The use of bioassay methods in assessing soil pollution with zinc

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Abstract. Increasing release of a new class of pollutants (nanoparticles) into the environment necessitates studying the applicability of classical biotesting methods and identifying the features of their application. We conducted a study on a site located in the development zone of forest-steppe chernozems with an area of about 100 m². The plot was divided into micro plots and nanoparticles. Zn solution was added to each surface at concentrations of 1000 mg/kg, 750 mg/kg, 500 mg/kg, 250 mg/kg and 100 mg/kg. In the course of field and laboratory studies, we identified germination, biomass and tolerance index of test cultures (Triticum aestivum, Raphanus sativus). A study of soil contamination with zinc nanoforms showed that the test plants Raphanus sativus and Triticum aestivum had different sensitivity to the effects of the toxicant. The germination rate showed the greatest dose-dependent dependence of Zn nanoparticles. Further growth and development of plants showed a gradual adaptation of plants to the effects of the stress factor and a gradual stabilization of the biomass and tolerance index.

1. Introduction
The release of pollutants is threatening the environment. Heavy metals and metalloids are significant polluters. They are chemicals that pose the highest threat to biological systems. Vigorous activity of industrial enterprises, high density of transport, use of fertilizers contributes to the accumulation of heavy metal ions. Unlike biodegradable organic pollutants, heavy metals do not decompose into less hazardous end products. Low concentrations of some chemical pollutants are necessary for life, but some of them, such as Hg, As, Pb and Cd, are biologically insignificant and very toxic to living organisms. Even the necessary metals can become toxic if they are present in concentrations above the acceptable level. The study of heavy metal ions influencing living organisms is currently an urgent environmental problem. Modern electric power, chemical, cosmetic and medical industries, which are associated with the production and use of metal nanoparticles, contribute to the accumulation of a new type of pollutant in the environment.

Bioassay is an informative group of methods for assessing potential dangers of chemical, physical or biological effects on natural environments, including soil biota. The principle of the bioassay method in a broad sense is based on the sensitivity of living organisms to exogenous effects. The essence of the method is to determine the effect of the tested substances on selected test objects in standard conditions with the registration of various behavioral, physiological or biological test reactions [1].
Since Zn ions are among the most widely used, the risk of its release into the soil is very high. However, due to the complex nature of the soil, the exact number of ions of a given metal in the environment is difficult to determine due to various limitations [2].

Zinc oxide in a nanodispersed form is particularly interesting not only because of its widespread use in various consumer goods, but also because of its frequent inclusion in agricultural products [3]. Zn ions are used as an active ingredient for insecticides. The antifungal and antibacterial properties of the metal have found their application in the production of granular macronutrient fertilizers [4, 5].

Plants are important receptors of soil solution. Therefore, when zinc enters the soil, plants are the most likely way for absorption, translocation and bioaccumulation of metal in the food chain [6].

The aim of the study was to study the information content of the phytotesting method in assessing the pollution of chernozems with zinc nanoparticles.

2. Materials and methods

We used Zn nanoparticles (NP) (size 90-150 nm, ζ potential = 25 ± 0.5 mV) obtained by the method of electric explosion of a conductor in an argon atmosphere (Advanced Powder Technologies LLC, Russia, http://www.nanosized-powders.com/).

The studies were conducted on a site located near the village of Vozdvizhenka, Ponomarevsky district, Orenburg region (coordinates 53.141111 ° N, 54.163333 ° E).

Chernozems are the object of the study. The site was divided into micro plots measuring 1m * 1m (1 m²). Nanoparticles were added to the soil surface of each plot by irrigation with a solution of the corresponding concentration. NPs solutions were prepared by adding the test metal at concentrations of 1000 mg/kg, 750 mg/kg, 500 mg/kg, 250 mg/kg and 100 mg/kg in deionized water (1000 ml), followed by dispersion on an ultrasonic disperser (UZDN, f -35 kHz, N-300 W, Russia), for 30 minutes.

The soils during the field experiment were contaminated with Zn nanoparticles, and a part of unpolluted soil a control plot.

In areas with different types of pollution, an experiment was carried out to determine the toxicity of soils contaminated with zinc nanoparticles in relation to higher plants (Triticum aestivum and Raphanus sativus). At each site, 50 seeds of test object plants were planted. The following indicators were used to assess the effect of soil pollution with various forms of zinc: germination, tolerance index and plant mass.

Morphometric data were determined for radish on day 10, for wheat on day 14. Processing the obtained results allowed us to calculate the tolerance index (IT %) of plants, which was defined as the ratio of the average value of the length of the root or stem of plants grown at a certain concentration of pollutant (experiment) to the average value of the length of the stem/root of control plants grown without metal (control) [7].

3. Results and discussions

The use of biotesting is based on the use of organisms of test objects: plants, animals and microorganisms. The study of the effect of soil pollution on the test function of plants of test objects under field experiments is complicated by the influence of environmental factors [1]. Therefore, plant species that are used and recommended for use in the diagnosis of pollution of chernozems and belonging to different classes of plants (monocotyledonous and dicotyledonous) are selected as test cultures. The plant germination rate is shown in Fig. 1.

The experimental results showed that the germination of plants in the control variant was significantly lower than 100 %, which is associated with the influence of heterogeneity of soil, climatic and biotic factors.

The introduction of zinc nanoforms into the soil led to a decrease in the germination of test plants, while Raphanus sativus was more sensitive to the pollutant.

The active intake of zinc ions in the soil solution, as well as the direct effect of nanoparticles on the root systems of plants, led to a decrease in the germination rate of both plant species to 44–45 %.
The effect of the contaminator manifested itself in a change in plant productivity and the biomass of Raphanus sativus sharply decreased to a minimum value (0.16 g) at a dose of nanoparticles Zn of 100 mg/kg (Fig. 2). An increase in the concentration of nanoparticles led to an increase in biomass with a maximum value of 500 mg/kg in the variant, and the response of the biomass indicator to the maximum dose of pollution was manifested in the stabilization of the indicator at the level of 0.4–0.45 g.

Figure 1. Field test seed germination

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Figure 2. Plant biomass Raphanus sativus

Figure 3. Plant biomass Triticum aestivum
The productivity index of Triticum aestivum when the pollutant was added to a concentration of 500 mg/kg increased slightly and assumed values of more than 0.12 mg, a further increase in concentration led to a decrease in biomass to 0.08 g (Fig. 3).

The calculation of the indicator of plant tolerance shows the resistance of plants to the effects of stress factors (Fig. 4).

Test cultures showed a different response to the effects of soil pollution. Since Raphanus sativus showed a significant decrease in the index of tolerance at a dose of 100 mg/kg nanoparticles of Zn. An increase in the dose of the pollutant caused a dynamics of the indicator similar to the value of plant biomass. A similar dynamics of indicators indicates the development of the toxic effect of zinc ions in relation to plants, which is manifested in a decrease in the rate of photosynthesis and the formation of ROS. Similar results were obtained in studies on a wide range of crops: lettuce, cucumber, corn, peas [8, 9, 10]. The tolerance index Triticum aestivum showed a greater ability of plants to compensate for the development of toxic effects and the value of the indicator gradually increased with increasing concentration of pollutant, which was also noted by studies of Korotkova et al. (2017) [11]. This could probably happen due to the inclusion of compensatory mechanisms of plants and the aggregation of nanoparticles with a decrease in their direct aggressive effect on the root systems of plants.

4. Conclusion
A study of the effects of soil contamination with zinc nanoforms showed that the test plants Raphanus sativus and Triticum aestivum have different sensitivity to the effects of the toxicant. The germination rate showed the greatest dose-dependent dependence of Zn nanoparticles. Further growth and development of plants showed a gradual adaptation of plants to the effects of the stress factor and a gradual stabilization of the biomass and tolerance index.

Thus, to assess the pollution of the soil cover by zinc nanoform, it is better to use the Raphanus sativus test culture and a complex of morphometric indicators.

![Figure 4. Plant tolerance index (difference with control)](image_url)
Acknowledgments
The research was supported by the Ministry of Science and Higher Education in accordance with the state assignment for Ural State Mining University No. 0833-2020-0008 ‘Development and environmental and economic substantiation of the technology for reclamation of land disturbed by the mining and metallurgical complex based on reclamation materials and fertilizers of a new type’. We obtain the scientific results with the staff of Center for the collective use by using funds of the Center for the collective use of scientific equipment of the Federal Scientific Center of biological systems and agricultural technologies of RAS as well (No Ross RU.0001.21 PF59, the Unified Russian Register of Centers for Collective Use – http://www.ckp-rf.ru/ckp/77384).

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