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MORPHOPHYSIOLOGICAL FEATURES OF WHEAT (*Triticum aestivum* L.) SEEDLINGS UPON EXPOSURE TO NICKEL NANOPARTICLES

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Abstract

The intensive development of nanotechnologies determines the need for the investigation on the patterns of biological impact of technogenic nanomaterials. The analysis of the researches reveals a wide range of toxicity manifestations when nanocompounds affect plants, which depends on the physical properties of the nanoparticles (dimensions, shape, catalytic activity, concentration). The relevance of the studies on the concentration effects of nanoparticles is due to the insufficient knowledge of their interaction with the plant cell, and, consequently, the need to determine the dose-effect relationship for each class of nanoparticles and various bio-objects. This paper presents the results of a comprehensive study of the effect of nickel nanoparticles (NP Ni^0, Δ50 = 5 nm in size) when used at different concentrations on growth, content of pigments, flavonoids and proline, photosynthesis and transpiration intensity of ten-day-aged wheat seedlings (*Triticum aestivum* L.). The calibrated seeds were pre-germinated for 2-3 days, up to the appearance of rootlets, in Petri dishes on filter paper impregnated with aquatic disperse systems of NP Ni^0 in concentrations of 0.01, 0.1, 1 and 10 mg/l. In the control, the seeds were germinated on distilled water. The germinated seeds were put into 500-millilitre vegetative pots for further growing in the aquatic dispersed NP Ni^0 systems of the above mentioned concentrations in the climate box until the 10-day age. Morphometric parameters assessed were the root length, the seedling height, the weight of the root and aboveground parts of the plants. To determine the content of photosynthetic pigments, flavonoids and proline, an average sample was collected from the leaves of 10 plants. The morphometric parameters under study depended on the doses of nickel nanoparticles in a disperse medium. NP Ni^0 at low concentrations (0.01 and 0.1 mg/l) did not change or stimulated growth, whereas at larger doses (1 and 10 mg/l) they suppressed the growth of roots and aboveground part of seedlings considerably. The root length decreased 2 times at 1 mg/l NP Ni^0 and 3 times at 10 mg/l NP Ni^0, the wet weight was 1.9 and 2.7 times lower, respectively, and the height declined 1.3 and 1.9 times. The content of chlorophyll a and b at 0.01 mg/l NP Ni^0 slightly increased and then decreased as the nanoparticle concentration increased, but no clear dose dependence was revealed. The amount of carotenoids gradually decreased with increasing NP Ni^0 concentration. The study of photosynthesis and transpiration showed a dose correlation of these indicators. NP Ni^0 at low concentrations (0.01 and 0.1 mg/l) increased the intensity of photosynthesis and transpiration significantly, at the concentration of 1 mg/l did not affect these processes, and at 10 mg/l concentration insignificantly suppressed these parameters. The amount of flavonoids decreased with increasing NP Ni^0 concentration; however, dose dependence was not observed. The lowest level of flavonoids, with a 75 % decrease, was at 0.1 mg/l NP Ni^0, and at 10 mg/l NP Ni^0 the amount of flavonoids decreased by 64 % as compared to the control. At the same time, the impact of nickel nanoparticles on wheat caused a rise in the level of proline from 22 to 130 %, with clear dose dependence on the nanoparticle concentration. Mass spectrometric studies revealed a significant accumulation of nanoparticles in plant organs, especially in the root system. In the roots of the experimental plants the nickel concentration was 50.89±1.67 μg/g per

dry weight, in the control plants this reached 3.8±0.15 μg/g. In the above-ground parts of plants the nickel concentration was an order of magnitude lower, 14.20±2.38 μg/g per dry weight for the test plants and 0.87±0.025 μg/g per dry weight for the control plants. Thus, our findings revealed the morphophysiological peculiarities of wheat seedlings grown on water dispersed systems of NP Ni^0 of 5 nm in size and showed a dependence of the majority of the studied parameters on NP Ni^0 concentration.

Keywords: *Triticum aestivum* L., nickel nanoparticles, accumulation, photosynthetic pigments, photosynthesis, transpiration, flavonoids, proline

Nanotechnologies are widely used in industry, medicine, and agriculture. However, some components of nanotechnology production are potentially dangerous for the environment, and their influence on biological objects has not been sufficiently studied [1]. The problem of the effect of nanoparticles on living organisms is related to the study of the mechanisms of their toxic effect and the cycle in nature. According to modern concepts, the complexity of interaction depends on the physical and chemical properties, the method of production, the size and structure of nanoparticles, as well as on the characteristics of biological objects, including plant species [2].

It is shown that substances in the form of nanoparticles have other properties and ability to penetrate into plants than the same substances in an ionic form [3]. Metal nanoparticles are characterized by excess surface energy and high reactivity; they actively enter the processes of aggregation and reactions with other chemical compounds [4]. In addition, when interacting with different cell structures and due to a prolonged action, nanoparticles can act as catalysts in reactions with the formation of both growth-promoting agents and inhibitors [5-6]. That is, plants make it possible to evaluate the specificity of nanoparticles action and their dose-dependent effects.

By now, the effect of TiO_2, Al_2O_3, Fe_2O_3, ZnO and CeO_2 nanoparticles on plant objects has been studied to a greater extent [7]. There is much less information on the action of nickel nanoparticles [8-10]. At the same time, there is a sufficient number of publications on the influence of nickel ions on the growth, development and physiological and biochemical parameters of plants [11, 12]. In terms of the production output of homogeneous metal powders with a high degree of purity, nickel nanoparticles are in the top five, along with iron, aluminum, copper and titanium nanoparticles [13]. They are widely used in medicine and biology [14, 15], are included in magnetic fluids and catalysts, are used to create high-speed optical devices [16, 17], and can also contaminate the environment during production, use, and disposal [18].

Nickel is considered an essential ultramicroelement for higher plants, since the activity of enzymes of various metabolic pathways, for example, urease, depends on its content. Low concentrations of nickel salts introduced into the nutrient solution have a positive effect on the growth and development of plants, including wheat [19]. Among heavy metals, nickel is highly toxic and causes significant failures in the structure and functioning of cells [20].

The present paper, for the first time, shows that the effect of low concentrations of nickel nanoparticles (Δ_{50} = 5 nm) in the range of 0.01-10 mg/l can cause significant changes in the structural and functional characteristics of wheat seedlings and mainly has a dose dependence.

The purpose of the study was to identify the morphological, physiological and biochemical features in wheat seedlings under the influence of nickel nanoparticles (NP Ni^0) at different concentrations.

Technuques. Ten-day seedlings of soft spring wheat (*Triticum aestivum* L.) of the Novosibirskaya 29 variety were used as the object of the study. The plants were grown under laboratory conditions in a climatic chamber (Labline Scientific Instruments, Poland) at a 12-hour photoperiod, a temperature of 23-

24 °C, and an illumination of 60 W/m^2. The experiment used seeds, the germination capacity of which was previously determined in accordance with RF State Standard GOST 12038-84 and was not less than 95%. The calibrated seeds were pre-germinated for 2-3 days (up to the appearance of rootlets) in disposable plastic Petri dishes with two layers of impregnated filtering paper. In the experiment, the suspensions of Ni^0 nanoparticles at a concentration of 0.01; 0.1; 1 and 10 mg/l were used, and in the control variant, distilled water was used. The germinated seeds were put into 500 ml vegetative pots, which were placed in the climatic chamber, for further growing until the 10-day age. In the experiment variants, suspensions of nickel nanoparticles of the concentrations indicated above were used for growing, and in the control variant, distilled water was used. Due to the aggregation of nanoparticles and the decrease in their concentration in free form, all the disperse systems in the vessels were replaced on a daily basis. Each vessel contained 25 plants; the experiment was carried out in 4 replications for each variant of the experiment.

Ni^0 nanoparticles were obtained using the method of laser ablation in distilled water from nickel bars (purity 99.95% by weight, grade Ni 3N5) (Girme", Russia). When the bar was exposed to radiation using an impulse Nd-YAG-laser LS-2134UTF (Lotis Tii, Belarus, Japan), ablation and spraying of the target material into the environment occurred. The thickness of the layer removed with one impulse was small and did not exceed a few tens of nanometers. Outside the target, the removed material was organized into nanoparticles [21]. According to the data obtained by transmission electron microscopy (Philips CM-12, Koninklijke Philips N.V., the Netherlands), the particle diameter was 2-12 nm with an average size of Δ_{50} = 5 nm and a specific surface area of 30 m^2/g. Necessary concentrations of disperse systems of NP Ni^0 were obtained by diluting the initial dispersion medium (DM) with distilled water followed by a 45-minute ultrasonic treatment at a frequency of 35 kHz in an ultrasonic bath (UZV-5.7/1 TTC, ZAO PKF Sapfir, Russia). The quantitative characteristics of nanoparticles absorption from the DM were determined by mass spectrometry with inductively coupled plasma in terms of Ni content in the samples of tissue from roots and above-ground parts (leaves + stem) of plants [22]. The roots before drying were washed twice with a 0.01% Na-EDTA solution, then were washed three times with distilled water in order to remove particles sorbed on the surface. Samples of roots and leaves dried to constant weight were ground in a porcelain mortar; then 0.1 g sample was taken for analysis. The samples were ashed in the microwave decomposition system Speedwave TM MWS-3+ (BERGHOF Products + Instruments GmbH, Germany) and analyzed using a mass spectrometer ELAN DRC-e (PerkinElmer, Inc., USA).

Morphometric parameters were evaluated according to the length of the root system and seedlings, the mass of the root and aboveground parts of the plants. The wet mass was determined by the standard weighing method. To assess the content of photosynthetic pigments, an average sample of 10 plants was formed (sample weight 0.4 g). The amount of chlorophylls and carotenoids was determined by spectrophotometry (spectrometer UV-1601PC, Shimadzu Corp., Japan) in alcohol extracts [23]. To measure the photosynthesis and transpiration rate, a portable infrared gas analyzer Li-6400 (LI-COR Biosciences, USA) with an open system was used, where a photodiode system (6400-02B LED) was used as an artificial light source, providing an illumination of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The temperature in the leaf chamber was maintained at 24 °C, the flow rate of CO_2 was 400 μmol/s, its content was 400 μmol/mol. Photosynthesis and transpiration rate was measured in the leaves of 10-day seedlings. The content of flavonoids was evaluated by spectrophotometry according to the reaction with alumi-

num chloride. The optical density of the solution was determined at $\lambda = 415$ nm using a UV-1601PC spectrophotometer (Shimadzu Corp., Japan). The number of flavonoids was calculated using a calibration curve constructed according to the routine (Sigma, Great Britain) [24]. The content of free proline in the shoots was evaluated using an acidic ninhydrin reagent by the method of L.S. Bates et al. [25].

Statistical processing of the data was performed using the Statistica 8 software (StatSoft, Inc., USA). The tables and figures show the arithmetic mean values (*M*) and their standard errors (±SEM) for morphological parameters from 100, for physiological-biochemical indicators and nickel accumulation from 4 biological replications. Differences were considered valid with an error probability of $p \leq 0.5$.

Results. A significant accumulation of nanoparticles in plant organs, especially in the root system, occurred at 10-day germination of wheat on a dispersion medium containing NP Ni^0 at a concentration of 10 mg/l. In the roots of the experimental plants, the nickel concentration was 50.89±1.67 μg/g per dry weight; in the control plants, this value was 3.8±0.15 μg/g. In the above-ground parts of plants, the nickel concentration was an order of magnitude lower: 14.20±2.38 μg/g dry weight in experimental plants, 0.87±0.025 μg/g dry weight in the control plants.

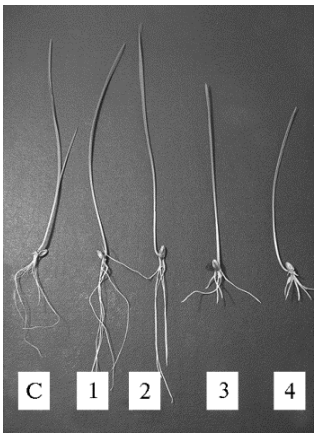

Fig. 1. 10-day seedlings of the soft spring wheat (*Triticum aestivum* L.) of the Novosibirskaya 29 variety under the action of Ni^0 nanoparticles at different concentrations: C — control; 1 — 0.01; 2 — 0.1; 3 — 1; 4 — 10 mg/l in the dispersion medium (laboratory test).

Accumulated nanoparticles caused visible changes in the morphometric parameters of the root system and the aboveground parts of the wheat seedlings (Fig. 1). The NP Ni^0 at concentrations of 0.01 and 0.1 mg/l did not change or even stimulated the growth processes, but the NP Ni^0 at higher concentrations (1 mg/l and 10 mg/l) significantly inhibited the growth of the roots and the aboveground parts (Table 1). The root length decreased by 2 times at 1 mg/l NP Ni^0 and by 3 times at 10 mg/l NP Ni^0, the wet weight decreased by 1.9 and 2.7 times, respectively, and the height decreased by 1.3 and 1.9 times.

D.F. Piccini et al. [11] also showed that the introduction of nickel nanoparticles of a size less than 100 nm at the concentration of 100 mg/kg had a toxic effect on the growth of the *Lepidium sativum* L. roots. The experiments on *Solanum lycopersicum* L. showed that nickel nanoparticles of 28 and 62 nm in size accumulate mainly in the roots, reduce the above-ground dry mass, and affect the Ca and K content in the leaves [9].

1. Morphometric parameters of the seedlings of the soft spring wheat (*Triticum aestivum* L.) of the Novosibirskaya 29 variety grown on a dispersion medium that contained nickel nanoparticles at different concentrations (*M*±SEM, laboratory test)

Concentration of nanoparticles, mg/l	Roots		Aboveground parts	
	length, cm	wet weight, mg	height, cm	wet weight, mg
Control	7.79±0.22	98.6±4.2	16.83±0.39	221.5±8.5
0.01	9.73±0.23*	129.7±6.4*	17.35±0.21	255.1±7.3*
0.1	8.33±0.16	112.6±8.2	17.02±0.19	233.1±10.2
1	4.12±0.07*	52.8±5.1*	12.89±0.28*	171.8±9.3*
10	2.56±0.07*	36.0±7.2*	8.03±0.34*	121.6±10.3*

* Differences with control are statistically significant at $p \leq 0.05$.

Inhibition of root growth is one of the earliest responses to the action of

heavy metals [26]. This feature is widely used to assess the degree of their toxicity at different concentrations [27]. Protective mechanisms and barriers that operate at the level of cells and tissues of the root reduce the ingress of heavy metals into shoots; the result is the accumulation of heavy metals in the roots in significant amounts, which affects the development and formation of the root system [28, 29].

Accumulated NP Ni^0 had the effect not only on the growth parameters but also on the physiological and biochemical characteristics of the leaf apparatus of wheat seedlings. In particular, when cultivating seedlings on the dispersion medium containing NP Ni^0, the amount of chlorophylls a and b at a 0.01 mg/l concentration of NP Ni^0 increased insignificantly. With an increase in the nanoparticles concentration, a decrease in this indicator was observed. The content of chlorophylls decreased statistically significantly ($p < 0.05$) by 30% as compared to the control only at a 10 mg/l concentration of NP Ni^0 (Fig. 2). Similar changes were observed previously when the pigment complex of wheat was exposed to platinum nanoparticles [30]. The amount of carotenoids at 0.1 and 1 mg/l concentrations of NP Ni^0 decreased by 19-20%, and at 10 g/l concentration by 35% (see Fig. 2).

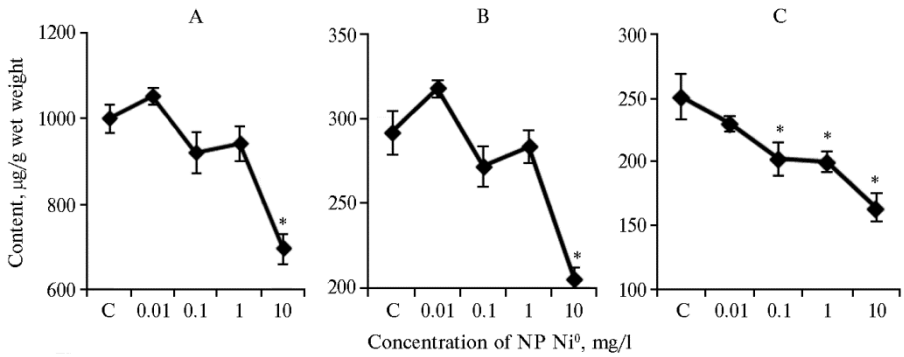

Fig. 2. The content of chlorophylls a (A) **and b** (B) **and the sum of carotenoids** (B) **in the leaves of the soft spring wheat (*Triticum aestivum* L.) of the Novosibirskaya 29 variety depending on the Ni^0 nanoparticles** (NP) **concentration in the dispersion medium** (laboratory test)
* Differences with control are statistically significant at $p \le 0.05$.

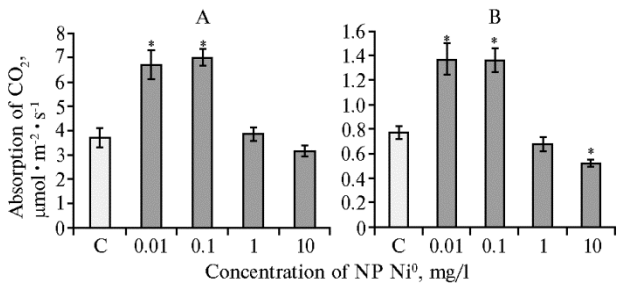

Fig. 3. Photosynthesis (A) **and transpiration** (B) **rate in the leaves of soft spring wheat (*Triticum aestivum* L.) of the Novosibirskaya 29 variety depending on the Ni^0 nanoparticles** (NP) **concentration in the dispersion medium** (laboratory test).
* Differences with control are statistically significant at $p \le 0.05$.

It is shown that the content of photosynthetic pigments decreases under the influence of most stress factors [19, 31]. For example, treatment of *T. vulgare* L. seeds with nickel nanoparticles of 57 nm in diameter with further 48-hour incubation using their solutions at concentrations of 0.0125-1 M caused a sharp decrease in the content of chlorophyll b in the leaves and led to an increase in the content of malonic dialdehyde in the root system of plants. This indicates the development of cytogenetic damage due to the oxidation of lipids in cell membranes. The dose dependence [10] was also observed.

The features of the photosynthetic pigments accumulation under the influence of Ni^0 NP as a whole were similar to the patterns of seedlings growth

(see Table 1). This is probably due to the strong correlation between photosynthesis and growth processes, which are regulated by metabolic and hormonal mechanisms [32].

A study of the integral functional characteristics of the leaf apparatus – photosynthesis and transpiration also revealed a dose-dependent effect. Nickel nanoparticles at low concentrations (0.01 and 0.1 mg/l) significantly ($p < 0.001$) increased the intensity of photosynthesis, at a 1 mg/l concentration did not change this indicator, and only at 10 mg/l concentration decreased it by 15% as compared to the control (Fig. 3, A). When measuring the intensity of transpiration, a similar dependence was found (see Fig. 3, B).

2. Biochemical parameters of the seedlings of soft spring wheat (*Triticum aestivum* L.) of the Novosibirskaya 29 variety grown on a dispersion medium that contained nickel nanoparticles at different concentrations (*M*±SEM, laboratory test)

Concentration of nanoparticles, mg/l	Sum of flavonoids		Proline content	
	μg/g of dry weight	to the control,%	mg/g of dry weight	to the control,%
Контроль	28.11±0.11	100	0.77±0.12	100
0.01	17.24±0.13*	61	0.95±0.09*	123
0.1	7.03±0.01*	25	1.15±0.21*	149
1	21.22±0.01*	75	1.28±0.25*	166
10	10.17±0.03*	36	1.79±0.26*	232

* Differences with control are statistically significant at $p \le 0.05$.

It is known that the stress-protective function under adverse effects is performed by flavonoids [33] and proline [34, 35], which are capable of binding metal ions with variable valency and thus limiting non-enzymatic free-radical processes.

In our experiments, the amount of flavonoids decreased with an increase in the concentration of NP Ni^0, but no clear dose dependence was observed (Table 2). The literature data on the change in the content of flavonoids under the influence of nanoparticles on plants are quite contradictory. Thus, when growing *Raphanus sativus* L. in the soil treated with cesium oxide nanoparticles at different concentrations, a considerable scatter of data in the variants of the experiment was observed, so the differences were not significant [36]. A decrease in the content of flavonoids in different organs of *Calendula officinalis* L. was observed under the influence of silver nanoparticles. The paper of C. Krishnaraj [38], on the contrary, shows a shift towards secondary metabolism and an increase in the content of flavonoids in *Bacopa monnieri* L. under the influence of silver nanoparticles.

At the same time, the effect of nickel nanoparticles led to an increase ($p < 0.05$) in the amount of proline in wheat leaves comparatively to the control. In this case, a dose dependence on the concentration of nanoparticles was observed (see Table 2), which agrees with the existing concept on the protector role of proline under stress [34].

Thus, this paper reveals the morpho-physiological peculiarities of wheat seedlings during their growing on aqueous disperse systems containing nickel nanoparticles ($\Delta_{50} = 5$ nm), with similar dependencies observed for the majority of the studied parameters (morphometric parameters, chlorophyll content, photosynthesis and transpiration rate), i.e. an increase at small concentrations of NP Ni^0 and a distinct decrease at higher concentrations of NP Ni^0. Among the compounds performing the protective function, a directly proportional increase in the content with an increase in the concentrations of NP Ni^0 is observed only in case of proline, while a decrease in the amount is shown for carotenoids and flavonoids. This suggests that, depending on the concentration, Ni^0 nanoparticles have a selective effect on the various metabolic processes. The obtained results can supplement the data on the justification of the permissible levels of contamination of plants and agrocoenosis by metal nanoparticles, and also be used to develop practical recommendations for diagnosing the negative impact of NP Ni^0 on plants.

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