Methods for assessing the toxic effects of heavy metals by bioassay methods

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Abstract. The article presents a method for assessing the toxic effects of heavy metals by means of bioassay, which is a complex of different approaches for assessing the state of different organisms under the influence of environmental factors. To determine the phytotoxicity of soil, theoretical methods, methods of model experiments, as well as methods of biological diagnostics can be used. It is the methods of biological diagnostics that allow us to study the toxic effect of heavy metals on the growth and development of plants.

1. Introduction
Today, the set of methods of bioassay is one of the most important modern research areas of pollution and environmental quality. Physical and chemical methods combined with ecological methods of bioassay constitute a comprehensive system of environmental monitoring.

Biotesting is a complex of different approaches for assessing the state of different organisms under the influence of environmental factors, both natural and anthropogenic. The fundamental indicator of their condition is the effectiveness of physiological processes that ensure the normal development of the body. In optimal conditions, the body reacts to the environment through a complex physiological system of buffer homeostatic mechanisms [1].

These mechanisms support the optimal flow of development processes. under the influence of adverse conditions, the mechanisms for maintaining homeostasis can be disrupted, which leads to a state of stress. Such violations may occur before changes in commonly used viability parameters occur. Thus, the bioassay methodology makes it possible to detect the presence of a stressful effect earlier than many commonly used methods.

2. Determination of soil phytotoxicity using watercress
To determine the phytotoxicity of soil, theoretical methods can be used, as well as methods of model experiments presented by quantitative chemical analysis, determination of enzymatic activity, biotesting, and determination of phytotoxicity. The obtained data can be processed using mathematical methods, methods of theoretical generalization, data systematization and comparative analysis. The validity and reliability of the results obtained is confirmed by research samples, the use of standard and approved methods, and the reproducibility and convergence of data [2].

The principle of biological diagnostics of soils is based on the idea that the soil as a habitat is a single system with populations of different organisms inhabiting it. There are two main groups of methods – chemical and biological (biotesting and bioindication).
The environmental hazard, or risk, should be assessed taking into account not only the nature and strength of the anthropogenic impact, but also the biological properties of the reacting system. Accordingly, there are two groups of environmental monitoring methods (monitoring the state of ecosystems): physical, chemical and biological (biomonitoring). Each type has its own limitations [3,4].

Analytical methods are often time-consuming and require expensive, sometimes scarce equipment and reagents, as well as highly qualified service personnel. But their main drawback is that these methods cannot guarantee a reliable assessment of environmental hazards, no matter how wide the range of substances analyzed. It is not the levels of pollution and impacts themselves that are important, but the biological effects that can be caused and about which even the most accurate chemical or physical analysis cannot provide information [5].

The indicators used in the practice of environmental and sanitary regulation (maximum permissible concentrations–MPC, maximum permissible doses–PD, maximum permissible levels – PD) are always based on Toxicological studies with testing of individual biological objects. They cannot account for changes in the toxicity of pollutants due to certain effects when combined with anthropogenic factors. These standards do not reflect the dependence of the toxic effect of pollution on the physical factors of the environment, do not take into account the processes of natural transformations of substances in the environment. Therefore, along with physical and chemical methods, it is necessary to use methods of biological control and diagnostics – bioindication and biotesting, which provide objective integral assessments of the quality of the environment and the basis for predicting the state of ecosystems.

Biological methods of environmental quality control do not require prior identification of specific chemical compounds or physical effects, they are quite simple to implement, cheap and allow you to monitor the quality of the environment in a continuous mode. However, once the General toxicity of soil or water samples has been identified, analytical methods should be applied to determine its causes. Traditional physical and chemical methods also allow us to assess the contribution of individual enterprises or other sources of pollution to the anthropogenic impact on nature [6].

Identification of the toxic effects of various pollutants in natural environments is a complex task, so it is currently relevant to simulate environmental pollution with different doses of individual toxicants and identify the range of their toxic effects.

Soil phytotoxicity is a property of the soil, due to the presence of pollutants and toxins, to suppress the growth and development of higher plants. This method is designed to determine the total toxicity and hazard class of production and consumption waste by phytotoxic action, regardless of the nature and degree of identification of the components.

This method is based on the ability of seeds to respond adequately to chemical effects by changing the intensity of root germination, which allows the length of the latter to be taken as an indicator of the test function. The criterion for harmful effects is the inhibition of the growth of seed roots. Most often, as a model test plant, cress seeds are used, which have good germination and rapid germination. According to the results of preliminary studies on environmental and hygienic assessment of waste of various chemical composition, cress seeds gave stable and reproducible data compared to seeds of other crops (peas, cucumbers, wheat, carrots, and others).

During the work, experiments were conducted on the germination of cress seeds on the soil (contact method) and soil extracts.

Seeds of experimental plants were sprouted in Petri dishes under laboratory conditions at an air temperature of + 22°C for 7 days. When germinating seeds by contact method, 40...50 grams of moistened soil with a toxic substance added to it were added to each Petri dish and 50 dry, healthy seeds were laid out. Control samples with plant seeds were grown on calcined river sand [7].

When sprouting seeds on soil hoods, filter paper was placed in each Petri dish and 50 dry, healthy seeds were placed on it, at approximately equal distances from each other. Filter paper was wetted with tested soil extracts until it was completely, but not excessively, moistened.

In the course of the experiment in a separate Petri dish was added to the tested soil extracts after drying the filter paper. Control samples with plant seeds were grown on distilled water.
At the end of the exposure period (7 days), the percentage of germination of seeds was determined, counting the number of sprouted and non-sprouted seeds, the length of stems and roots of seedlings was measured (and compared) in control and experimental samples, and the object of measurement for each seed is the root of the maximum length. Dried seedlings were also weighed. The results of weighing and measurements were made out in the form of a table. Statistical processing of experimental data was performed in accordance with the method [5].

Enzymes are biological catalysts of protein nature, which play an important role in metabolism, regulating biochemical processes. They are synthesized by microflora, higher plants and enter the soil with their lifetime secretions, after the death and lysis of microbial cells and plant residues. Enzymes released into the soil remain active for a considerable time due to the fixation of silty and dusty fractions of the soil, its organic matter [8].

The enzymatic activity of soils determines the intensity and direction of biochemical processes that affect the fertility and quality of the soil, and is one of the important indicators of its biological activity. The activity of enzymes in the soil is influenced by various factors that inhibit or activate their action, including anthropogenic influence [3].

The principle of the method for determining the activity of soil enzymes is based on taking into account the amount of substrate processed during the reaction or the resulting reaction product under optimal conditions of temperature, medium acidity and substrate concentration [9].

Enzymes catalyze redox reactions, which play a leading role in biochemical processes in the cells of living organisms, as well as in the soil. The most common enzyme of this class is catalase, which decomposes into water and molecular oxygen toxic to the cell hydrogen peroxide, formed during the respiration of living organisms as a result of various biochemical reactions of oxidation of organic substances. Thus, catalase activity is an important indicator of soil Genesis.

Hydrolases perform hydrolysis reactions of various complex organic compounds, acting on various bonds: ester, glycoside, peptide, and others. This class includes such enzymes as invertase and urease, whose activity is an important indicator of soil biological activity and is widely used for assessing anthropogenic impact.

Decomposition of organic nitrogen compounds is carried out with the direct participation of extracellular enzymes. Ammonia, which is formed under the action of urease, serves as a source of nutrition for plants.

Enzymatic activity is considered as an integral expression of the action of environmental factors and mutually dependent processes occurring in soils. The use of the indicator of enzymatic activity is effective in assessing the ecological state of soils, especially when it comes to the ability to resist man-made impacts [8].

3. Determination of soil phytotoxicity using watercress
Phytotest (that is, a plant object for determining soil phytotoxicity) is able to respond to chemical effects by reducing the intensity of root germination, and therefore act as indicators of toxicity.

In connection with the above, it is interesting to investigate the phytotoxic effect of soils with a high content of heavy metals in the biotest on the germination of seeds of a model plant, as well as to determine whether there are correlations between the content of heavy metals in the soil and the germination of seeds.

Soil phytotoxicity was determined using watercress by soil extraction method. As a control fitoestrogeny, in addition to the control distilled water was used from a sample of soil without additives. Phytotoxicity of the studied soils was assessed by three indicators – root length, stem length and germination.

Concentrations of the mobile form of cadmium up to 5 and more than 20 mg/kg had a pronounced stimulating effect relative to the control values for the root length indicator, but such doses of mobile cadmium did not have a stimulating effect on the indicators of stem length and germination. When the content of mobile cadmium is 12…20 mg/kg, there is a pronounced inhibition effect on all indicators.
In General, according to the results of biotesting and phytotesting of soils with additives of various doses of cadmium, additives of 12…20 mg/kg and more than 25 mg/kg of cadmium in terms of mobile form can be considered the most toxic for test objects.

In terms of root length and germination in this case, there is a significant inhibitory effect at the content of the mobile form of copper 2…5 and 70…90 mg/kg. In terms of the length of the stem, there is a slight stimulating effect relative to the control values in the entire range of the studied contents of mobile forms of copper [3,8].

Mechanisms of plant resistance to excess of heavy metals can be manifested in different ways: some species are able to accumulate high concentrations of heavy metals, but show tolerance to them; others seek to reduce their intake by maximizing their barrier functions. For most plants, the first barrier level is the roots, where the largest amount of heavy metals is retained, the next is the stems and leaves, and, finally, the last is the organs and parts of plants responsible for reproductive functions (most often seeds and fruits, as well as root and tubers).

At the same time, the following regularity in their content is established for copper and zinc: roots-grain-straw. For lead, cadmium and strontium, it has a different form: roots-straw-grain. Therefore, the most informative indicator of phytotoxicity in this case can be considered as the root length. In the aggregate, the most toxic samples are those containing a mobile form of copper of about 5 mg/kg, which is significantly lower than for cadmium.

4. Conclusion
When detecting anthropogenic environmental pollution, along with other methods, techniques based on the assessment of the state of individual individuals exposed to the polluted environment are used. Methods of bioassay have sufficient sensitivity, since living organisms can perceive lower concentrations of substances than any analytical sensor, and in this regard, biological material may be subject to toxic effects that are not recorded by technical means.

The proposed method for determining soil phytotoxicity using watercress as a test plant on soil extracts is currently one of the most accurate. Using this method, the dependence of phytotoxicity indicators (germination, length of seedlings and roots) on the content of the mobile form of metals is established.

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