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METHODS FOR STUDYING AND ASSESSING SOIL POLLUTION

Biotesting of Soil Ecotoxicity in Case of Chemical Contamination: Modern Approaches to Integration for Environmental Assessment (a Review)

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Abstract—The main directions of using biotesting to assess the environmental risk of pollution—(a) predictive (predicting possible effects of chemicals and determining safe levels of their use) and (b) diagnostic, allowing one to assess the real hazard or damage at the moment—are considered. The historical stages of ecotoxicology development are analyzed. An idea is given about the variety of test systems and methods for assessing ecotoxicity and criteria for selecting test species in biotest batteries. Examples of the use of OMICS technologies, molecular biomarkers, nanoecotoxicology, and ecotoxicogenomics in the assessment of soil toxicity are presented. In world practice, in order to compare the results of standard tests, reference (standard, artificial) soil recommended as a reference sample according to the ISO11268 protocol. Attention is focused on the relevance of soil assessment based on the biotic concept of modern environmental control. The advantages and disadvantages of some methods and indices of the ecological state of soils based on the use of reactions of living systems to environmental pollution (in particular, the so called integral indicator of the biological state of the soil (IIBS), the functional diversity of the microbiome (FDM), and the state index according to the TRIAD methodology) are characterized. At the present stage, the best way to integrate the results of biotesting into the overall assessment of soils is an interdisciplinary TRIAD methodology, which implies a set of chemical, bioindication (in situ) and toxicological (ex situ) studies.

Keywords: ecotoxicity, biodiagnostics, biotesting, biosensors, battery of biotests, chemical pollution, bioavailability, soil assessment, environmental quality, integral indices, TRIAD methodology

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INTRODUCTION

In the past 30 years, the assessment of soil ecotoxicity has received a reliable methodological justification in the quality control of terrestrial ecosystems. The biotic concept has become dominant in the ecological control of natural media [10]. The widespread use of biological diagnostics, along with bioindication observations in situ, involves laboratory assessment of the ecotoxicity of samples during ex situ biotesting. Biotesting is a recognized ecotoxicological approach aimed at protecting ecosystems from anthropogenic impact. Over the past few decades, biotesting has been used to assess the quality of natural environments and human-made objects, such as industrial waste and industrial preparations applied in various sectors of the national economy, including chemical, bacterial, and humate-composite materials and sorbents for the rehabilitation of disturbed soils.

Soil quality is defined as one of the most complex components of environmental quality [40]. The quality of water and air implies mainly the purity of substances, which directly affects the consumption and health of humans and animals or natural ecosystems [42, 48]. The definition of soil quality involves more complex concepts. It is not limited only to the degree of contamination [33]. In a broad sense, soil quality is usually defined as "the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health," while the concept of "animal health" includes human health [49]. Soil quality can be assessed both for agroecosystems, where productivity is the main, but not exclusive, ecosystem service, and for natural ecosystems. Chemical pollution by traditional (heavy metals, pesticides, petroleum products, PAHs) [2, 6, 8, 48] or relatively new (pharmaceuticals and antibiotics, nanomaterials, microplastics) [64, 65] toxicants pose a great hazard to the sustainable functioning of soil ecosystems and the quality of the environment as a whole. The characteristic of the ecological quality of all three environmental components (water, air, soil), according to a number of regulatory documents, includes an assessment of toxicity [9, 25–29, 59–63, 76–81].

Historical aspects of ecotoxicology. The beginning of the development of soil ecotoxicology is associated with observations of the effects of pesticides on soil invertebrates in the 1960s [51, 53]. That period was characterized by great attention to the effects of chemicals in general on the environmental objects. In this regard, the American journalist Rachel Carson is often brought to mind, who first attracted attention to the negative consequences of the increasing use of synthetic pesticides in the postwar years with the publication of her book *Silent Spring* in 1962. This book warned that the struggle battle for the harvest by chemical means inevitably leads to a threat to human health, and the first signals should already be seen in the effects of pesticides, especially DDT, on birds—singing birds fall silent because of the uncontrolled spread of chlorinated pesticides accumulating in the food chain. The publication of this book was a bright event and an important trigger for the birth of a new science, ecotoxicology. In 1969, a significant impetus to its development was given by Rene Truhart, who combined elements of a number of natural sciences (chemistry, biochemistry, physiology, population genetics, etc.) and defined the key concepts, subject, and methods of research of the new science.

R. Carson's book attracted universal attention to environmental pollution and turned out to be an important, but not the only impetus for the development of toxicological research. This was facilitated by the mass introduction of technologies and the use of chemical products in various fields since the beginning of the 19th century, which necessitated the need to assess the consequences of such anthropogenic impacts. For the first time at the legislative level, biotesting was included in the operational control of water pollution in the United States, where the United States Environmental Protection Agency (US EPA) was created to protect the environment and human health. Subsequently, such organizations designed to develop standards and monitor their implementation, began to legislatively stimulate the introduction of biotests into practice in other countries.

In the 1980s and 1990s, the issues of methodological support for ecotoxicological studies to assess the risk of chemicals were successfully solved. During this period, many ecotoxicity tests were developed using individual species of living organisms. They were based, first of all, on an integral characteristic—the survival rate of beings.

A great contribution to the introduction of biotests into the environmental practice of our country was made by the Russian school of hydrobiology under the leadership of Professor Stroganov from the Lomonosov Moscow State University [15]. In 1990, the USSR State Committee for the Environmental Protection approved the first regulatory document in the field of ecotoxicological assessment of water bodies *Methodological Guide for Water Biotesting* (RD 118-02-90) providing for the use of a small set of hydrobionts: algae (*Scenedesmus quadricauda*, *Chlorella vulgaris*), invertebrates (*Daphnia magna*, *Ceriodaphnia affinis*), fish (*Poecilia reticulatus*). In 1991 The *Rules for the Protection of Surface Waters* regulating the treatment and discharge of wastewater into water bodies with the use of toxicological control by biotesting methods were approved [12]. The principles developed in this methodological document are currently used in legislative acts in order to protect not only water but also soil resources.

Diversity of test systems and methods for assessing ecotoxicity. An indicator of ecotoxicity is the degree of change in certain parameters of a living system at various levels of organization, which is recorded by various methods. These can be biochemical, biophysical methods, visual counting, various types of microscopy. Thus, during the study of response of mycobiota representatives to the chemical pollution using light microscopy, changes in the germination of spores under the impact of heavy metal salts and oil products were identified for a number of micromycetes (*Phoma* spp., *Fusarium oxysporum, Stemphylium* sp., *Trichoderma garcian, Penicillium frequentans, Mucor racemosum*) [18, 21]. The transformation of the morphobiological structure of the biomass of microscopic fungi under the impact of waste from the production of mineral fertilizers has been established by the method of luminescent microscopy using a specific dye [17]. By seeding on solid media, the presence of pollutants can be recorded by changes in the radial growth rate of colonies of micromycetes (species of the *Phoma, Fusarium,* and *Thielaviopsis* genera) [17, 21]. The effect of toxic substances is controlled by the accumulation of biomass during the cultivation in liquid media [16, 17, 21, 52].

In the late 1990s and early 2000s, biotests standardized by international organizations (OECD—Organization for Economic Cooperation and Development and ISO—International Organization for Standardization) appeared in soil ecotoxicology; they were based on accounting for mortality and reproduction rates of enchytraeids [79], earthworms [78, 80] and collembolas [77]. Later, behavioral tests (avoidance) were approved for the same organisms [62], and a bioaccumulation test was also approved for earthworms and enchytraeids [100]. A series of regulatory documents regulating the use of higher plants appeared [76, 81, 84, 86] and microorganisms [60, 85] appeared.

Test organisms transferred from the environment to controlled conditions of laboratory cultivation must meet a number of requirements. As a rule, it is indicated that they should be represented by species widely distributed in natural conditions, easily available in large quantities throughout the year, represent the most genetically homogeneous population, and be free from pathogens and parasites. The most important thing is that standardized test-organisms should have a high sensitivity to toxicants, which, according to the requirements of standard methods of toxicity measurements, should be regularly monitored in the laboratory for reactions to model toxicants. Ideally, they should be sensitive to a wide range of toxins (or to a group of compounds) for the purposes of their identification in media, and the observed reaction should be reproducible.

Batteries of biotests. Eventually, the so-called biotest batteries—series of test systems based on the reactions of different types of living organisms to the same target area—are becoming more and more popular [38, 54, 94, 101]. More complicated complex modular systems focused on measuring structural and functional indicators characterizing bioavailability and accumulation of toxicants have also become popular [4, 5].

Biotest batteries are considered particularly efficient for the analysis of mixed environmental pollution. They allow us to combine test responses into one assessment, which makes it possible to classify plots according to their degree of contamination and compare the effects of various commercial products when detecting toxicity in samples with mixed contamination [57]. This period is associated with the emergence of integrative multi-marker concepts: Multi-Marked Bioindication Concept (MMBC). For example, a modern experimental assessment of the hazard class of waste is based on the use of a battery of two test systems with the participation of organisms of different taxonomic affiliation according to the current *Criteria for Assigning Waste to Hazard Classes I-V according to the Degree of Negative Impact on the Environment* (approved by the Order of the Ministry of Natural Resources of the Russian Federation No. 536 of December 4, 2014) [9].

At the same time, discussions about the size of the battery of biotests, the validity of the inclusion of certain tests, and the significance of the sensitivity of individual test species are still in progress. The results of the response of one test species with high and wide sensitivity within a battery are given decisive importance: if at least one species has detected toxicity, then the sample is usually classified as toxic. Other approaches show the possibility of using a limited number of test species chosen with due account for their specific sensitivity to pollutants participating in the contamination of the plot. This is justified by an increase in the profitability of the battery composition [72, 73].

In recent years, the need for the concept of specific tests, the so-called "site-specific ecotoxicological tests," the results of which can be combined into one integrated index, has been experimentally substantiated [16, 102].

In order to improve both discrimination and a full-fledged conclusion about the contamination degree of plots, it is advisable to use a larger number of test species in biotests integrated into batteries, including those that are highly sensitive to certain types of pollution [54, 73]. For example, the US Environmental Protection Agency recommends screening using CALUX ® analysis (Chemically Activated LUciferase eXpression) to detect dioxins and dioxin-like compounds in soils and sediments (https://www.epa.gov/sites/default/files/2015-12/documents/4435.pdf).

Our twenty-year experience of ecotoxicological research indicates the need to adapt the measurement methods existing in the register of the Federal Information Fund for Assuring the Measurements' Uniformity of Russian Federation to specific types of soil pollution, since the sensitivity of test crops differs significantly [16, 23].

The criteria for selecting test types in the battery include, inter alia, practicality, determined by the feasibility and cost-effectiveness of the test; acceptability, including aspects such as standardization, reproducibility, and statistical reliability of the test method; and environmental significance, including sensitivity. Van Gestel et al. [102] believe that in order to obtain a balanced battery of tests, it is necessary to ensure the representativeness of the ecosystem or biotopes under study, namely, to include organisms representing different functional groups, different taxonomic groups, and different ways of exposure, on one hand, and the representativeness of the responses of test species, which means their actual relevance for the normal functioning of populations and communities (survival, reproduction), on the other hand [102].

The inclusion of representatives of the main trophic groups (producers, consumers, and reducers) in the battery of biotests to a certain extent reflects the ecosystem approach to assessing the risk of pollution and increases the reliability of biodiagnostics of the quality and sustainable functioning of ecosystems [16]. Such an approach, certainly, cannot replace field tests in natural ecosystems, but it complements it, and allows us to obtain signals of trouble in the advanced mode [2].

Molecular biomarkers. In 1990, the need to assess the effects of chemicals on living organisms, the interest in studying the bioavailability of pollutants and the need to assess it accelerated the development of new methods in which sensitive and potential early warning tools for negative effects were biochemical test functions (biomarkers) [66, 96]. Such early biomarker effects are observed in many species of soil invertebrates (isopods) [50], as well as in microorganisms. In particular, various targets of the toxic effect of organic and inorganic pollutants on fungal cells are known: (1) inhibition of enzymatic activity; (2) oxidative stress or interaction with systems, which usually protect from the harmful effects of free radicals; (3) toxic metals displacing or replacing metal ions in metal enzymes, which become inactivated; and (4) disturbance of the integrity of membranes [3, 34, 41].

Chemicals acting on the membranes of fungi—the first physicochemical barrier that meets them—are able to cause quantitative and qualitative changes in their lipid composition [14]. In particular, the analysis of lipid profiles of fungi showed that heavy metals (Ni, Cu, Zn ions) affect the composition of lipid components and change the flowability of *Curvularia lunata* membranes [82, 83]. Pb ions cause significant damage to the cytoplasmic membrane, reducing the proportion of phosphatidylcholines and unsaturated fatty acids in the composition of phospholipids of the mycelium of *Paecilomyces marquandi* [95].

Such biomarkers can act as a sensitive indicator of early warning of possible effects at higher levels of biological organization, and can also provide information about the mode of action of a chemical.

Advances in molecular biology contribute to the development of new biosensors for the study of bioavailability and (eco)toxicity of both heavy metals and nanoparticles. In nanoecotoxicology, metal-specific bacterial biosensors based on recombinant microorganisms are already used in combination with a set of multitrophic biotests on invertebrates, algae, and bacteria [65].

Scientific interest is attracted to the combination of various stress factors, and not only to the interaction between various chemicals (toxicity of mixtures) but also to the toxicity caused by the combined action of chemical agents and other stress factors [99, 103].

Ecotoxicogenomics. OMICS technologies are of increasing interest as tools in assessing the environmental significance of the effects of chemical stress, environmental vulnerability of living systems at the genetic level.

When comparing various biochemical test parameters [96], it was shown that responses at the gene level (DNA damage) are most sensitive to cadmium. Genomics, proteomics, and transcriptomics significantly increase the set of ecotoxicology tools. Currently, ecotoxicogenomics is considered as a tool to better understand the molecular mechanisms of action of chemicals and the mechanisms of resistance to pollution forming, in particular, resistance to metals or pesticides [103].

Ecotoxicogenomics can also help to reveal the mechanisms of the impact of various particles on organisms, as was shown by the example of metal nanoparticles [65] or microplastic particles [64]. Ecotoxicogenomics tools can be useful in econanotoxicology, but it still requires significant efforts and further research before they can be applied in the practice of assessing the ecotoxicological risk of soil contamination.

Approaches to the assessment of ecotoxicity of soils. The impact in test systems is measured via imitation of possible routes of entry of harmful substances into organisms, so that water is the main media for the tested objects. Hence, hydrobionts—protozoa, algae, crustaceans, and other organisms or their elements—are mainly used as sensitive biological sensors.

The study of the toxicity of solid components of the environment (soils, bottom sediments, soils, waste, etc.) by the reactions of hydrobionts is considered an indirect way of affecting biosensors [17]. This approach has been called eluate, since its implementation uses aqueous extracts (leaching extracts, eluates of solid objects). Unfortunately, some types of pollutants exhibit hydrophobic properties, accumulating in soil or sediments. In such cases, biotesting in test systems based on the reactions of hydrobionts and the analysis of water extracts may not always reflect the toxicity of a given soil or sediment sample.

It is obvious that soil studies, as well as other solid substrates, should be carried out with the help of native test species. This approach, in which the solid mass is analyzed in direct contact with soil-dwelling organisms, has been called application, or contact (sometimes called substrate), biotesting [16].

To assess the quality and toxicity of bulk soil samples, the most informative approaches are based on the use of invertebrates [59, 77–80] and higher plants, among which there are many traditionally applied species of monocotyledonous and dicotyledonous plants [75, 76, 81, 84, 86, 88].

Reference soil. In an effort to standardize the method and facilitate comparison of the results of standard tests, a reference sample is used—standard soil (Reference/Standard/Artificial soil) according to the ISO 11268-2 protocol. It consists of peat (10%), kaolin clay (20%), and quartz sand (70%) with the addition of some $CaCO_3$ (pH 6.0). According to its properties, this soil resembles loamy sand. Some manuals allow the use of 5% peat [101].

The use of reference soil is important for comparing the toxicity of different preparations and predicting their impact on natural soils. At the same time, the idea that soil type is important in determining the toxicity of chemicals has been accompanied by a growing understanding of the concept of bioavailability: only a part of the total amount of a chemical in the soil is available for absorption by organisms and, therefore, is an active agent relevant for risk assessment. This has been demonstrated more than once in experimental works [11, 16, 19, 89].

In soils that differ in acidity and the contents of clay and organic substances affecting the availability of lead, the same dose of lead (2000 mg Pb/kg soil) proved to be differently harmful to earthworms *Eisenia andrei* [39]. After 28 days of exposure, all earthworms died in some samples, whereas earthworm mortality was not observed in other soils, and only a part of earthworms died in the third group of soils. In a soddy-podzolic soil from two fields differing in the organic carbon content, and with the same level of polymetallic contamination with a complex of lead, copper, and zinc salts, the test plants of white mustard *Sinapis alba* differed sharply in growth indicators and biomass accumulation [19].

Heavy metals have different effects on the accumulation of biomass of fungi and bacteria, as well as the structure of micromycete communities in soils of the same type differing in their humus contents. Under the impact of heavy metals, the proportion of melanized forms of fungi that are, as a rule, resistant to adverse impacts [107] increased by 25.9% in the humus-rich soil and by 45.7% in the humus-poor soil [20].

Biotesting of ecotoxicological risk. In the demand for biotests for environmental control and pollution risk, two directions can be highlighted: prognostic and biodiagnostics (assessment of soils at the moment).

The prognostic approach is aimed at predicting the possible effects of chemicals in order to regulate their use and control their appearance on the market. For this purpose, laboratory bioassays are conducted to determine the safe levels of specific chemicals (usually, new preparations) entering soils and other natural media.

The second approach is diagnostic; it allows us to assess the real environmental risk or damage and, in the case of detection of toxic pollution, make management decisions to restore disturbed soils and reduce the risk of chemical pollution.

The prognostic approach is almost entirely based on the principles of human toxicology and assumes that the potential effect of a chemical on ecosystems can be assessed by its toxicity relative to standardized test cultures of sensitive species under controlled laboratory conditions. In order to get a correct idea of the potential hazard of the chemical to the ecosystem, the testing is carried out in a series of tests with a set of species. When determining safe levels of chemicals, acute and chronic toxicity assessments are carried out. In the variants of acute toxicity assessment, survival is assessed in short-term experiments, while chronic toxicity is assessed in long-term experiments; as a rule, fertility rate is assessed.

A set of toxicometric indicators have been determined, with the help of which the toxicity is quantified, reflecting the concentrations of the active substance that cause mortality or deviation from the control of the values of any other test functions by a certain amount for a certain period of exposure of test organisms in the sample of the object under study. Toxicity is quantified using concentration parameters such as LC_{10} and LC_{50} (concentrations causing the death of 10% and 50% of test organisms in the exposed samples, respectively), EC_{10} and EC_{50} (concentrations causing a decrease by 10% and 50%, respectively, of any test functions, for example, growth or the number of young individuals produced), as well as NOEC and LOEC (concentrations, respectively, not causing observed effects and causing minimal observed effects) [25–29, 59–63].

Thus, with a predictive approach, the results of toxicity tests are used to establish thresholds or safe levels of chemicals in the soil. If it is possible to obtain data on toxicity in several test systems based on responses of different species (ideally ≥8) then a statistical method of species sensitivity distribution (**SSD**) is used [22, 87].

Earlier in our work, critical values of a number of soil pollution indicators (Co, Cr, Zn, U, Ra) were obtained from the dumps of uranium mines (village of Kajy-Say, Kyrgyzstan), providing a given permissible probability of environmental risk based on the SSD method applied to the toxicity of elements detected in the soil for certain types of soil micromycetes [22].

The diagnostic approach when using biotesting provides an effective tool for assessing the degree of toxicity and the ecological quality of soils monitoring. These assessments are critical to the planning and successful implementation of rehabilitation activities.

Thus, both to predict the effects of the use of specific substances and to diagnose the quality of soils, toxicity tests are used; in both cases, not one, but a series of tests is recommended.

Ways to integrate biotesting results into environmental assessment. Depending on the type of impact on ecosystems, the toxicity, microbiological, other biological, and physicochemical parameters of the soil changes to varying degrees. There are many indices and assessment systems that offer one or another degree of integration of different ecosystem parameters [6, 7, 13, 30, 45]. Recently, within the framework of the concept of soil quality (or soil health, SH), a holistic approach was developed. It gives an idea of the interaction between the main components of the soil system [40]. However, the authors themselves state that it is not easy to implement a holistic approach, since the soil is a complex system in which physical, chemical, and biological characteristics and processes are involved and interact. The approach within the framework of the SH concept can be considered as a desirable, but unrealistic tool for practical use at this stage [40].

The use of biotic indicators in the integrated assessment of soils has been progressing markedly in recent decade; a number of proposals for the generalization of various indices fine have found their practical application.

Integral indicator of the biological state of the soil. One example of a comprehensive assessment is the integral indicator of the biological state of the soil (IIBSS), proposed as a criterion for the degree of disturbance of the ecological functions of the soil [6]. It is based on a point assessment of individual indicators of the state of the biota for a particular sample (or variant of the experiment) relative to the maximum observed value in a series of samples (or variants of the experiment), then the average assessment score of a number of studied indicators is calculated from the sum of the relative values of the indicators (points). The integral indicator of the ecological and biological state of a particular soil is calculated by the formula:

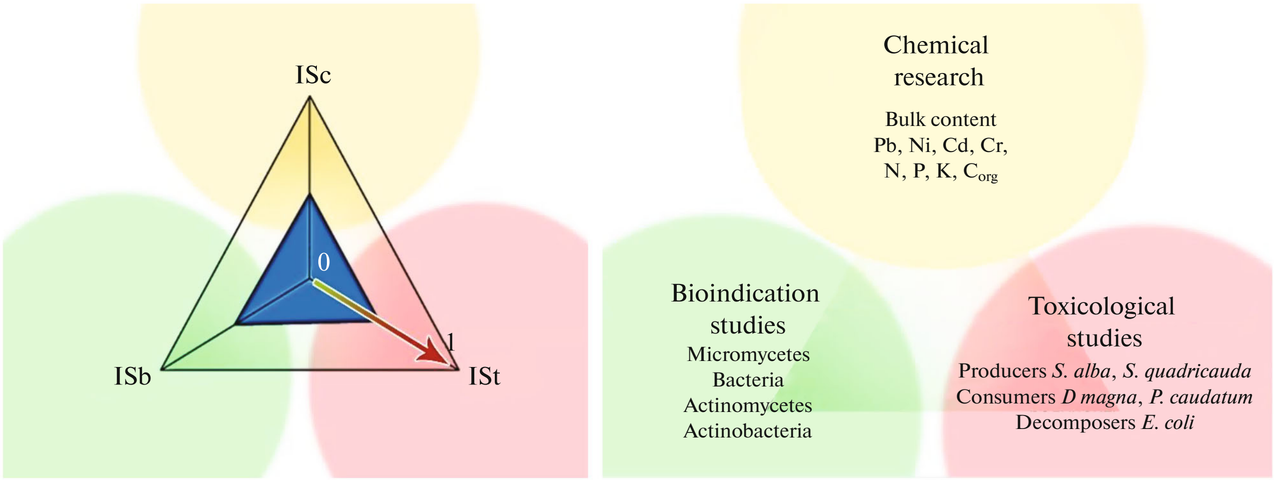

Fig. 1. Graphical representation of the integral index (IS) of soil state (left) and the studied indicators of urban soils (urbanozems) at different plots in Kirov (right) (according to [13]). Legend: Control, ISc, ISb, and ISt are the indices of soil state calculated according to the chemical, bioindication, and toxicological indicators, respectively.

$$\mathrm{IBSS} = \frac{\mathrm{S}_{\mathrm{av}}}{\mathrm{S}_{\mathrm{av\,max}}} \times 100\%.$$

where S_{av} is the average assessment score of all indicators, and $\mathrm{S}_{\mathrm{av.\,max}}$ is the maximum assessment score of all indicators [6].

In addition to the indicators, the IBSS may also include the assessment of toxicity based on the results of biotesting. When diagnosing pollution, the authors suggest taking the value of each of the indicators in uncontaminated soil for 100% and expressing the values of the same indicator in polluted soil as a percentage in relation to it. It is considered that if the values of the IBSS decrease by less than 5%, then the soil performs its ecological functions normally; a decrease in the IBSS by 5–10% attests to the disturbance of information ecofunctions; by 10–25%, biochemical, physicochemical, chemical, and holistic ecofunctions; by more than 25%, physical ecofunctions. The disadvantages of this approach include the lack of consideration for real changes in the chemical and physical properties of disturbed soils.

Integral index of the ecological state of the ecosystem. The original methodology is used to assess the ecological state of freshwater ecosystems [1], which can be applied to soil assessment. The Integral Index of the Ecological State of an Ecosystem (IIESE) is a combination of two components reflecting the ecological state by chemical and biological indicators. IIESE is defined as

$$\mathrm{IIESE} = \frac{\sum B_i + \sum H_i}{N_b + N_h},$$

where B_i and H_i are the analyzed biotic and hydrochemical indicators expressed in relative units (scores) and N_b and N_h are the numbers of the biotic and hydrochemical indicator, respectively [1].

This technique is also based on the analysis of dimensionless values (scores), which are established on the basis of expert assessments. The curve of dependence between the anthropogenic load and the IIESE is a typical S-shaped dose–response function with a characteristic inflection point characterizing the critical level of anthropogenic load [2, 22].

Index of functional biodiversity of the microbiome. An original method of assessing the activity of the soil microbiome is presented in the literature by an integral indicator of “soil health” based on the functional biodiversity (FBD) indicators of the soil microbial community. These indicators are calculated on the basis of the consumption spectra of substrates in multisubstrate testing [4, 5].

TRIAD methodology. A comprehensive assessment of the state of natural media involves the so-called TRIAD methodology. In 2017, it was included in the system of international standards—ISO 19204:2017 *Soil quality—Procedure for Site-Specific Ecological Risk Assessment of Soil Quality (Soil Quality TRIAD Approach)* [43, 61]. The TRIAD paradigm was formulated by Peter Chapman in relation to the assessment of sediment pollution as an algorithm that allows assessing potential harmful effects on the ecosystem taking into account simultaneously the concentrations of chemicals, bioavailability of pollutants, and ecotoxicological parameters of the observed ecosystems [43, 44]. It is based on the methodology of the interdisciplinary level and takes into account the data of chemical (ISc), bioindication (ISb) and toxicological (ISt) studies [43, 47, 89–91] (Fig. 1).

The calculation of the state indices (ISc, ISt, and ISb) takes place in several stages by comparing the

Table 1. Correspondence of the integral index of the soil state determined on the basis of the TRIAD methodology to the categories of soil quality state of load

IS values	Soil quality category	Load	Soil state
$IS = 0$	I	Permissible	Background
$0 < IS < 0.30$	II	Low	Slightly disturbed
$0.30 \le IS < 0.50$	III	Moderate	Moderately disturbed
$0.50 \le IS \le 0.79$	IV	High	Strongly disturbed
$0.79 \le IS \le 1$	V	Very high	Irreversibly disturbed

obtained values for the sample with background data or the maximum permissible concentrations (MPC) [47, 93].

When calculating the soil condition index by bioindicators, the ISb in each test sample is compared with that in the background sample. Similarly, the assessment of toxicological indicators for the ISt index is carried out. The indices of the state of toxicological and bioindication parameters are calculated on the basis of arithmetic averages of all measured indicators. For all components, functions of a certain type are used to convert to a normalized scale (from 0 to 1) [13, 93].

As biotic (toxicological and bioindication) indicators are most informative from the point of view of maintaining a stable state of ecosystems and performing ecological functions by soils, such as habitat for living organisms [16], when calculating the integral state indices according to the triad of indicators—chemical, toxicological, and bioindication (ISc, ISt and ISb, respectively)— it is proposed to use weighting coefficients equal to 1.5 and 2.0 [47]. Then, the formula for calculating the integral state index (**IS**) has the form:

$$IS = \frac{ISc + 1.5ISt + 2.0ISb}{1.0 + 1.5 + 2.0}.$$

In the work of Pukalchik et al. [13], gradations of IS are proposed, the ranges of values of which correspond to a five-level scale and characterize the corresponding degree of anthropogenic load on soils and the ecological state of soils [2, 30] (Table 1).

As can be seen from this table, the polar values (0 and 1) correspond to the gradations "good" and "bad"; i.e., the higher the index, the greater the difference from the background and, therefore, the soil suffers from the greater chemical load.

The results of the biotesting processed according to the algorithm of the TRIAD methodology are included in the integral index for assessing the state of soils in polluted and background areas of urbanozems in Kirov and in mountainous ecosystems of Kyrgyzstan near the storage place of radioactive waste [98], for determining the degree of soil recovery after the use of remediation preparations [89, 90], for substantiating the need for remediation of soils contaminated with heavy metals at six areas within a radius of 2 km from an abandoned mine in North Korea, where gold (Au), lead (Pb), and zinc (Zn) were mined [58]. In the absence of background territories, similar criteria and tests were applied to assess soils and compare the environmental risk of contamination of two plots near a landfill in Northern Spain, studied upstream and downstream the river relative to the landfill site [55], as well as in many other works [11, 32, 67, 70].

CONCLUSIONS

Biotesting is a classic experimental methodological assessment of the toxicometric parameters of living systems (whole organisms or their parts) after exposure to the analyzed objects. The methodological basis of biological testing developed in the field of medical toxicology has provided a tool for laboratory assessment of toxicity not only in medicine and veterinary medicine. The use of biotesting methods has long spread beyond the boundaries of the area defined as the sphere of development of standards for the content of chemicals in the environment and the initial assessment of the properties of new substances. Biotesting is used all over the world to analyze the ecological state of natural media subjected to the harmful impact of human-made factors, as well as to determine the degree of danger of production and consumption waste [9, 25–29, 45].

Biotesting provides information on the danger to stable functioning of ecosystems in advance, before the appearance of visible changes in biota, whereas bioindication is aimed at recording changes in the state of biocenoses under the impact of harmful factors in the natural environment.

Since the publication of the first schemes and tools for assessing and monitoring soil quality in the 1990s, more than 60 national and regional approaches have appeared; they have mainly been developed in North America, Europe, and China. The main attention in these approaches is paid to the characteristics of soil fertility, which is considered as their capacity to provide nutrients and water to plants, as well as to the absence of toxic substances (www.fao.org). In this regard, some authors consider it necessary to supplement the characteristics of soil quality optimal for crop growth with indicators of biodiversity and functional activity of the soil microbiota [7, 20]. Thus, the microbial biomass of the soil and its respiratory activity can serve as indicators of its change under different impacts [36, 60] and thus characterize the soil health. These indicators are included in the environmental monitoring programs of soils and terrestrial ecosystems in a number of European countries [7, 36].

The practical relevance of biotesting methods is reflected in the modern regulatory documentation of the relevant regulatory authorities. Certification of the methods for measuring toxicity, which ensures the established accuracy indicators, has become possible largely due to the introduction of a strict procedure for standardization of the assessment methods and test organisms (GOST R 8.563–2009). In Russia, in various fields of economic activity (agricultural, medical, and environmental), sets of biotests are used in accordance with methodological guidelines, manuals, and corresponding orders for soil quality assessment by relevant ministries.

To create a standard biotesting technique, it is necessary to go through a strict procedure that includes regulation of the species of test organisms, which should ensure a certain level of sensitivity of the test culture and creating optimum conditions for the test regulated by the methodology. Although the first biotesting methods were certified relatively recently (in the 1990s), w a significant number of standard biotests have already been entered into the federal Register (FR) as recommended for practical environmental control. One can learn more about them on the website (https://fgis.gost.ru/fundmetrology/registry/16). These documents are practical guidelines aimed at introducing ecotoxicological control methods into laboratory research.

Toxicity assessment is an important but not the only component of an integrated assessment of the ecological quality of soils. The optimal way to integrate the results of biotesting into the integral assessment of soils is the TRIAD methodology that takes into account the results of quantitative chemical analysis of the content of pollutants and bioindication, i.e., observations of the state of representatives of biota in natural conditions (in situ) and indicators of soil toxicity in relation to standardized test cultures in laboratory conditions (ex situ). This interdisciplinary variant of data integration is not free from controversial points in the calculation algorithm (in particular, at the stage of assigning the so-called weighting coefficients to biotic indicators [47]). However, the TRIAD methodology has been widely tested in many countries on soils with different contamination levels and nature of the contaminants for two decades since Chapman's work [44]. Its implementation in the form of the international standard ISO 19204-2017 [61] provides an efficient tool for assessing and comparing the ecological quality of soils and predicting the effects of chemicals intentionally or unintentionally entering the soil.

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REFERENCES

1. *Bioindication of the Ecological State of the Plain Rivers*, Ed. by O. V. Bukharin and G. S. Rozenberg (Nauka, Moscow, 2007) [in Russian].
2. E. L. Vorobeichik, O. F. Sadykov, and M. G. Farafontov, *Ecological Standardization of Technogenic Pollution of Terrestrial Ecosystems (Local Level)* (Nauka, Yekaterinburg, 1994) [in Russian].
3. E. A. Gorbunova and V. A. Terekhova, "Heavy metals as a stress factor for fungi: their action at the cellular and organism levels," Mikol. Fitopatol. **29** (4), 63–69 (1995).
4. M. V. Gorlenko and P. A. Kozhevin, *Multisubstrate Testing of Natural Microbial Communities* (MAKS Press, Moscow, 2005) [in Russian].
5. M. V. Gorlenko, O. S. Yakimenko, M. V. Golichenkov, and N. V. Kostina, "Functional biodiversity of soil microbe colonies affected by organic substrates of different kinds," Moscow Univ. Soil Sci. Bull. **67**, 71–78 (2012).
6. K. Sh. Kazeev, S. I. Kolesnikov, and V. F. Val'kov, *Biological Diagnostics and Indication of Soils: Study Methods* (Rostov-on-Don, 2003) [in Russian].
7. V. I. Kiryushin, "Methodology for integrated assessment of agricultural land," Eurasian Soil Sci. **53**, 960–967 (2020). https://doi.org/10.1134/S1064229320070066
8. G. N. Koptsik, "Problems and prospects concerning the phytoremediation of heavy metal polluted soils: a review," Eurasian Soil Sci. **47**, 923–939 (2014). https://doi.org/10.1134/S1064229314090075
9. *Criteria for Assessment of Wastes to I-V Hazard Classes According to the Degree of Negative Impact on Environment (Approved by Order of the Ministry of Natural Re-*

sources and Ecology of Russian Federation No. 536 of December 4, 2014) (Moscow, 2014) [in Russian.

10. A. P. Levich, "A biological concept of environmental control," Dokl. Biol. Sci. **337**, 360–362 (1994).
11. N. V. Mayachkina and M. V. Chugunova, "Biotesting of soils using their ecotoxicological assessment," Vestn. Nizhegorodsk. Univ. im. N.I. Lobachevskogo, No. 1, 84–93 (2009).
12. *Regulations on Protection of Surface Waters* (USSR State Committee for Nature Protection, Moscow, 1991) [in Russian].
13. M. A. Pukalchik, V. A. Terekhova, O. S. Yakimenko, K. A. Kydralieva, and M. I. Akulova, " "Triad method for assessing the remediation effect of humic preparations on urbanozems," Eurasian Soil Sci. **48**, 654–663 (2015).
14. O. A. Rozentsvet, E. V. Fedoseeva, and V. A. Terekhova, "Use of lipid biomarkers in the ecological assessment of soil biota: fatty acid analysis (a review)," Usp. Sovrem. Biol. **139** (2), 161–177 (2019). https://doi.org/10.1134/S0042132419020078
15. N. S. Stroganov, *Toxic Analysis of Aquatic Environment* (Nauka, Moscow, 1971), p. 14.
16. V. A. Terekhova, "Soil bioassay: problems and approaches," Eurasian Soil Sci. **44**, 173–179 (2011).
17. V. A. Terekhova, "The importance of mycological studies for soil quality control," Eurasian Soil Sci. **40**, 583–587 (2007).
18. V. A. Terekhova, *Use of Micromycetes for Ecological Assessment of Aquatic and Terrestrial Ecosystems* (Nauka, Moscow, 2007) [in Russian].
19. V. A. Terekhova, E. V. Prudnikova, A. P. Kiryushina, M. M. Karpukhin, I. O. Plekhanova, and O. S. Yakimenko, "Phytotoxicity of heavy metals in contaminated podzolic soils of different fertility levels," Eurasian Soil Sci. **54**, 964–974 (2021). https://doi.org/10.1134/S1064229321060132
20. V. A. Terekhova, E. V. Fedoseeva, Ju. V. Belfeg, A. P. Kiryushina, A. G. Rychagova, and N. V. Verkhovtseva, "Structure of microbial complexes in modelling of polymetallic pollution and remediation of agrosoddy-podzolic soils," Moscow Univ. Soil Sci. Bull. **76**, 33–40 (2021).
21. V. A. Terekhova and L. G. Shved, "Change of morphobiochemical characteristics of aquatic fungi affected by heavy metals," Ekologiya, No. 6, 77–79 (1994).
22. V. A. Terekhova, V. K. Shitikov, A. E. Ivanova, and K. A. Kydralieva, "Assessment of the ecological risk of technogenic soil pollution on the basis of the statistical distribution of the occurrence of micromycete species," Russ. J. Ecol. **48**, 417–424 (2017).
23. M. A. Timofeev, V. A. Terechova, and P. A. Kozhevin, "Biotesting for Cd pollution in soils," Moscow Univ. Soil Sci. Bull. **65**, 179–182 (2010).
24. O. F. Filenko, E. F. Isakova, and A. V. Chernomordina, "Specific effect of potassium bichromate on generations and model populations of lower crustaceans," in *Current Problems in Aquatic Toxicology* (Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, 2004), pp. 176–194.
25. *FR.1.39.2007.03222. Biological Control Methods. Toxicity Analysis of Water and Water Extracts of Soils, Sediments of Sewage Waters, and Wastes by Mortality and Fertility Dynamics of Daphnia* (Akvaros, Moscow, 2007) [in Russian].
26. *FR.1.31.2020.38716. Biological Control Methods. Measurement of Biological Activity of Humic Acids by Phytotesting on Fitoskan–2* (Moscow State Univ., Moscow, 2008) [in Russian].
27. *FR.1.39.2006.02506. PND F 14.1:2:3.13-06 (16.1:2.3:3.10-06). Toxicity Analysis of Wastes, Soils, Sediments of Sewage, Surface, and Ground Waters by Biotesting Using Unicellular Ciliates Paramecium caudatum Ehrenberg* (Moscow State Univ., Moscow, 2006) [in Russian].
28. *FR.1.39.2007.03221. Toxicity Analysis of Water and Water Extracts of Soils, Sediments of Sewage Waters, and Wastes by Mortality and Fertility Dynamics of Ceriodaphnia* (Akvaros, Moscow, 2007) [in Russian].
29. *FR.1.39.2007.03223. Biological Control Methods. Toxicity Analysis of Water and Water Extracts of Soils, Sediments of Sewage Waters, and Wastes by Dynamics of Fluorescence Level of Chlorophyll and Abundance of Algae Cells* (Akvaros, Moscow, 2007) [in Russian].
30. A. S. Yakovlev and O. A. Makarov, "Environmental assessment, environmental regulation, and land reclamation: general terms and definitions," Ispol'z. Okhr. Prir. Resur. Ross., No. 3 (87), 64–70 (2006).
31. I. Acosta Rodríguez, V. M. Martínez-Juárez, J. F. Cárdenas-González, and M. D. G. Moctezuma-Zárate, "Biosorption of arsenic(III) from aqueous solutions by modified fungal biomass of *Paecilomyces* sp.," Bioinorg. Chem. Appl. **2013**, 376780 (2013). https://doi.org/10.1155/2013/376780
32. M. O. Aljahdali and A. B. Alhassan, "Ecological risk assessment of heavy metal contamination in mangrove habitats, using biochemical markers and pollution indices: a case study of *Avicennia marina* L. in the Rabigh lagoon, Red Sea," Saudi J. Biol. Sci. **27**, 1174–1184 (2020). https://doi.org/10.1016/j.sjbs.2020.02.004
33. S. S. Andrews, D. L. Karlen, and J. P. Mitchell, "A comparison of soil quality indexing methods for vegetable production systems in Northern California," Agric. Ecosyst. Environ. **90** (1), 25–45 (2002). https://doi.org/10.1016/S0167-8809(01)00174-8
34. P. Baldrian, "Effect of heavy metals on saprotrophic soil fungi. Soil Heavy Metals," Soil Biol. **19**, 263–281 (2010). https://doi.org/10.1007/978-3-642-02436-8
35. A. Barman, D. Gohain, U. Bora, and R. Tamuli, "Phospholipases play multiple cellular roles including growth, stress tolerance, sexual development, and virulence in fungi," Microbiol. Res. **209**, 55–69 (2018). https://doi.org/10.1016/j.micres.2017.12.012
36. E. Blagodatskaya and Y. Kuzyakov, "Active microorganisms in soil: critical review of estimation criteria and approaches," Soil Biol. Biochem. **67**, 192–211 (2013). https://doi.org/10.1016/j.soilbio.2013.08.024
37. L. Bondarenko, A. Kahru, V. Terekhova, G. Dzhardimalieva, P. Uchanov, and K. Kydralieva, "Effects of humic acids on the ecotoxicity of Fe_3O_4 nanoparticles and Fe-ions: impact of oxidation and aging," Nano-

materials **10** (10), 2011 (2020).
https://doi.org/10.3390/nano10102011

38. W. Brack, R. Altenburger, U. Ensenbach, M. Moder, H. Segne, and G. Schuurmann, "Bioassay-directed identification of organic toxicants in river sediment in the industrial region of Bitterfeld—A contribution to hazard assessment," Arch. Environ. Contam. Toxicol. **37**, 164–174 (1999).
39. K. D. Bradham, E. A. Dayton, N. T. Basta, J. Schroder, M. Payton, and R. P. Lanno, "Effect of soil properties on lead bioavailability and toxicity to earthworms," Environ. Toxicol. Chem. **25** (3), 769–775 (2006). https://doi.org/10.1897/04-552R.1
40. E. K. Bünemann, G. Bongiorno, Z. Bai, R. E. Creamer, G. De Deyn, R. de Goede, and L. Brussaard, "Soil quality—A critical review," Soil Biol. Biochem. **120**, 105–125 (2018). https://doi.org/10.1016/j.soilbio.2018.01.030
41. M. Calonne, J. Fontaine, D. Debiane, F. Laruelle, A. Grandmougin-Ferjani, and A. Lounès-Hadj Sahraoui, "The arbuscular mycorrhizal *Rhizophagus irregularis* activates storage lipid biosynthesis to cope with the benzo[a]pyrene oxidative stress," Phytochemistry **97**, 30–37 (2014). https://doi.org/10.1016/j.phytochem.2013.10.014
42. M. R. Carter, E. G. Gregorich, D. W. Anderson, J. W. Doran, H. H. Janzen, and F. J. Pierce, "Concepts of soil quality and their significance," in *Developments in Soil Science*, Vol. 25: *Soil Quality for Crop Production and Ecosystem Health* (Elsevier, Amsterdam, 1997), Ch. 1, pp. 1–19.
43. P. A. Chapman, "Decision making framework for sediment assessment developed for the Great Lakes," Hum. Ecol. Risk Assess. **8** (7), 1641–1655 (2005). https://doi.org/10.1080/20028091057538
44. P. M. Chapman, "The sediment quality Triad approach to determining pollution-induced degradation," Sci. Total Environ. **97**, 815–825 (1990). https://doi.org/10.1016/0048-9697(90)90277-2
45. P. M. Chapman, B. G. McDonald, and G. S. Lawrence, "Weight-of-evidence issues and frameworks for sediment quality (and other) assessments," Hum. Ecol. Risk Assess. **8** (7), 1489–1515 (2002). https://doi.org/10.1080/20028091057457
46. M. Chodak, B. Klimek, H. Azarbad, and M. Jaźwa, "Functional diversity of soil microbial communities under Scot's pine, Norway spruce, silver birch and mixed boreal forests," Pedobiologia **58** (2–3), 81–88 (2015). https://doi.org/10.1016/j.pedobi.2015.04.002
47. A. S. Dagnino, A. Sforzini, F. Dondero, S. Fenoglio, E. Bona, and J. Jens, "A weight-of-evidence approach for the integration of environmental "triad" data to assess ecological risk and biological vulnerability," Integr. Environ. Assess. Manage. **4** (3), 314–326 (2008).
48. D. A. Davidson, "Soil quality assessment: recent advances and controversies," Prog. Environ. Sci. **2**, 342–350 (2000).
49. J. W. Doran and M. R. Zeiss, "Soil health and sustainability: managing the biotic component of soil quality," Appl. Soil Ecol. **15** (1), 3–11 (2000).
50. D. Drobne, A. Jemec, and Z. Pipan Tkalec, "In vivo screening to determine hazards of nanoparticles: nanosized TiO_2," Environ. Pollut. **157**, 1157–1164 (2009). https://doi.org/10.1016/j.envpol.2008.10.018
51. P. J. Edwards, "Soil pollutants and soil animals," Sci. Am. **220**, 88–99 (1969). https://doi.org/10/1038/scientificamerican0469-88
52. E. V. Fedoseeva, V. M. Tereshina, O. A. Danilova, E. A. Ianutsevich, O. S. Yakimenko, and V. A. Terekhova, "Effect of humic acid on the composition of osmolytes and lipids in a melanin-containing phytopathogenic fungus Alternaria alternata," Environ. Res. **193**, 110395 (2021). https://doi.org/10.1016/j.envres.2020.110395
53. C. J. S. Fox, "The effect of five herbicides of the numbers of certain invertebrates on the number of grassland soil," Can. J. Plant Sci. **44** (5), 405–409. 1964). https://doi.org/10.4141/cjps64-080
54. P. Grenni, A. B. Caracciolo, L. Patrolecco, et al., "A bioassay battery for the ecotoxicity assessment of soils conditioned with two different commercial foaming products," Ecotoxicol. Environ. Saf. **148**, 1067–1077 (2018). https://doi.org/10.1016/j.ecoenv.2017.11.071
55. L. Gutiérrez, C. Garbisu, E. Ciprián, J.M. Becerril, M. Soto, J. Etxebarria, and L. Epelde, "Application of ecological risk assessment based on a novel TRIAD-tiered approach to contaminated soil surrounding a closed non-sealed landfill," Sci. Total Environ. **514**, 49–59 (2015). https://doi.org/10.1016/j.scitotenv.2015.01.103
56. M. Hagner, M. Romantschuk, O. P. Penttinen, A. Egfors, C. Marchand, and A. Augustsson, "Assessing toxicity of metal contaminated soil from glassworks sites with a battery of biotests," Sci. Total Environ. **613–614**, 30–38 (2018). https://doi.org/10.1016/j.scitotenv.2017.08.121
57. S. Hartwell, "Demonstration of a toxicological risk ranking method to correlate measures of ambient toxicity and fish community diversity," Environ. Toxicol. Chem. **16** (2), 361–371 (1997).
58. Y. K. Hong, D. H. Yoon, J. W. Kim, M. J. Chae, B. K. Ko, and S. C. Kim, "Ecological risk assessment of heavy metal-contaminated soil using the triad approach," J. Soils Sediments **21** (8), 2732–2743 (2021).
59. *ISO 11268-2:2012. Soil Quality—Effects of Pollutants on Earthworms—Part 2: Determination of Effects on Reproduction of Eisenia fetida/Eisenia andrei* (International Standardization Org., Geneva, 2012).
60. *ISO 16072:2002. Soil Quality—Laboratory Methods for Determination of Microbial Soil Respiration* (International Standardization Org., Geneva, 2002).
61. *ISO 19204:2017. Soil Quality—Procedure for Site-Specific Ecological Risk Assessment of Soil Contamination (Soil Quality TRIAD Approach)* (International Standardization Org., Geneva, 2017).
62. *ISO 17512-1:2008. Soil Quality—Avoidance Test for Determining the Quality of Soils and Effects of Chemicals on Behavior—Part 1: Test with Earthworms (Eisenia fetida and Eisenia andrei)* (International Standardization Organization, Geneva (2008).

63. *ISO 17616:2019. Soil Quality—Guidance on the Choice and Evaluation of Bioassays for Ecotoxicological Characterization of Soils and Soil Materials* (International Standardization Org., Geneva, 2019).
64. X. Jiang, H. Chen, Y. Liao, Z. Ye, M. Li, and G. Klobučar, "Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant *Vicia faba*," Environ. Pollut. **250**, 831–838 (2019.
65. A. Kahru, H.-C. Dubourguier, I. Blinova, A. Ivask, and K. Kasemets, "Biotests and biosensors for ecotoxicology of metal oxide nanoparticles: a minireview," Sensors **8**, 5153–5170 (2008). https://doi.org/10.3390/s8085153
66. J. E. Kammenga, R. Dallinger, M. H. Donker, H.-R. Köhler, V. Simonsen, R. Triebskorn, and J. M. Weeks, "Biomarkers in terrestrial invertebrates for ecotoxicological soil risk assessment," Rev. Environ. Contam. Toxicol. **164**, 93–147 (2000).
67. A. M. Karjalainen, J. Kilpi-Koski, A. O. Väisänen, S. Penttinen, C. A. van Gestel, and O. P. Penttinen, "Ecological risks of an old wood impregnation mill: application of the triad approach," Integ. Environ. Assess. Manage. **5** (3), 379–389 (2009). https://doi.org/10.1897/IEAM_2008-072.1
68. B. M. Klimek, M. Niklińska, A. Jaźwa, I. Tarasek, Ł. Tekielak, and Ł. Musielok, "Covariation of soil bacteria functional diversity and vegetation diversity along an altitudinal climatic gradient in the Western Carpathians," Pedobiologia **58** (2–3), 105–112 (2015). https://doi.org/10.1016/j.pedobi.2015.04.005
69. B. Klimek, A. Sitarz, M. Choczyński, and M. Niklińska, "The effects of heavy metals and total petroleum hydrocarbons on soil bacterial activity and functional diversity in the Upper Silesia industrial region (Poland)," Water, Air Soil Pollut. **227** (8), 1–9 (2016). https://link.springer.com/article/10.1007/s11270-016-2966-0
70. A. Klimkowicz-Pawlas, B. Maliszewska-Kordybach, and B. Smreczak, "Triad-based screening risk assessment of the agricultural area exposed to the long-term PAHs contamination," Environ. Geochem. Health **41**, 1369–1385 (2019). https://link.springer.com/article/10.1007/s10653-018-0220-y
71. K. Kydralieva, L. Bondarenko, V. Terekhova, N. Chistyakova, S. Patsaeva, L. Rabinskiy, and G. Dzhardimalieva, "Characterization and bioactivity of magnetite-based nanocomposites," Mater. Today: Proc. **34**, 317–321 (2021). https://doi.org/10.1016/j.matpr.2020.05.363
72. C. Lors, J. F. Ponge, M. Martínez Aldaya, and D. Damidot, "Comparison of solid and liquid-phase bioassays using ecoscores to assess contaminated soils," Environ. Pollut. **159**, 2974–2981 (2011). https://doi.org/10.1016/j.envpol.2011.04.028
73. S. Manzo, S. Schiavo, P. Aleksi, and A. Tabaku, "Application of a test battery integrated index for a first screening of the ecotoxicological threat posed by ports and harbors in the southern Adriatic Sea (Italy)," Environ. Monit. Assess. **186** (11), 7127–7139 (2014). https://link.springer.com/article/10.1007/s10661-014-3915-2.
74. M. Niklińska and B. Klimek, "Effect of temperature on the respiration rate of forest soil organic layer along an elevation gradient in the Polish Carpathians," Biol. Fertil. Soils **43** (5), 511–518 (2007.
75. O. Nikolaeva and V. Terekhova, "Improvement of laboratory phytotest for the ecological evaluation of soils," Eurasian Soil Sci. **50**, 1105–1114 (2017). https://link.springer.com/article/10.1134/S1064229317090058.
76. *OCSPP 850.4230: Early Seedling Growth Toxicity Test, EPA 712-C-010* (US Environmental Protection Agency, Washington, DC, 2012).
77. *OECD Guidelines for the Testing of Chemicals 232. Collembolan Reproduction Test in Soil* (Organization for Economic Cooperation and Development, Paris, 2009).
78. *OECD Guidelines for the Testing of Chemicals No. 207. Earthworm, Acute Toxicity Tests* (Organization for Economic Cooperation and Development, Paris, 1984).
79. *OECD Guidelines for the Testing of Chemicals No. 220. Enchytraeid Reproduction Test* (Organization for Economic Cooperation and Development, Paris, 2004).
80. *OECD Guidelines for the Testing of Chemicals No. 222. Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)* (Organization for Economic Cooperation and Development, Paris, 2004).
81. *OECD, Test No. 208. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test. OECD Guidelines for the Testing of Chemicals: Section 2* (Organization for Economic Cooperation and Development, Paris, 2006).
82. K. Paraszkiewicz, P. Bernat, and J. Długoński, "Effect of nickel, copper, and zinc on emulsifier production and saturation of cellular fatty acids in the filamentous fungus *Curvularia lunata*," Int. Biodeterior. Biodegrad. **63** (1), 100–105 (2009). https://doi.org/10.1016/j.ibiod.2008.03.015
83. K. Paraszkiewicz, P. Bernat, M. Naliwajski, and J. Długoński, "Lipid peroxidation in the fungus *Curvularia lunata* exposed to nickel," Arch. Microbiol. **192** (2), 135–141 (2010). https://doi.org/10.1007/s00203-009-0542-3
84. G. Persoone, B. Blahoslav, I. Blinova, et al., "A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters," Environ Toxicol. **18** (6), 395–402 (2003). https://doi.org/10.1002/tox.10141
85. *New Microbiotests for Routine Toxicity Screening and Biomonitoring*, Ed. by G. Persoone, C. Janssen, and W. De Coen (Springer-Verlag, Boston, MA, 2000).
86. *Phytotoxkit. Seed Germination and Early Growth Microbiotest with Higher Plants. Standard Operational Procedure* (Nazareth, MicroBioTests, 2004).
87. *Species Sensitivity Distributions in Ecotoxicology*, Ed. by L. Posthuma, G. W. Suter, and T. Traas (CRC Press, Boca Raton, FL, 2002).
88. E. V. Prudnikova, A. Neaman, V. A. Terekhova, M. M. Karpukhin, E. L. Vorobeichik, I. A. Smorkalov, E. A. Dovletyarova, K. Navarro-Villarroel, R. Ginocchio, and P. Peñaloza, "Root elongation method for the quality assessment of metal-polluted soils: whole soil or soil-water extract?" J. Plant Nutr. Soil Sci. **20**, 2294–2303 (2020). https://doi.org/10.1007/s42729-020-00295-x

89. M. A. Pukalchik, V. A. Terekhova, M. M. Karpuchin, and V. M. Vavilova, “Comparison of eluate and direct soil bioassay methods of soil assessment in the case of contamination with heavy metals,” Eurasian Soil Sci. **52**, 507–514 (2019). https://doi.org/10.1007/s11368-017-1841-y
90. M. Pukalchik, F. Mercl, M. Panova, K. Břendová, V. Terekhova, and P. Tlustoš, “The improvement of multi-contaminated sandy loam soil chemical and biological properties by the biochar, wood ash, and humic substances amendments,” Environ. Pollut. **229**, 516–524 (2017). https://doi.org/10.1016/j.envpol.2017.06.021
91. V. Ribé, E. Aulenius, E. Nehrenheim, U. Martell, and M. Odlare, “Applying the Triad method in a risk assessment of a former surface treatment and metal industry site,” J. Hazard. Mater. **207–208**, 15–20 (2012). https://doi.org/10.1016/j.jhazmat.2011.07.120
92. K. Ritz, H. I. Black, C. D. Campbel, J. A. Harris, and C. Wood, “Selecting biological indica- tors for monitoring soils: a framework for balancing scientific and technical opinion to assist policy development,” Ecol. Indic. **9** (6), 1212–1221 (2009). https://doi.org/10.1016/j.ecolind.2009.02.009
93. E. Semenzin, A. Critto, M. Rutgers, and A. Marcomini, “Integration of bioavailability,ecology and ecotoxicology by three lines of evidence into ecological risk indexes for contaminated soil assessment,” Sci. Total Environ. **389**, 71–86 (2008). https://doi.org/10.1016/j.scitotenv.2007.08.032
94. S. Sforzini, L. Oliveri, S. Chinaglia, and A. Viarengo, “Application of biotests for the determination of soil ecotoxicity after exposure to biodegradable plastics,” Front. Environ. Sci. **4**, 68 (2016). https://doi.org/10.3389/fenvs.2016.00068
95. M. Słaba, P. Bernat, S. Różalska, J. Nykiel, and J. Długoński, “Comparative study of metal induced phospholipid modifications in the heavy metal tolerant filamentous fungus *Paecilomyces marquandii* and implications for the fungal membrane integrity,” Acta Biochim. Pol. **60** (4), (2013). https://doi.org/10.1016/j.biortech.2015.08.045
96. D. J. Spurgeon, H. Ricketts, C. Svendsen, A. J. Morgan, and P. Kille, “Hierarchical responses of soil invertebrates (earthworms) to toxic metals stress,” Environ. Sci. Technol. **39**, 5327–5334 (2005). https://doi.org/10.1021/es050033k
97. V. A. Terekhova, M. A. Pukalchik, and A. S. Yakovlev, “The TRIAD approach to ecological assessment of urban soils,” Eurasian Soil Sci. **47**, 952–958 (2014). https://doi.org/10.1134/S1064229314090129
98. N. Totubaeva, Z. Tokpaeva, K. Kojobaev, A. Usubalieva, and V. Terekhova, Pol. J. Environ. Stud., 2022 (in press).
99. M. Valko, H. Morris, and M. T. D. Cronin, “Metals, toxicity and oxidative stress,” Curr. Med. Chem. **12** (10), 1161–1208 (2005). https://doi.org/10.2174/0929867053764635
100. C. A. M. van Gestel, “Physico-chemical and biological parameters determine metal bioavailability in soils,” Sci. Total Environ. **406**, 385–395 (2008). https://doi.org/10.1016/j.scitotenv.2008.05.050
101. C. A. M. van Gestel, “Soil ecotoxicology: state of the art and future directions,” ZooKeys **176**, 275–296 (2012). https://doi.org/10.3897/zookeys.176.2275
102. C. A. M. van Gestel, C. D. Léon, and N. M. van Straalen, “Evaluation of soil fauna ecotoxicity tests regarding their use in risk assessment,” in *Soil Ecotoxicology* (CRC Press, Boca Raton, FL, 1997), pp. 291–317.
103. N. M. van Straalen, “Peer reviewed: ecotoxicology becomes stress ecology,” Environ. Sci. Technol. **37** (17), 324A–330A (2003). https://pubs.acs.org/doi/pdf/10.1021/es0325720.
104. N. M. van Straalen and M. E. Feder, “Ecological and evolutionary functional genomics-how can it contribute to the risk assessment of chemicals?” Environ. Sci. Technol. **46**, 3–9 (2012). https://doi.org/10.1021/es2034153
105. N. M. van Straalen, T. K. S. Janssens, and D. Roelofs, “Micro-evolution of toxicant tolerance: from single genes to the genome’s tangled bank,” Ecotoxicology **20**, 574–579 (2011). https://doi.org/10.1007/s10646-011-0631-3
106. M. Wieczerzak, J. Namieśnik, and B. Kudłak, “Bioassays as one of the green chemistry tools for assessing environmental quality: a review,” Environ. Int. **94**, 341–361 (2016). https://doi.org/10.1016/j.envint.2016.05.017
107. N. N. Zhdanova, A. I. Vasilevskaya, L. V. Artyshkova, et al., “Changes in micromycete communities in soil in response to pollution by long-lived radionuclides emitted in the Chernobyl accident,” Mycol. Res. **98** (7), 789–795 (1994). https://doi.org/10.1016/S0953-7562(09)81057-5

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