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SOIL BIOLOGY

Soil Bioassay: Problems and Approaches

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Abstract—The methodological and organizational problems of the practical application of soil bioassay to monitor the state of soils for environmental, agricultural, and sanitary–epidemiological purposes are analyzed. To improve the efficiency of the integral valuation of soil toxicity, soil bioassay should be performed with a set of organisms (sensors) representing the major trophic levels of the ecosystems, i.e., producers, consumers, and decomposers.

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INTRODUCTION

The theoretical organization of soil ecological control and monitoring system is based on the concept of the functional role of soils in biogeocenoses and the biosphere [18, 19]. The need for the biological diagnostics of the soil quality arises from the close interdependence between the biotic and abiotic soil components. Biotic criteria can give us information about the transformation of soil ecosystems, the state of the organisms in them, and their response to various impacts. The analytical control of soil pollution by chemical methods can only show us the presence of certain concentrations of pollutants in the soil. They may cause different consequences in the regions differing in their environmental conditions and in the species composition of the living organisms. This information is insufficient for predicting the structural and functional changes in the state of the soil biota and, hence, in the entire ecosystem.

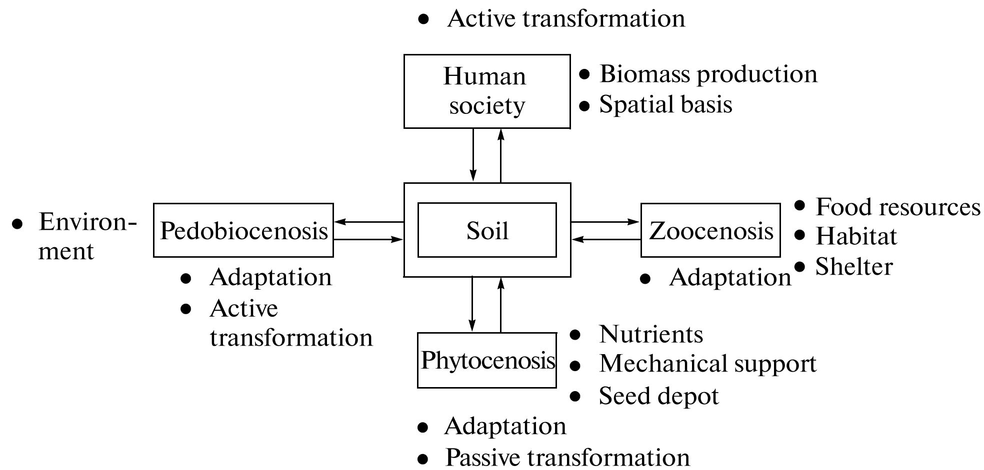
At present, after a recession in the late 1970s, investigations into the biological control of soils are being actively developed. This can be confirmed by the dynamics of the publications in this field as reflected in the bibliographic database of the ISI Web of Science (http://isiknowledge.com/). According to this system, from 1977 to 2009, more than 4500 works devoted to biodiagnostics, bioindication, and bioassay methods were published. Among them, there were only 120 works devoted to the biological monitoring of soils (with the words biodiagnostics, bioindication, bioassay, and soil in the titles), i.e., four works per year on the average. However, these numbers could be much higher if all the works of Russian authors devoted to these problems were available for the bibliographic analysis of the Web of Science.

The approaches to the ecological assessment of soils are specified by the diversity of the soil functions on the planet. In turn, the role of soils in ecosystems is conditioned by the diverse demands of the living organisms, on the one hand, and their impact on the soil systems, on the other hand (figure) [1]. The principles of the systems of the biotic control of the soil properties were created in relation to the diversity of the soil functions in various spheres of human activity, including agriculture, sanitation and epidemiology, and environmental protection [9].

The soils of agrocenoses. Soil has always played the role of the major life-support system, the main means of agricultural production, and the place of human settlements.

For agricultural lands, the assessment of the soil's fertility—the capacity of the soil to satisfy the plants' demand for nutrients, moisture, air, and biotic and physicochemical environments—is of major importance. Soil fertility sustains crop yields and the biological productivity of virgin vegetation. A distinction is made between natural and artificially created soil fertility. To monitor the soil fertility in the Russian Federation, as well as in many other countries, including the United States, Canada, and Germany, a classification of soils with respect to their suitability for agricultural production is used (Edict of the Government of the Russian Federation no. 1564-r from October 1). This classification is based on the grain equivalent. Arable soils are subdivided into several classes with respect to their potential fertility. For example, class I corresponds to the grain equivalent of 5.5–6.1 t/ha. In 2002, the mean potential soil fertility in Russia was equal to 2.3 t/ha, which corresponds to class V.

Soil quality and geochemical characteristics; excessive concentrations of toxic agents, pesticides, and fertilizers; and other factors affect the germination and ripening of crops, the biomass development, and the quality of the agricultural produce. The choice of the chemical and biological indices to be determined



Interaction of the soil with living organisms: the diversity of the demands and impacts (to [1]).

depends on the character of the potential sources of pollution in the studied area, the composition of the chemicals applied to the fields, and the specificity of the environment. The relevant normative documents (Sanitary Regulations and Norms (SanPiN) 2.1.7.1287-03; State Standard (GOST) nos. 17.4.2.01-81, 17.4.3.06-86, and 17.4.2.03-86; and

SanPiN 2.1.7.1287-03) establish common hygienic requirements for the quality of agricultural soils with due account for their specificity, the soil-climatic conditions, and the background contents of the chemical compounds and elements in the soils. In other standards, indices of the sanitary-chemical, sanitary-bacteriological, sanitary-helminthological, and sanitary-entomological states of the soils are characterized with respect to the particular land uses.

Soils of settlements. Soils can accumulate and transform natural and technogenic organic compounds and, thus, perform a sanitary function. To ensure human health and epidemiological safety, the soil quality valuation in settlements is performed in accordance with the sanitary–epidemiological demands. In the Russian Federation, they are specified in SanPin 2.1.7.1287-03 approved by the Federal Sanitary Inspector and regulate the requirements for the soil quality in residential blocks, construction areas, recreation zones, and other places where soil contamination may affect human health and living conditions.

This document regulates a number of biotic (sanitary-toxicological) indices: the presence of enteric infections, parasitic disease agents, pathogenic bacteria, enteroviruses, etc. Permissible values of the bacteriological, parasitological, and entomological parameters for clean soils are also specified in it.

Natural soils. The generation, sustenance, and preservation of the biodiversity in natural ecosystems are important integral functions of soils in the biosphere. Being an environment for numerous organisms, soils restrict the living activity of some species and stimulate other species. Dozens of thousands of specific soil habitats exist in the world; they differ in their structural arrangement and environmental parameters. The great diversity of soils with respect to their nutrient supply, water capacity, pore space, acidity, redox conditions, and other properties is a factor stimulating the adaptation of organisms and ensuring their biodiversity.

However, a system of regulatory norms concerning the application of bioassay methods to control the state of soils under natural conditions has yet to be developed. This is explained by the incompleteness of the concept of the ecological regulation in the field of environmental protection and rational use of natural resources. The regulations and norms in this field are irregularly distributed: among the 500 official documents, 104 documents concern the use and protection of water resources, and only 2 documents are devoted to soils [12].

In the main environmental document of the Russian Federation—the Federal Law On Environmental Protection—the notions of environmental quality and permissible loads on the environment are specified. With respect to natural ecosystems, only general requirements for their quality are suggested; they provide the basis for the environmental quality assessment with the aim to preserve natural ecosystems and the genetic pool of wildlife species. The particular norms take into account the chemical (maximum permissible concentrations), physical (radioactivity levels, thermal pollution, etc.), and biological indices characterizing the state of the environment.

At present, the legislative basis for the ecological evaluation and control of soils in their natural biogeocenoses is insufficiently developed. At the same time, biologists have already developed efficient methods of the biological control of soils and other environmental components.

Remarkable achievements in this field have been made by hydrobiologists and phytocenologists. Soil as a multilevel hierarchical system and a heterogeneous medium represents an object for which the appropriate assessment of the ecological quality is a very difficult challenge. The great spatial diversity of soils complicates the problem [5]. The spatial and temporal (seasonal, annual, etc.) variability in the activity of soil biotic complexes should be taken into account. To a certain extent, the reliability of the estimates of the ecological toxicity of soils on the basis of the chemical and biological indices of the soil quality can be improved if we increase the number of repeated measurements [11].

BIOASSAY OF THE ECOLOGICAL TOXICITY OF SOILS

A bioassay is an efficient method to estimate the potential danger of the chemical, physical, or biological impacts on the soil. This is an experimental method in which standardized laboratory test systems are used. Changes in the biologically important indices of these systems under the impact of the tested samples are determined. On this basis, the samples are arranged according to their toxicity levels. The test systems represent spatially limited complexes of sensitive biological elements (sensors) placed into the studied environment.

To specify the elements of test systems, the terms test object and test culture (or test organism) are often used in Russian sources. The test object represents the studied object (sample) that affects the test culture. Changes in the test culture under the impact of the test objects are referred to as the test responses (or test reactions). A test culture is a laboratory population of some species (test organism) artificially cultivated on a nutrient medium under standard conditions. It is used to estimate the toxicity of the tested objects. In fact, the living population (test culture) serves as a sensitive indicator of the signals from the tested objects. In the English literature, such populations are often referred to as sensors. The sensitivity of the sensors is estimated with respect to a reference toxicant (an analogue of a standard sample in analytical chemistry) whose parameters are standardized. The term test organism can be used to identify the taxonomic position of the species used as a sensor.

The goals of a bioassay may be different in the different spheres of its application [10, 24, 32]. A bioassay is needed to estimate the general toxicity and the mutagenic and carcinogenic potentials of the studied objects. The impact in test systems is usually measured via the imitation of the uptake of some harmful substance by the organisms. Most often, water environments are tested, and various aquatic organisms—protozoa, algae, crustaceans, mollusks, fish, etc.—are used as sensors. The toxicity of the solid-phase components of the environment (soils, parent materials, wastes, etc.) exerts an indirect impact on the sensors (biosensors) [12]. In this case, water extracts or pore waters from these media are used; also, a bioassay can be performed for suspensions.

A larger part of the bioassay methods is based on the analysis of water extracts; it is referred to as an *eluate bioassay.* The impact of insoluble pollutants may be evaluated in the course of direct contact between the sensor and the object; the sensors are applied onto the object. This group of bioassay methods can be referred to as a *contact or applicator bioassay.* Microorganisms participating in the decomposition of organic matter and humification processes are often used as sensors.

One of the promising methods to trace the changes in the functional state of the microbial community of soils under the impact of chemical pollution is the method of multisubstrate testing. This method is based on the study of the changes in the spectrum of the consumed substrates [4]. At present, it is applied for the analysis of soil water extracts. For the more adequate assessment of soil samples, the solid soil phase should also be tested. To ensure the wide-scale application of this method for practical purposes, it should be thoroughly tested and included in the State Registry of Methods for Ecological Control. Also, it is important to organize the production of standardized equipment and test cultures.

As a rule, the biological methods are highly sensitive; they may detect lower concentrations of the pollutants than various analytic devices, and their informative value for the environmental impact assessments is higher than that of the physicochemical methods. It is believed that a bioassay gives information about some disturbance in the natural system even before this disturbance manifests itself in the system. A bioassay is a method that complements the bioindication and various chemical methods. Its advantages are quite evident. First, this method makes it possible to determine the negative changes in a system even upon very weak anthropogenic loads on it. Second, it gives an integral evaluation of the harmful action of different factors, including the physical and chemical impacts, on the biota. Third, it is a quantitative method. In this context, it should be noted that the notion of toxicity is a quantitative notion. Its estimate is based on the median values of lethal concentrations (LC_{50}). In toxicology, toxicity is defined as the reciprocal of this value determined during 48 h [17]: $T = 1/LC_{50}^{48}$.

The practical importance of the bioassay methods. The great interest in bioindication and bioassay methods is stimulated by the demands of practice, including the creation of a system of control over the environment. It is important to have reliable and adequate methods to assess the environmental quality, especially in relation to large-scale bioecological and sociotechnological changes on the planet (including climate fluctuations, species invasions, transport flows, new sources of electromagnetic radiation, etc.). The global degradation of the environment is largely due to the strong contamination of the soils. Various human impacts on the environment (including extensive land plowing and the expansion of agricultural lands, urbanization, transport, and industrialization) transform the biotopes; they also lead to a rapid rise in the concentration of xenobiotic substances.

Thus, a bioassay can be used to control the quality of natural media (soils and water); it can also be used in other spheres, e.g., for the experimental determination of the class of danger of industrial and municipal wastes. In Russia, its use is regulated by Edict no. 511 (2001) of the Ministry of Natural Resources Criteria for Determining the Class of Danger of Harmful Wastes. The results of the bioassay are used in the certification of various biopreparations, sorbents of oil products and other toxicants, and for quality control of soil and water bioremediation [21].

In our work, methods of bioassays have been applied for the purposes of the ecological certification of the microbiological preparations recommended for purification of natural objects from oil products in oil-polluted areas of the Salym oil field in Siberia, in the Usinskii district of the Komi Republic [26], and in the Baltic Sea [16, 36]. These methods have also been used for the quality control of soil rehabilitation procedures.

Bioassays as study methods are widely used by specialists in different branches of science: in ecological toxicology, for the analysis of waters and soils; in humanitarian and veterinarian medicine, for the analysis of inner tissues of higher organisms; in agriculture, for rapid tests concerning the toxicity of forage; in chemistry, for the initial estimate of the biological properties of new substances, etc. In recent years, the ecological toxicity of various nanomaterials, including those that may become potential soil pollutants, has been studied [27, 33, 34].

Methodological and organizational problems of soil bioassays. There are several sets of biotests certified for use in the agricultural, medical, and environmental protection fields in Russia. Their use is regulated by the corresponding normative documents. The methods of the ecotoxicological analysis in three different spheres—the control of agrocenoses (the evaluation of food safety and soil fertility), sanitary and epidemiological control (the evaluation of the impacts harmful for human health), and the ecological control of natural ecosystems (the evaluation of their biodiversity and sustainability)—are properly registered and documented.

There are several tens of methods of bioassaying, and only about ten of them are included into the Federal Register (FR) and into the Federal Register of Documents on Environmental Protection (FRDEP) as methods recommended for practical application in the ecological control of environmental media, including soils.

A list of these methods with their codes in the federal registers and with the indication of the authors (developers) of the particular methods is given below.

FR 1.39.2007.03222. A method for determining the toxicity of water and water extracts from soils, sewage sludge, and other wastes on the basis of data on the death rate and fertility rate of daphnia (the Akvaros JSC).

FR 1.39.2007.03221. A method for determining the toxicity of water and water extracts from soils, sewage sludge, and other wastes on the basis of data on the death rate and fertility rate of ceriodaphnia (the Akvaros JSC).

FR 1.39.2007.03223. A method for determining the toxicity of water and water extracts from soils, sewage sludge, and other wastes on the basis of data on the changes in the fluorescence of chlorophyll and the number of algal cells (the Akvaros JSC.).

FR 1.39.2006.02506. FRDEP 14.1:2:3.13-06 (FRDEP 16.1.1:2.3:3.10-06). A method for determining the toxicity of wastes, soils, sewage sludge, and surface and ground waters on the basis of a bioassay with *Paramecium caudatum* Ehrenberg infusorians as the sensor (the Faculty of Soil Science, Moscow State University).

FR 1.39.2006.0250. FRDEP 14.1:2.14-06 (FRDEP 16.1:3.11-06). A method for determining the toxicity of highly saline surface and sewage waters, soils, and waste products on the basis of data on the survival rate of the salt-tolerant crustacean *Artemia Salina* L. (Faculty of Soil Science, Moscow State University; Biological Faculty, Moscow State University; and the Ecoterra Center).

FR 1.39.2007.04104. FRDEP 16.3.12-07. A method for determining the toxicity of ash and slag dumps on the basis of data on the survival rates of paramecia and ceriodaphnia (Faculty of Soil Science, All-Russia Research Institute of Thermal Engineering).

FRDEP 14.1:2:3:4.10-04 (FRDEP 16.1:2.3:3.7-04). A method for determining the toxicity of fresh surface, ground, potable, and sewage waters and water extracts from soils and sewage sludge on the basis of data on the changes in the optical density of a *Chlorella vulgaris* Beijer culture (Krasnoyarsk State University).

FRDEP 14.1:2:4.12-06 (FRDEP 16.1:2:3:3.9-06). Methods for determining the toxicity of water extracts from soils; sewage sludge; solid wastes; and potable, sewage, and natural waters on the basis of data on the death rate of *Daphnia magna* Straus (Krasnoyarsk State University).

FRDEP 14.1:2:3:4.11-04 (FRDEP 16.1:2.3:3.8-04). A method for determining the toxicity of water and water extracts from soils, sewage sludge, and wastes on the basis of data on the changes in the bioluminescence of the Ecolum test system as determined using a Biotox-10 device (the Ecologic Prospective JSC).

FRDEP 16.2:2.2-98. A method for determining the toxicity of soils and bottom sediments on the basis of data on the chemotaxis of infusorians (the Spectrum-M JSC).

FRDEP 14.1:2:3:4.2-98. A method for determining the toxicity of water on the basis of data on the chemotaxis of infusorians (the Spectrum-M JSC).

FRDEP 14.1:2:4:15-09 (16.1:2:2.3:3.13-09); FR 1.31.2009.06301. A method for measuring the index of toxicity of soils, subsoils, waters, and wastes on the basis of in vitro changes in the motility of mammal gametes (Faculty of Soil Science, Moscow State University; Institute of Ecological Soil Science, Moscow State University; BMK-Invest Ltd; Biognosis JSC; Ecoterra JSC; and the Severtsov Institute of the Problems of Ecology and Evolution, Russian Academy of Sciences).

As we can see, the methods used in the ecotoxicological control are mainly based on the response of various aquatic organisms (crustaceans, green protococcus algae, and holotrichs) to toxic agents [6, 14, 15, 20–23, 25, 27, 29]. Indices of the death rate, fertility rate, growth suppression, infusorian motility, and some others are applied in the assessments of the toxic impacts. Also, luminescent bacteria (Ecolum preparations obtained by the method of lyophilic drying) are used. In this case, a decrease in the intensity of the luminescence is indicative of the presence of toxic agents.

To analyze soils in agrocenoses, the seeds of higher plants are usually used. The following parameters are measured: the germination capacity, the energy of the germination, the sprouting density, the sprouting rate, the root length, the length of the shoots, the root mass, and the mass of the shoots. According to the recommendations, the best results are provided by small seeds (watercress, radish, mustard, wheat, etc.) with a relatively small supply of nutrients, because such seeds are more sensitive to external impacts. However, large seeds are also used. In dependence on the cultivated crops, monocotyledonous (gramineous) or dicotyledonous plants can be applied. A more integral approach has been suggested by Belgian authors (MicroBioTests Inc., Belgium). According to it, the seeds of three species with two dicotyledonous species (*Lepidium sativum* and *Sinapis alba*) and one monocotyledonous species (*Sorgum Saccharatum*) are used. The degree of the soil's phytotoxicity is judged from the inhibition of the growth and development of young plants on the studied soil in comparison with those on the standard control soil (ISO 11269-1 standard).

Soil as a heterogeneous medium with a large number of various nutrients is a difficult object for bioassaying. The results of soil bioassaying are largely determined by the procedures of the samples' preparation for the tests, the conditions of the test, and the choice of the test cultures (sensors). For examples, it is known that the high content of biogenic elements in soil water extract hampers the use of green protococcus algae as sensors. Changes in the salinity of soil water extracts affect the response of *Artemia salina* to the pollutants, etc.

To improve the efficiency of soil bioassaying, the procedures of the soil sampling and the sample preparation should be optimized with due account for the chemical and aggregate state of the soil samples [2].

Many works performed in the recent period are devoted to the comparison of the efficiency of various methods of bioassaying [28–31, 33]. Data on the analysis of widespread pollutants (heavy metals, oil and oil products, chloroorganic compounds, etc.) prove that the sensitivity of the test systems depends on their chemical nature.

We have compared the sensitivity of the standard bioassay procedures to different pollutants in the course of the experimental determination of the toxicity for more than 500 samples of soils and wastes of different geneses [3]. Oil-polluted samples exerted the strongest toxic impact on the test cultures such as the *Daphnia magna* crustaceans and the *Paramecium caudatum* protozoa. The Daphnia proved to be more sensitive to oil products, whereas the protozoa were more sensitive to soil pollution with heavy metals.

It is evident that the standard methods of the analysis of soil water extracts do not completely reflect the potential danger of the soil samples, because some part of the toxic components may be bound in them in insoluble forms.

Hence, specific methods should be selected for different groups of pollutants with due account for the range of sensitivity of the particular test cultures. The spectrum of the methods should be widened. The most adequate assessment of the soil toxicity can be obtained with the use of soil-dwelling organisms (pedobionts) as sensors. Contact methods of the determination of the soil quality on the basis of the response of microorganisms are being developed. Unfortunately, the authors of these methods do not try to introduce them into practice in some standard formats.

For scientific purposes, some other species are used for bioassaying, e.g., enchytraeidae and earthworms [8, 13]. There are international standards and guidelines concerning the application of earthworms for bioassay purposes. As a rule, they are used to control the quality of pesticides and to certify new pesticides [35]. However, these methods are not used for the assessments of the degree of soil pollution. To apply them for this purpose, they have to be standardized and included in the corresponding federal registries. The certification of the methods of soil bioassaying and their metrological support should be performed in order to improve the biological control of soils.

In many cases, the same species of microorganisms are used as sensors; hence, the same test reactions are evaluated. However, the results are interpreted differently. This can be confirmed by the existing approaches to the identification of danger classes of wastes by different institutions. Thus, according to the edict of the Ministry of Natural Resources of the Russian Federation from June 15, 2001 (no. 511) about the identification of danger classes of wastes, five such classes are specified. According to the Sanitary Rules (SR 2.1.7.1386-03) adopted on June 30, 2003, only four danger classes are established for toxic wastes. Moreover, in some cases, similar test systems used by different institutions somewhat differ in the methods of the sample preparation for bioassaying (in particular, the ratio of water to the solid-phase components upon the preparation of water extracts varies from 1 : 2 to 1 : 5), which leads to ambiguity of the obtained results.

CONCLUSIONS

Bioassaying is applied by different services responsible for the ecological control of the soil quality, including the environmental protection, agricultural, and sanitary-epidemiological services. Certain organizational difficulties and interdepartmental barriers complicate the unambiguous use of this method and the interpretation of the results.

The spectrum of the test organisms is relatively wide; none of the species can be used as a universal sensor because of the selective actions of the potential toxicants. If we follow the traditional approach and choose two test organisms from different taxa, we shall not be able to give an adequate assessment of the ecological toxicity of the environmental media. In foreign countries, the practice of using organisms representing major links of the trophic chain—producers, consumers, and decomposers—is considered to be feasible for the rapid analyses of the levels of toxicity. This is a reasonable approach, and it is expedient to put it into practice in the Russian Federation. The minimum set of test cultures (sensors) should be expanded; at least three sensors are required for reliable conclusions. It is known that the trophic chain in natural biogeocenoses includes several major groups of organisms: autotrophic plants synthesizing organic matter from inorganic compounds (producers), parasitic organisms feeding at the expense of autotrophic plants (consumers), and organisms feeding on the decomposing residues of other organisms and favoring their mineralization (reducers). This concept should be taken into account upon the improvement of the existing system of biological tests. A spectrum of sensors representing the organisms from the three major trophic levels should be used. The quality of the tested sample should be judged from the response of the most sensitive sensor or from the summary effect of the three tests, as is recommended for the determination of the integral index of the soil biological quality [7].

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