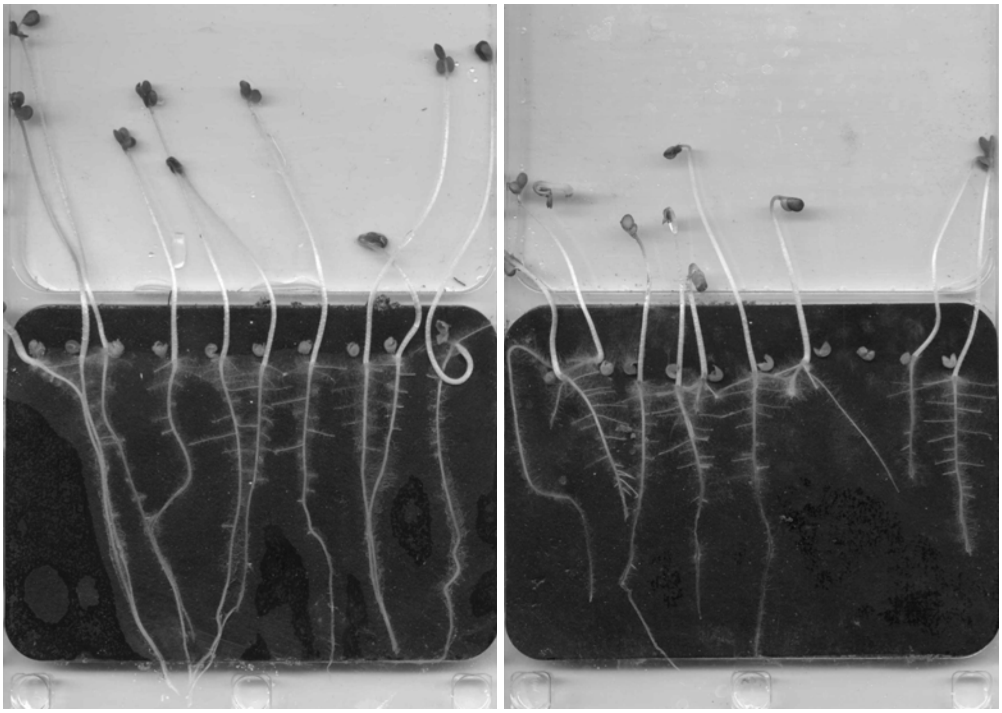

Figure 3. Inhibition of the growth of white mustard seedlings

The phytotoxic effect of soil samples was related to their location, and primarily concerned the area of the former dump and its surroundings. The research also demonstrated that the percentage inhibition of the crustaceans growth in the tested soil was the highest at the same sites. The mean percentage mortality rate for each replication of soil in the centre of the former dump was 21.7%, in comparison to 3.33% in the control soil. An ecotoxicological assessment performed in 2008 did not reveal phytotoxic effects in the form of inhibition of seed germination and roots growth in relation to all test plants under examination, regardless of the place of collecting soil samples. However, a toxic effect of soil on the crustaceans under examination continued, although it occurred to a limited extent. The highest mortality rate of these organisms, *i.e.* at the level of 75%, was observed at a distance of 125 m from the centre of the former dump. Additionally, the research established growth inhibition of crustaceans, the average size of which was 229.4 µm in this site, in comparison to 423.3 µm at the beginning of the measurement zone.

To sum up, it should be emphasized that the aim of this study was to provide an introduction to relatively new research methods in environmental studies. There is no doubt that biomonitoring will have great practical significance in this context.

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