

Determining the common patterns of the action of nanoparticles of various compositions and structures on physiological and biochemical processes in plants

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Abstract. Technogenic and natural nanomaterials are alien to living systems. The negative effect of nanomaterials may be due to their accumulation in intracellular organelles. The properties of nanoparticles are determined not only by their size, which manifests itself in the activation of a thermodynamic variable, but also by their chemical structure and shape, their ability to aggregate. The effect of metal nanoparticles and oxides of cobalt and titanium on the development and growth of plants has been studied. An important established fact is the ability of oxide nanoparticles, unlike metals themselves, to accumulate in living systems. Transmission electron microscopy, carried out by means of electron microscopic analysis, has revealed interstitial bioaccumulation of nanoparticles of cobalt oxide and titanium oxide in the form of aggregates 80-300 nm in size. If nanoparticles quickly aggregate, they are less dangerous for organisms than single nanoparticles, since a large aggregate of nanoparticles cannot get inside the cell. The rate and dynamics of deposition of nanoparticles of metals and their oxides in water are different. Metal NPs are deposited much more slowly than titanium dioxide. Moreover, the safety of NPs depends on their size and concentration. Biogenic nanoparticles with a size of 35-75 nm have high biological activity, biocompatibility and environmental safety. There is a direct correlation between the energy produced in cells, which is necessary for seed viability, and an increase in the number of protons under the action of metal nanoparticles, which leads to an increase in the permeability of cell membranes and the activity of enzymes and phytohormones.

1 Introduction

Publications on nanotechnology are in demand and their number is increasing every year. The use of NPs in medicine, agriculture and forestry is a feature of modern work [1-4].

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The effect of nanoparticles on plants is important. Plants are able to adsorb nanoparticles in large quantities, which determines the possibility of their transfer through the food chain and, ingestion by humans. However, nanoparticles can also have a positive effect on increasing yields, creating inducers of stress resistance in agricultural plants, etc. [5-6]. Advantages of created nano-preparations based on nanoparticles are their increased bioavailability, the absence of accumulation in various living systems, and reduction in the risks of side effects. The toxicity of nanoparticles depends on many factors and is determined not only by concentration, especially by size, but also by shape. Spherical particles have a lower cytotoxicity than dendritic and spindle-shaped particles.

Nanoparticles also affect animals. On the one hand, they affect the liver, lungs, the digestive duct, kidneys, brain of laboratory animals. On the other hand, under certain conditions, they increase the productivity of farm animals [7-8] by reducing the risk of oxidative stress and chronic diseases. The sizes of nanoparticles for penetration into living organisms and accumulation are different. In some works [9-10] the translocation of nanoparticles from the lumen of alveoli to the interstice of lungs was shown in rodents. This explains the role of inhaled nanoparticles in the pathogenesis of various diseases.

One of the main properties for agriculture may be the ability of nanoparticles to penetrate the cell membrane [11], overcoming the hematoencephalic barrier [12]. The increased specific surface of NPs leads to an increase in the chemical potential at interfaces and a huge increase in reactivity and solubility. Such properties of NPs are manifested in various effects on living systems, including plants. An important established fact is the ability of oxide nanoparticles, unlike metals themselves, to accumulate in living structures. Moreover, the safety of metal NPs is determined by their size. NPs of biogenic metals (35-75 nm in size) have high biological activity, biocompatibility and environmental safety.

Researches to identify the mechanism of action of technogenic nanoparticles on plants, animals and humans is currently of paramount importance. Biogenic metals and their compounds themselves are biologically active substances that act as microfertilizers and stimulators of plant growth and development. It is noted [13] that, when seeds of various plants are soaked in solutions with NPs at a concentration of 0.01-10 g/ha, stimulation of respiration intensity and morphophysiological parameters is observed. The accumulation of biopolymers, the mass of dry residue increase. Seedlings maximize accumulation of biomass in all experiments, depending on the genetic orientation of hybrids and plants. Nanoparticles also affect the intensity of forest growth and its restoration [14].

It is possible to identify the main factors that change the formation of new properties of nanosystems. There are two of them: a change in characteristics of the thermodynamic state of the system in comparison with the macro classical state and the formation of quantum-dimensional effects, while the characteristic dimensions of the structural components decrease. Thus, nanosystems have the so-called size effect from a certain moment, i.e. properties of nanosystems depend on the concentration and size of nanoparticles. The described size effect [15] is noticed in the case when the size of the block of the microstructure and the critical length that characterizes the phenomenon of the nanosystem coincide (the path length of phonons and electrons, the size of magnetic domains, the radius of the critical dislocation loop, etc.), which is typical for the structure of nanoparticles. One of the manifestations of stress, including nanoparticles, is the phenomenon of an adaptive response, which is a universal response of cells to a stimulus in "small" doses. The form of the response, that is, the mechanism of action of nanoparticles, depends on the biocompatibility of nanoparticles to a living system, and, consequently, on the structure.

2 Materials and methods

Determining the influence of nanoparticles on plant objects by morpho-physiological and vital indicators.

Lab researches were carried out in the Center for Nanomaterials and Nanotechnologies for the Agro-Industrial Complex of the Russian Federation at Ryazan State Agrotechnological University and Departments of General Chemistry and Biological Chemistry with a course of clinical laboratory diagnostics at Ryazan State Medical University. Vetch and rice seeds were used in the experiment. Laboratory viability and germination readiness were determined on the basis of "Seeds of agricultural crops. Methods for determining the viability and germination readiness", GOST 12038-84, as well as "Determining the impact of nanoparticles on plant objects for food and feed purposes by vital and morpho-physiological indicators" [16].

The research concerned seeds of vetch and rice of one year of harvest, not treated with a disinfectant and certified for compliance with class 1 in accordance with the Regulations on Certification. Seeds were germinated on domestic microbiological agar in the form of a gel-like cultivation medium.

When preparing suspensions, nanoparticles were weighed on an analytical balance ViBRA HT, Japan, with an accuracy of ± 0.0001 g. Weighed nanoparticles were added to the prepared flask with water, 1,000 ml in volume. Suspensions were prepared in an ultrasonic bath (model PSB-5735-5) according to TU 931800-4270760-96) for 10 minutes. The power of the ultrasonic bath was 300 W, at a frequency of 23.740 kHz. The prepared suspensions were added to a gel-like medium prepared for research, which, if necessary, was placed in Petri dishes. Seeds were subsequently sown in these containers. Suspensions were prepared at different concentrations with a content of nanoparticles from 0.01 to 100.0 g per hectare of seed sowing. The method for determining the impact of biologically active nanoparticles on seeds was based on the assessment of the determined vital and morphophysiological characteristics of seedlings, including germination energy on the 3rd day; the viability on the 7th day; lengths of 7-day-old underground and aerial parts of seedlings; masses of 7-day-old underground and aerial parts of seedlings.

This method was used to study the effect of nanoparticles uniformly distributed in the cultivation medium. This made it possible to determine changes in such morphological, physiological and vital parameters of seedlings, which are due to the stimulating or depressing effect of nanoparticles of various concentrations. Based on the results of the research, the most significant concentrations and the degree of their stimulating effect were highlighted.

The method to assess the solubility of nanoparticles in water and biological fluids.

Physiological saline was chosen as the physiological fluid. Suspensions of nanoparticles in such a solution were placed in a thermostat at a temperature of $37 \pm 0.5^\circ$ C for a certain time. Then, an aliquot taken from this solution was centrifuged (5,000 rpm) for 30 min to separate the solid phase. On a spectrophotometer (PD-303 Apel, Japan), the concentration of cobalt and iron ions in the resulting solution was determined. The data obtained were used to calculate the degree (wt %) of dissolution of nanoparticles. The gravimetric method was used to determine the degree of solubility of titanium dioxide. The dependence of solubility on both the size and composition of nanoparticles was revealed.

The method to determine bioaccumulation of nanoparticles in seedlings using electron microscopy.

Electron microscopic analysis was carried out according to the following method. Vetch and rice seeds were germinated for 10 days in a specially prepared biologically active medium, to which iron, cobalt, cobalt oxide, and titanium dioxide nanoparticles were

previously added. For comparison, control lots of seeds were prepared. In that case, the viability was carried out without adding nanoparticles to the biological medium.

On the 5th and 10th day of the experiment, seedlings were selected, fixed and examined by electron microscopy. The distribution of metals in the samples was carried out on electron microscopes (SEM) Merlin, Carl Zeiss and Neon 40 (Germany) and transmission electron microscopes "JEM-1400", "JEOL" (accelerating voltage 80-120 kW) using electron-microscopic analysis.

3 Results and Discussion

Biological activity of metal nanoparticles.

The study of the effect of Fe, Co, Cu, Zn nanoparticles on the development and growth of corn, cucumber, wheat, rice, vetch made it possible to determine their biological activity in the concentration range of 0.01-100 g/t of seeds. The regularity of that dependence was typical for all studied plants. It was determined in laboratory studies that the biological activity was high and the effect of the so-called "low doses" was observed for nanoparticles with a particle size of 35-75 nm obtained by a chemical method. The best concentrations in determining biological activity were observed when determining the germination readiness at concentrations of 0.5 and 5.0 g/t of seeds, the weight of a 3-day-old seedling equal to 0.05 and 1.0 g/t, the weight of a 7-day-old seedling equal to 0.1 and 1.0 g/t, the root weight equal to 0.5 g/t. At the content of nanoparticles above 100 g/t, the dose-effect dependence was no longer determined, and all indicators remained at the control level, that is, no inhibition of seedlings was noted even at a concentration of 500 g/t (Figure 1).

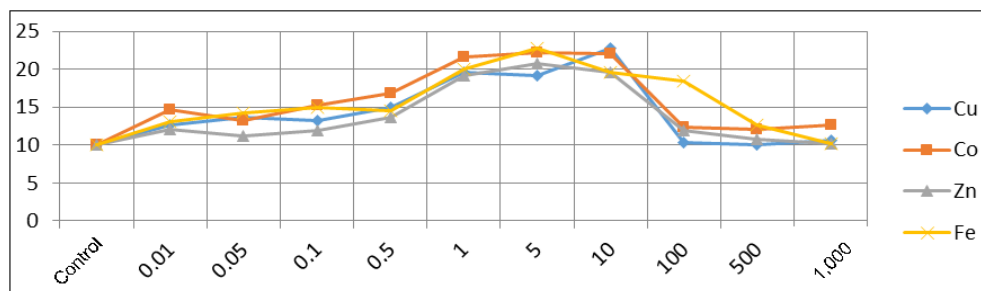


Fig. 1. The size of roots of 3-day-old vetch seedlings with 40-70 nm nanoparticles.

Biological activity of metal oxide nanoparticles.

Metal oxides, on the contrary, reduce the morpho-physiological parameters of plants. The vital parameters of vetch and rice seeds when interacting with nanoparticles of cobalt and titanium oxides are shown in Table 1. The germination readiness of rice in all variants with cobalt oxide was lower than the control, reaching its minimum at CoO concentration of 10 g/t. The viability also differed from the control. The maximum decrease (20.9 %) was at CoO 100 g/t. The viability and germination readiness of vetch under the influence of low concentrations of cobalt oxide nanoparticles exceeded the control by 2-3 %, and with increasing concentrations, the vital indicators began to decrease, starting from a concentration of 10 g/t and became lower compared to the control value. So, in the case of CoO, the viability decreased by 32.5 % and germination readiness by 21-36.8 %. However, under the influence of titanium oxide nanoparticles (Figure 2), the viability of vetch and the germination readiness changed slightly. The same regularity was observed in the effect of nanoparticles of cobalt oxide and titanium dioxide on rice seeds.

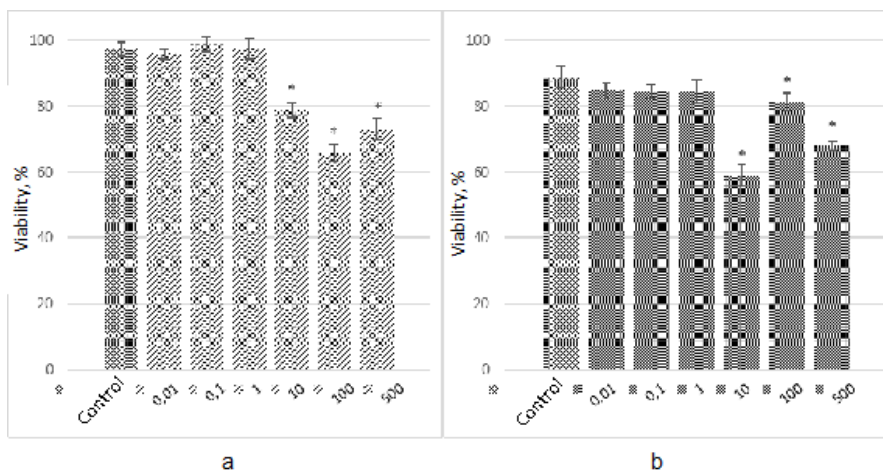


Fig. 2. The viability of vetch (a) and rice (b) seeds on a gel-like polysaccharide medium with cobalt oxide nanoparticles (Note: * - significant differences compared to control ($p < 0.05$)).

Table 1. Metric indicators of vetch and rice seedlings germinated on a gel-like cultivation medium with oxide nanomaterials.

Variant	Vetch (3-day-old); n = 20		Rice (3-day-old); n = 20	
	The length of the aerial part of the seedling, mm	The length of the underground part of the seedling, mm	The length of the aerial part of the seedling, mm	The length of the underground part of the seedling, mm
Control	22.7 ± 2.68	28.1 ± 1.68	19.0 ± 3.08	11.2 ± 3.76
CoO 0.01	27.2 ± 2.91*	25.0 ± 2.21*	12.0 ± 2.99**	9.1 ± 2.76
CoO 0.1	18.9 ± 3.28*	22.7 ± 2.82*	14.0 ± 2.11*	12.0 ± 2.69
CoO 1.0	23.3 ± 2.91	25.3 ± 2.24*	14.5 ± 3.0*	11.1 ± 3.29
CoO 10	22.3 ± 3.84	25.5 ± 2.97*	14.9 ± 2.6*	11.6 ± 2.88
CoO 100	19.6 ± 3.24*	25.0 ± 2.83*	14.3 ± 2.5*	12.5 ± 3.31
CoO 500	18.4 ± 4.34*	20.2 ± 3.28*	10.6 ± 3.03**	8.0 ± 3.32*
TiO ₂ 0.01	20.0 ± 4.79	25.6 ± 3.26	16.7 ± 3.19	11.4 ± 3.89
TiO ₂ 0.1	20.7 ± 4.39	25.2 ± 4.65	13.2 ± 3.85*	14.0 ± 4.09
TiO ₂ 1.0	21.8 ± 4.47	29.6 ± 4.18	16.6 ± 4.39	15.5 ± 6.2*
TiO ₂ 10	23.0 ± 4.07	27.0 ± 2.90	18.8 ± 4.46	13.3 ± 5.24
TiO ₂ 100	21.8 ± 3.14	29.4 ± 3.43	13.4 ± 3.57*	14.7 ± 4.79*
TiO ₂ 500	21.9 ± 3.61	27.0 ± 3.52	13.1 ± 3.54**	12.8 ± 4.4

Note: * - significant differences compared to control ($p < 0.05$); ** - significant differences compared to control ($p \leq 0.01$).

An increase in the concentration of CoO nanoparticles from 0.1 to 10.0 contributed to an increase in the weight of both the underground part (27.0 % more) and aerial one (21.9-34.7 % more) of a 7-day-old vetch seedling compared to the control (Figure 3). But a higher concentration caused a decrease in root weight by 36.1 % below the control. The weight of the aerial part of rice seedlings at any concentration of cobalt oxide nanoparticles was lower than the control, significantly at CoO 100, where it was 18.3 lower than the control. The weight of the underground part was also lower than the control with the highest result of 22.7 %. TiO₂ nanoparticles inhibited the development of both underground and aerial parts of the rice seedling. At concentrations of 100 g/t, the inhibition was 20.3-26.1 %.

CoO nanoparticles affected the vital parameters of rice seeds similarly to TiO₂, but inhibition was observed to a lesser extent.

Table 2. Weight indices of 7-day-old seedlings obtained on a cultivation gel-like medium with oxide nanoparticles.

Variant	Vetch; n= 3 5		Rice; n = 35	
	The weight of the aerial part of the seedling, g	The weight of the underground part of the seedling, g	The weight of the aerial part of the seedling, g	The weight of the underground part of the seedling, g
Control	0.0751 (0.0683; 0.0823)	0.0370(0.0342; 0.0415)	0.0321 (0.0296; 0.0352)	0.0088 ± 0.0014
CoO 0.01	0.07955 (0.0768; 0.0839)	0.04005 (0.0389; 0.0411)	0.0300 (0.0264; 0.0317)	0.0076 ± 0.0015
CoO 0.1	0.08475 (0.0827; 0.0873)	0.0490 (0.0482; 0.0501)*	0.0272 (0.0244; 0.0310)*	0.0071 ± 0.0013*
CoO 1.0	0.0927 (0.0882; 0.0934)*	0.0728 (0.0709; 0.0736)**	0.0313 (0.0285; 0.0340)	0.0068 ± 0.0009*
CoO 10	0.1243 (0.1162; 0.1288)**	0.0689 (0.0615; 0.0744)**	0.0284 (0.0247; 0.0330)	0.0070 ± 0.0012*
CoO 100	0.0999 (0.0958; 0.1064)*	0.0494 (0.0468; 0.0512)*	0.0252 (0.0236; 0.0379)*	0.0069 ± 0.0011*
CoO 500	0.0654 (0.0625; 0.0718)	0.0242 (0.0225; 0.0263)*	0.0246 (0.0233; 0.0270)*	0.0068 ± 0.0009*
TiO ₂ 0.01	0.0860 (0.0832; 0.0912)	0.04195 (0.0392; 0.0436)	0.0268 (0.0257; 0.0278)*	0.0068 ± 0.0011*
TiO ₂ 0.1	0.0937 (0.0914; 0.1007)*	0.04375 (0.0394; 0.0492)*	0.02835 (0.0275; 0.0295)	0.0074 ± 0.0014*
TiO ₂ 1.0	0.10145 (0.0956; 0.1049)**	0.0316 (0.0288; 0.0337)**	0.02725 (0.0259; 0.0293)	0.0074 ± 0.0014*
TiO ₂ 10	0.1095 (0.1046; 0.1136)**	0.0444 (0.0416; 0.0485)*	0.02565 (0.0247; 0.0268)*	0.0066 ± 0.0014
TiO ₂ 100	0.1005 (0.0975; 0.1036)**	0.03805 (0.0352; 0.0428)	0.0263 (0.0251; 0.0273)*	0.0065 ± 0.0013*
TiO ₂ 500	0.1072 (0.1037; 0.1092)**	0.04455 (0.0379; 0.0487)	0.0272 (0.0256; 0.0279)*	0.0062 ± 0.0009**

Note: * - significant differences compared to control ($p < 0.05$); ** - significant differences compared to control ($p \leq 0.01$)

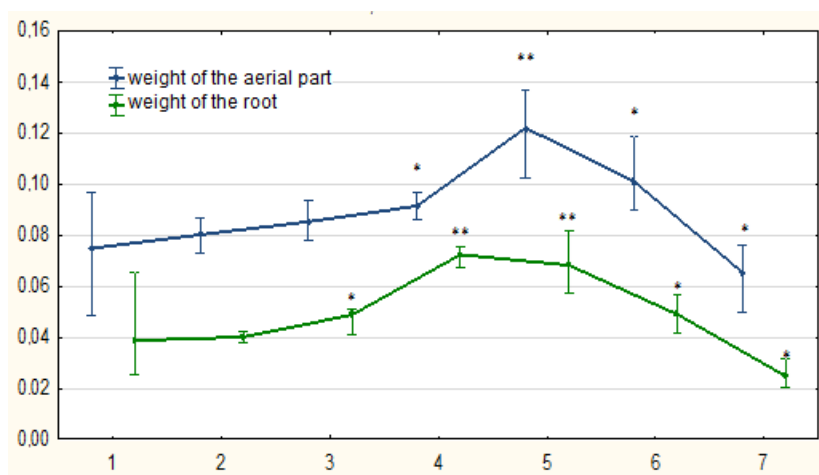


Fig. 3. Changes in weight indices of 7-day-old vetch seedlings on a gel-like polysaccharide medium with CoO nanoparticles (Note: * - significant differences compared to control ($p < 0.05$); ** - significant differences compared to control ($p \leq 0.01$)).

Analyzing the given data, it can be argued that the length of the aerial and underground parts of the rice seedling was lower than the control at all concentrations of oxide nanoparticles. 3-day-old seedlings were least inhibited by 10 g/ha CoO, and 7-day-old seedlings were least inhibited by 0.01 g/ha and 1.0 g/ha. The underground part of 3-day-old seedlings exceeded the control only at 1.0 g/ha concentration of TiO₂.

The weight indicators of 7-day-old rice seedlings were lower than the control in all variants. The weight of the aerial part was 15.5-20.36 % lower and that of the underground one was 13.6 - 26.0 % lower. Morphometric parameters of 3-day-old seedlings decreased by 20.3 % at low concentrations of CoO particles and became 44 % lower than the control at high concentrations.

Thus, cobalt and titanium oxides are not growth stimulants for either vetch or rice, but due to their energy characteristics, they increase morpho-physiological parameters at concentrations below 10 g/t and are not dangerous for plants. At higher concentrations, inhibition is possible (Table 2). The nature of the action of oxides on the viability of seeds and the development of seedlings is the same, and it may be determined by the same mechanism of action. Thus, metal and oxide nanoparticles differ in the mechanism of their action on growth and development, in particular, due to biocompatibility.

Factors affecting the possibility of accumulation of nanoparticles in plants.

Predicting the possibility of accumulation of nanoparticles is based on the possibility and degree of dissolution of solid nanoparticles in water and biological fluids. This characteristic determines their migration in plant tissues and organs [17] and depends on the influence of the environment on the solubility of nanoparticles.

The solubility of cobalt oxide in water is up to 10 wt.% and it is up to 22 wt.% in saline. The nanosized titanium dioxide and 35-60 nm metal particles did not show solubility in water and phosphate buffer. While particles with a size of 18-20 nm had some solubility (wt.%), increasing with time and reaching the following data after 24 hours in water: Co - 11.6, Fe - 12.1, and 11.8, 12.8 in saline, respectively. As previously shown, nanoparticles of 20 nm and below are hyperactive at low concentrations and are dangerous for both plants and animals [18].

Application of electron microscopy for interstitial bioaccumulation of nanoparticles.

Transmission electron microscopy, carried out by means of electron microscopic analysis, revealed interstitial bioaccumulation of nanoparticles of cobalt oxide and titanium oxide in the form of aggregates 80–300 nm in size. Titanium oxide nanoparticles 25–60 nm in size showed the greatest tendency to bioaccumulation and the formation of dense agglomerates of complex shape with sizes up to 300 nm. Titanium oxide formed medium-sized, about 80 nm agglomerates [20-21]. If nanoparticles quickly aggregate, they are less dangerous for organisms than single nanoparticles, since an aggregate of nanoparticles with a diameter of more than 200 nm cannot enter the cell. The rate and dynamics of deposition of metal and metal oxides nanoparticles in water differ. Metal NPs are deposited much more slowly than titanium dioxide.

With the help of electron microscopy, data were obtained indicating the ability of nanoparticles to penetrate from the environment into plant tissues and accumulate in them, which can be recommended for use in practice by laboratories assessing the safety of nanotechnological products. In the medium with cobalt and titanium oxides, germinated samples of vetch revealed the accumulation of nanoparticles in the cell structure of tips, which apparently inhibits the development of plants. It is necessary to consider the data obtained, which reliably confirm that many particles, getting into plants, are not cytotoxic, that is, they do not lead to death or mutation of living cells. At the same time, these nanoparticles do not participate in chemical reactions in cells, which leads to accumulation of unchanged nanoparticles. However, accumulating in this way and eventually getting into

the human body together with plants, nanoparticles can lead to disorders in the immune system [22-28].

Based on the data of elemental analysis of the distribution of metals and electron microscopic studies, evidence was obtained that iron and cobalt do not accumulate in the considered plants. Metal NPs can be considered as biocompatible structures and sources of additional energy.

4 Conclusion

The cell surface is covered with many receptors that can form bonds with various ligands outside the cell, including nanoparticles, and transmit a signal into the cell space. Such a signal can trigger various biochemical reactions, and can also lead to internalization of nanoparticles by receptor-mediated endocytosis. Probably, as a result of this process, the membrane receptors bind to corresponding proteins that nanoparticles use as transport, a vesicle is formed, and the subsequent release of particles inside the cell occur. Further, the particles that enter the cell are incorporated into metabolic processes, interacting with various organelles: lysosomes, the Golgi complex, mitochondria, and the endoplasmic reticulum. Thus, nanostructures have a resource of direct influence on the processes of growth and development of a plant.

Oxide NPs accumulate in the structure of plants, forming large agglomerates, although oxides are not cytotoxic, but they inhibit plant development. Metal nanoparticles do not form large aggregates, do not accumulate in the structure of plants, and convert their own and additional energy with an increase in the number of protons due to their high reducing ability. The transmembrane electrochemical potential, directly dependent on the number of protons, causes a proportional increase in the physiological parameters of the germination and development of plants and metal NPs with high biological activity and biocompatibility.

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