Influence of different forms of iron on the morphobiological indicators of Pinus sylvestris

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Abstract. The wide distribution of iron in the environment of various forms of iron makes it relevant to study the characteristics of their influence on plant growth. In the present paper, the growth features of Pinus sylvestris – Scots pine were studied - during the cultivation of stratified seeds in a medium containing different forms of iron. It was established that the cultivation of pine seeds in a medium containing 0.1 M of iron in any form, inhibits the development of the root system. When cultivated in the environment containing iron nanoparticles at a concentration of 6.25 mmol / l, this led to a decrease in the root length to 3.71 ± 0.06 mm. On the contrary, the presence of iron nanoparticles in the medium for the cultivation of pine caused an increase in the length of the main and adventitious roots, as well as the number of adventitious roots. The content of iron microparticles in the medium for the cultivation of pine caused only a decrease in the length of the main root and adventitious roots. At the same time, at concentrations from 0.39 to 25 mmol / l, an increase in the number of adventitious roots occurred, which indicates compensatory reactions.

1. Introduction

The work resulted in the identification of the upper tolerance limit in the series “Fe microparticles → Fe nanoparticles → Fe ions”, as well as significant changes in the root system of Pinus Sylvestris L. when they are exposed in variants with different concentrations.

Currently, as a result of the widespread development of the oil and gas industry, the risk of environmental pollution by heavy metals is increasing. The atmosphere of the Orenburg region is polluted with large amounts of zinc, lead, sulfur, copper, iron, which increase the effect of sulfur dioxide, and the soil in the immediate vicinity of the plant is more polluted by heavy metal salts, which are the most toxic among chemical elements. An increase in their content in the soil always negatively affects the condition of the plants. The action of metals is manifested at the morphobiological and physiological levels. However, nanomaterials are widely used in crop production, particularly in forestry. Essential metal nanoparticles are used to stimulate plant seeds. The list of nanomaterials is regularly supplemented with new substances and their spectrum of action, which inevitably leads to their effect on living systems, in particular, on plants [1-3].
Currently, ecologists, physiologists and other scientific researchers are faced with the problem of poorly understood nanomaterials, which makes it impossible to assess the potential risks of using nanomaterials [4,1,5,6-8].

The ambiguity of the action of nanoparticles of essential metals is manifested in the toxic, stimulating action, selective permeability, individual adaptive reactions of plants [9-12].

2. Materials and methods

The object of the study was the seeds of Pinus Sylvestri s L. of the first class, collected on the territory of the Orenburg region and having passed the preliminary stratification.

The experiments were carried out using iron nanoparticles (80 ± 15 nm.), Iron microparticles (<99.5%), and also iron sulfate. Nanoparticles were obtained at the Institute of Energy Problems of Chemical Physics, Russian Academy of Sciences (Moscow) on a MiGen installation using the method of high-temperature condensation. At the first stage of the study, weighed the test substances (100 mmol / l of each substance) was diluted with distilled water (10 ml) and dispersed by treating with 35 kHz ultrasound in a Sapphire TTC type source (CJSC PCC "Sapfir", Russia) minutes. Then cooked fourfold dilutions.

The dilution scheme presented in the multiplicity of dilution is indicated.

Quartz sand pretreated was used as a substrate for germination. washed and sifted to obtain a fraction of 0.5-2.0mm. In order to exclude impurities in cultured medium, sand was soaked in 15% HCl solution for a day, then washed 30 times with distilled water until neutral pH was reached, the control was performed using an "Expert-001" liquid analyzer (Limited Liability Company "Econix-expert" Russia). After that, the sand was dried, screened, calcined in a drying cabinet at a temperature of 135°C for an hour. At the final stage of preparation of the cultivation medium, sand was weighed, placed in sealed containers, and the prepared lysols and suspensions were moistened. Control samples were moistened with distilled water. Biotesting was performed in accordance with State Standard 15150 in laboratory conditions [13-15]. The air temperature in the laboratory corresponded to the limits of +18 to 25°C. Relative humidity 80%, atmospheric pressure 84-106 kPa. Lighting is natural and artificial. The experiment was carried out according to the following scheme: a previously prepared substrate was placed in containers, then the seeds of Pinus sylvestris L. were buried by 2 cm and irrigation was carried out with pre-prepared suspensions or solutions. Irrigation of control samples was carried out with distilled water [7,16,17]. After that, the containers were placed in a thermostat with an optimal germination regime for a period of 14 days, if necessary, the substrate was moistened with the test solutions. Diagnostic signs were: germination, morphological features [3,16,18].

It has been established that microparticles and iron ions are phytotoxic to a certain extent for seeds of Pinus sylvestris L. The ionic form causes complete growth inhibition. A significant decrease in morphological characters occurred at lower concentrations compared with micro and nanoparticles.

Thus, a decrease in seed germination occurred already with Pinus sylvestris L. seeds with iron ions (100 mmol / l) revealed, and a concentration of 30 mmol / l reduced the germination rate to 12.9 ± 5.6%, the germination rate in the control sample was 96.6 ± 3.1%. When analyzing the biometric characteristics of the shoots and roots, certain changes were noted. In particular, the concentration of iron ions of 30 mmol / l led to a blockage of root development and a reduction in the length of the shoots to 5.3 ± 2.1 mm, and the ion concentration of 5.5 mmol / l reduced the length of the roots to 14.2 ± 0.3 mm, while the length of the roots in the control sample amounted to 212.3 ± 4.1 mm. The length of the shoots of the prototype is reduced in comparison with the control 2 times.

Investigated forms Indicators of action of suppression of germination of the reduction of the length of the shoot reduction of the length of the root.

3. Results and discussion

The results of the study show that there is a decrease in the effective parameter by an average of 50% of the control, which characterizes the upper limit of tolerance to iron ions. The experiment indicates a pronounced toxic effect.
In the experiment in the study of micro- and nanoparticles with a similar concentration, it was found that the effect of the substances does not adversely affect the germination and germination of the seeds of Pinus sulvestris L., the effect of a stimulating effect is observed. The result of the experiment shows the high tolerant properties of Pinus sulvestris L. to the action of micro- and iron nanoparticles. The conducted studies confirm the expediency of applying the presowing treatment of seeds of Pinus sulvestris L. with biologically active iron nanopowders.

The main objective of this study was to study the morphobiological reactions of Pinus sulvestris L. to the action of various forms of iron (Table 1).

Table 1. The influence of various forms of iron on the laboratory germination of pine seeds.

| Concentration | Ions $\bar{x} \pm S\bar{x}$ | Percentage of control $Cv$ $\bar{x} \pm S\bar{x}$ | Nanoparticles $\bar{x} \pm S\bar{x}$ | Percentage of control $Cv$ $\bar{x} \pm S\bar{x}$ | Microparticles $\bar{x} \pm S\bar{x}$ | Percentage of control $Cv$ $\bar{x} \pm S\bar{x}$ |
|---------------|-----------------------------|----------------------------------------|-----------------------------|----------------------------------------|-----------------------------|----------------------------------------|
| 100           | 0                           | 0                                      | 76.2 $\pm$ 8.54            | 77.9                                   | 6.2                         | 81.1 $\pm$ 3.4                       | 82.9                                   | 5.8                                   |
| 25            | 0                           | 0                                      | 120.6 $\pm$ 7.6            | 123.2                                  | 5.4                         | 83.9 $\pm$ 5.2                       | 85.7                                   | 6.0                                   |
| 6.25          | 3.7 $\pm$ 0.06              | 3.8                                    | 100.2 $\pm$ 7.51           | 100.2                                  | 4.8                         | 80.7 $\pm$ 3.1                       | 82.5                                   | 4.7                                   |
| 1.56          | 62.64 $\pm$ 7.1             | 64.0                                   | 96.4 $\pm$ 7.9             | 100.5                                  | 5.1                         | 84.6 $\pm$ 3.9                       | 86.5                                   | 4.8                                   |
| 0.39          | 78.54 $\pm$ 6.6             | 80.3                                   | 107.2 $\pm$ 6.8            | 109.6                                  | 4.7                         | 90.1 $\pm$ 4.2                       | 92.1                                   | 5.1                                   |

The results confirm the data on a similar issue of domestic scientists, who also note the phytotoxicity of iron nanoparticles [4,17,19]. Researchers confirm that the main effect of metal nanoparticles is on the root system of plants [13,14,20]. Since the root barrier plays a significant role in the formation of tolerance to excess copper. Studies on the tolerance of corn and radish to the effects of copper and its nanoparticles have determined that their concentration of 2000 mg/l negatively affects the germination of corn seeds and inhibits root growth. The concentration of zinc nanoparticles for radish - 50 mg/l, rape - 20 mg/l also led to the suppression of root growth [6]. However, world experience has been gained confirming not only toxic, but also phytostimulating effect.

When pine was cultivated in a medium containing iron ions at concentrations (by iron) of 0.025-0.1 mmol / l, inhibition of the development of the root system was observed. Cultivation in a medium containing iron nanoparticles at a concentration of 6.25 mmol / l resulted in a decrease in the root length to 3.71 ± 0.06 mm. On the contrary, the presence of iron nanoparticles in the medium for the cultivation of pine caused an increase in the length of the main and adventitious roots, as well as the number of adventitious roots. The toxic effect of iron nanoparticles was manifested only at a concentration of 100 mmol / l.

The content of iron microparticles in the medium for the cultivation of pine caused only a decrease in the length of the main root and adventitious roots. At the same time, at concentrations from 0.39 to 25 mmol / l, an increase in the number of adventitious roots occurred, which indicates compensatory
reactions. The study made it possible to identify the phytotoxic effect of iron on the germination of seeds, the formation and growth of the root system and, as a result, the growth of the shoot. When assessing the parameter “growth and development of the root system”, it is necessary to reveal an increase in the activity of iron in the series: microparticles — nanoparticles — ions. In addition, concentrations were established at which the germination and growth rate of the root system was reduced to values close to zero. These values can be considered the upper tolerance of tolerance of pine seedlings in relation to different forms of iron.

Analysis of the growth of pine seedlings in an environment with the content of various forms of iron revealed significant changes in biometric indicators. The content of iron ions and microparticles in the culture medium was toxic at any concentrations, nanoparticles - only at the maximum concentration (100 mmol / l). The toxic effect was manifested in changes in the morphometric parameters of seedlings.

4. Conclusion
In the course of the research, the low tolerance of the pine to the action of ions and iron microparticles was revealed. In vitro studies of pine seedlings with the presence of iron revealed significant morphobiological changes in the root system. It was established that the presence of iron nanoparticles in the medium did not alter the germination of seeds, but at the maximum experimental concentration (100 mmol / l) reduced the length of the shoot and suppressed the formation of the root system. Another factor testifying to the low tolerance of pine - to the effect of iron is a certain suppression of the frequency of germination, the length of the shoot and the roots in the experiment with the presence of iron ions in the medium. In a variant of the experiment with the presence of iron microparticles in the culture medium, an insignificant suppression of the growth of the root system was revealed.

The obtained data are consistent with the data of domestic and foreign authors on the effect of metal nanoparticles on grass species and algae. Scientists found that the presence of copper nanoparticles in a cultured medium, whose dimensions did not exceed 120 nm, did not affect seed germination, but at high concentrations there was a pronounced effect of suppressing the formation of the root system and stem, as well as a decrease in root mass and an increase in its diameter compared to control. Similar studies in herbaceous plants have shown that the phytotoxic effect of nanoparticles still manifests itself in inhibiting seed germination, reducing the length of shoots and roots, reducing the rate of photosynthesis and respiration, as well as morphological and enzymatic changes.

References
[1] Brooks R R 1998 Plants that hyperaccumulate heavy metals (Wallingford: CAB International) p 384
[2] Da Costa M V J, Sharma P K 2016 Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in Oryza sativa Photosynthetica 54 110
[3] Kalyakina R G, Ryabukhina M V, Ryabinina Z N, Anhalt E M 2018 Ecological and biological features of conifers in an urbanized environment: monograph (Orenburg: Publishing Center OSAU) p 171
[4] Belava V N, Panyuta O O, Yakovleva G M et al 2017 The effect of silver and copper nanoparticles on the wheat—pseudocercosporaehorpotrichoidespathosystem Nanoscale Res Lett. 12 250
[5] Cyrusová T, Petrová Š, Vaněk T et al 2017 Responses of Wetland Plant Carexvulpina to Copper and Iron Nanoparticles Water Air Soil Pollut pp 228–258
[6] Din M I Arshad F, Hussain Z et al 2017 Green Adeptness in the Synthesis and Stabilization of Copper Nanoparticles: Catalytic, Antibacterial, Cytotoxicity, and Antioxidant Activities Nanoscale Res Lett. 12 638
[7] Mayskiy R, Ryabukhina M and Dodova M 2017 Ecological and economic modelling of the insects elimination effectiveness in the coniferous forests of the Orenburg region Vestnik Bashkir State Agrarian University 1(41) 101–3

[8] Ryabinina Z N, Ryabukhina M V, Kolodina M V 2017 Dose-dependent effects of the action of sulfur nanoparticles on the growth response and seed production of typical steppe phytocenoses Nano Hybrids 13 156–161

[9] Kasana R C, Panwar N R, Kaul R K et al 2017 Biosynthesis and effects of copper nanoparticles on plants Environmental Chemistry Letters 15(2) 233–240

[10] Kalyakina R G, Ryabukhina M V, Maiski R A 2018 Influence of Orenburg gas condensate field development on ecological and biological condition of landscape-botanical complexes IOP Conference Series: Materials Science and Engineering electronic edition 012194

[11] Melekhov I S 1980 Forest studies: uch. for universities (Moscow: Forest industry) p 408

[12] Ryabukhina M V, Filippova A V, Maiski R A 2017 Ecological potential and carbon sequestration of forest areas Baituganov oil field in Orenburg region Bulletin of the Nizhnevartovsk state University 4 105–110

[13] Maiski R A, Ryabukhina M V, Kalyakina R G 2018 Ecological and technological aspects of increasing sustainability of vegetation cover of Caspian oil and gas provinces IOP Conference Series: Materials Science and Engineering electronic edition 012193

[14] Maiski R A, Pavlova Yu A, Proskura V S 2017 Economic efficiency of environmental projects and activities Vestnik UGNTU. Science, education, Economics. Series: Economics 4(22) 40–47

[15] Medvedev M V 2001 Biotransformation of the organic matter of forest soils in the area of aerotechnogenic pollution of the Kostomuksha mine Forestry 3 25–27

[16] Nekrasova G F, Ushakova O S, Ermakov A E et al 2011 Effects of copper(II) ions and copper oxide nanoparticles on Elodea densa Planchn Russell J EcoL. 42 458

[17] Yang X E, Long X X, Ye H B et al 2004 Cadmium tolerance and hyperaccumulation in new Znhyperaccumulating plant species (Sedum alfrediiHance) Plant Soil 259 181–189

[18] Taran N, Storozhenko V, Svetlova N et al 2017 Effect of Zinc and Copper Nanoparticles on Drought Resistance of Wheat Seedlings Nanoscale Res Lett. 12 60

[19] Ryabukhina M V, Maiski R A, Salikhova R Kh 2017 Environmental risks of landscape botanical complexes and minimization of technogenic influence exerted by objects of oil&gas production in steppe zone of the Southern Urals IOP Conference Series: Materials Science and Engineering. International Conference on Construction, Architecture and Technosphere Safety (ICCATS 2017) 012167

[20] Rajput V D, Minkina T, Suskova S et al 2018 Effects of Copper Nanoparticles (CuO NPs) on Crop Plants: a Mini Review BioNanoSci. 8 36