Biological effects of iron nanoparticles entering the soil

L V Galaktionova1,2, N A Terehova2, E A Osipova2, N F Gusev.1, S V Lebedev1,2 and I A Gavrish1

1Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russia
2 Orenburg State University, Orenburg, Russia

E-mail: info@fbras.ru

Abstract. The active development of nanotechnologies, accompanied by wide use of nanoparticles in various industries, necessitates to study its effect on higher plants. The studies were conducted in the summer of 2019 in a territory located near the village Vozdvizhenka of Ponomarevsky district (Orenburg region, Russia). The plot was divided into micro-plots and nanoparticles of Fe3O were introduced onto the surfaces at doses of 1000 mg/kg, 750 mg/kg, 500 mg/kg, 250 mg/kg and 100 mg/kg. During the experiment, test plants were sown, and then we determined a set of their vital and morphometric indicators. The results allow us to conclude that of the entire set of parameters, the most sensitive test object when setting the germination test is Raphanus sativus. According to certain morphometric indicators, the most informative is the use of Lepidium sativum in the experiment. In general, the nanooxide in small doses used in the study increased the productivity of plants, which indicates the need for further studies to assess the prospects of its use in crop production.

1. Introduction
In the modern world, there is an active development of nanotechnology, accompanied by the expansion of the scope of nanoparticles in various industries. Consequently, there is an increase in their production. So the production of iron nanopowders is 1250 tons per year [1].

In the USA, emulsified iron nanoparticles are used to restore contaminated soils [2], to purify groundwater from organochlorine pesticides and heavy metal ions [3]. Application of nanoscale zero valent iron (NZVI) for groundwater remediation in Europe [4], according to the study conducted by Yoon H. Et al. (2019), showed that the effect of aqueous solutions of plant iron nanoforms increased biomass due to an increase in photosynthesis rate by 38 % [5]. Scientists from Mexico studied the effects of magnetite Fe3O4 nanoparticles on the germination and early growth of Quercus macdougallii, found that these particles increase growth, dry biomass, and chlorophyll concentration and therefore can be used to restore forests [6].

Such features of iron nanoparticles make their use as nanofertilizers promising. At the same time, there are concerns that the widespread use of iron nanopowders can lead to negative environmental consequences in the environment [7]. Scientists from China and the USA found that at high concentrations of iron nanopowders (> 500 mg/kg), the transfer of active iron from the root to the shoot is blocked and this slows down the growth of rice seedlings [8]. In addition, iron nanoparticles possess cytotoxicity and significantly alter the taxonomic and functional composition of the microbial communities of the rhizosphere [7].
Thus, previous studies show that the reaction of plants to the effects of iron nanoparticles is poorly studied and depends on the biological characteristics of plants and environmental conditions. Changes in the vital and morphometric parameters of test cultures (Triticum aestivum, Raphanus sativus, Lepidium sativum) grown under conditions of soil contamination with nanoparticles of iron oxide (Fe₃O₄) became the goal of our research.

2. Materials and methods
We used Fe₃O₄ nanoparticles (NP) (size 80-110 nm) obtained by the method of electric explosion of a conductor in an argon atmosphere (Advanced Powder Technologies LLC, Russia, http://www.nanosized-powders.com/) (Fig.1).

Studies were conducted on a site located near 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 1 m² (1 m²) (Fig. 2). 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 Fe₃O₄ nanoparticles, and a portion of unpolluted soil was used as a control.

In areas with different types of pollution, an experiment was carried out to determine the phytotoxicity of soils contaminated with zinc nanoparticles in relation to higher plants (Triticum aestivum cultivar Uchitel, provided by the Federal Scientific Center of BTS RAS (Orenburg), Raphanus sativus cultivar Sarsa RS and Lepidium sativum Vesenny varieties produced by GAVRISH LLC http://www.gavrish.ru/). 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 concentrations of iron nanooxide: germination, index of tolerance and plant productivity.

Morphometric data were determined for radish and watercress 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 root or stem length of plants grown at a certain concentration of nanooxide (experiment) to the average stem/root length of control plants grown without a pollutant (control) [9].

3. Results and discussions
The effect of soil pollution by nanoparticles of iron oxide was studied starting from the first seedlings, since laboratory and field germination of plants did not coincide (Fig. 3).

Figure 3. Seed germination of test cultures in the study areas

The plant germination rate in the control variant of the experiment was lower than 95 % declared by the manufacturer, and for the Raphanus sativus test culture it decreased by more than 20 %. In general, this test object showed the lowest germination rates for all variants of experience. Despite the potential toxicity of iron oxide nanoparticles, in some variants of pollution an increase in plant germination is higher than the control values: Triticum aestivum at a dose of 100 mg/kg, and Raphanus sativus – 250 mg/kg. Increasing the dose of nanooxide reduced seed germination in experimental plots.

The determination of morphometric indicators made it possible to calculate the index of plant tolerance (IT, %) (Fig. 4).

Calculation of IT shows the successful adaptation of plants to the effects of stress factors, and the difference with the control allows us to assess how the values of the experimental options exceed the control. Plants of Triticum aestivum showed a low ability to level the toxic effects of nanoparticles Fe$_3$O$_4$ at a concentration of 100 mg/kg in the soil; an increase in concentration caused an increase in plant length, which is also described in the works of Korotkova et al. (2015). Raphanus sativus and Lepidium sativum showed greater resistance to the influence of pollutant in all variants of pollution, as morphometric parameters of plants exceeded the values of this indicator in the control.

Studies on the effect of Fe$_3$O$_4$ indicate that the mechanism of action of nanoparticles happens due to the action of iron ions and their direct absorption by plant root systems [11]. Therefore, their toxicity manifests itself with an increase in the concentration of the contaminator. The biomass data of test plants show a steady upward trend in all pollution variants, with the exception of the maximum dose of nanooxide, which reduces productivity for Lepidium sativum to the values of the control variant, and for Raphanus sativus by more than 60 % of the control (Fig. 5).
4. Conclusion
The study of the effect of iron nanooxide on vital and morphometric parameters of test plants allowed us to conclude that out of the entire set of parameters, the most sensitive test object when setting the germination test is Raphanus sativus. And according to morphometric indicators, use of Lepidium sativum in the experiment is the most informative.

In general, nanooxide used in the study in small doses (100–250 mg/kg) increased the productivity of plants, which indicates the need for further studies to assess the prospects of its use in crop production.

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