

ASSESSMENT OF THE CONTRIBUTION OF TITANIUM-CONTAINING NANOMATERIALS USING THE TEST SYSTEM OF *SECALE CEREALE L.* FOR SCREENING STUDIES OF POTENTIAL HAZARD

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Introduction. Doping is a widely used method of modifying nanoparticles to enhance their electrical, optical, and biological activity. Doping with heavy metals can increase the initial toxicity of the nanomaterial, and therefore negatively affect the health of workers and cause pollution of environmental objects.

The aim of the research – to assess the potential toxic effect of additional doping of titanium-containing nanopowders with heavy metals (silver) on the plant test system *Secale cereale L.*

Materials and methods of research. In a model laboratory experiment, the phytotoxicity of titanium dioxide doped with silver (nanocomposite $\text{TiO}_2 + \text{Ag}$, mass fraction $\text{Ag} \sim 4\%$), titanium dioxide complex doped with silver (nanocomposite $\text{TiO}_2 + \text{Ag}$, mass fraction $\text{Ag} \sim 8\%$) and nanopowder of titanium dioxide (TiO_2), synthesized by the method of thermal decomposition in a glucose-citrate buffer (1 glucose: 4 sodium citrate) using the example of *Secale cereale L.* (rye) seeds.

Results. The determination of morphometric (biometric) indicators of the test object (*Secale cereale L.*) demonstrated that the growth processes of the studied plants were inhibited in almost all variants with nanomaterials.

Conclusions. It was established that the growth processes of the studied plants are inhibited in almost all variants with titanium-containing nanomaterials. Under the exposure to titanium dioxide and nanocomposite $\text{TiO}_2 + \text{Ag}$ (4.0 %) there is a decrease in the value of the test function in the experiment in comparison with the control in the range from low to medium when used as a test object as seed rye seedlings, and bovine spermatozoa.

Key words: titanium-containing nanomaterials, doping, phytotoxicity

Introduction

During the last decade, considerable scientific attention has been focused on the unique properties of nanomaterials resulting from quantum size confinement and surface effects. In particular, the limitation of the quantum size affects the energy structure and physical properties of doped (alloyed) nanomaterials and nanodevices. In turn, doping of nanomaterials provides a flexible way to tune material properties while maintaining high surface areas. Doped nanomaterials are expected to make a significant contribution to nanotechnology for practical applications in the fields of

electronics, photonics, optics, medical sciences, homeland security, etc. [1]. Doping of various materials, especially semiconductors, is known as a powerful tool for improving properties and finding appropriate applications in industry, in particular, in nanoelectronics and nanophotonics [2]. Luminescence phenomena are actively studied in doped nanoparticles for the purpose of radiation detection, in particular, in infrared detection and dosimetry. Near-infrared luminescent nanoparticles are particularly promising for biological imaging, as higher resolution imaging can be obtained using autofluorescence. Doped

insulator nanomaterials, including carbon nanotubes, represent a new type of highly efficient diagnostic luminescent material. As a new type of biological markers, insulator nanoparticles are less toxic than semiconductor nanoparticles and are promising for the detection, diagnosis, and treatment of cancer.

Therefore, doping is a widely used method of modifying nanoparticles to enhance their electrical, optical, and biological activity [3]. For example, lanthanide-doped nanoparticles have been developed as a new class of luminescent nanomaterials. Unlike conventional bulk phosphors, nanoparticles provide a convenient platform for tuning optical emission and facilitate integration with other functional groups such as biological molecules. Therefore, such materials are very promising in bio- and information technologies, energy, etc. Impurities of doping (alloying) substances, such as Cu^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , rare-earth and transition elements, play an important role in changing the electronic structure, in particular, in terms of modulation capabilities of the base material. Doping can also enhance the antimicrobial effect [4]. In particular, silver doped with polymeric chitosan and iron oxide provides high antimicrobial efficiency against *E. coli*, *B. subtilis* and *S. aureus* bacteria. In turn, titanium dioxide (TiO_2) doped with silver and nitrogen can increase the antibacterial properties of TiO_2 nanoparticles against *E. coli* and *B. subtilis* when irradiated with fluorescent light after cultivation for 24 hours. In another study, Ag-doped TiO_2 nanoparticles were also toxic to *E. coli* bacteria. The TiO_2 +Ag composite coating showed complete eradication of methicillin-resistant *S. aureus* within 24 hours under all cultivation conditions.

Thus, doped titanium-containing nanomaterials, in particular, silver-doped titanium dioxide

nanopowder, can contribute to the destruction of pathogenic microorganisms and be used in disinfectants. At the same time, it should be noted that doping with heavy metals can increase the initial toxicity of the nanomaterial, and therefore cause environmental pollution and negatively affect the health of workers. It should also be noted that data from a limited range of studies show that some nanomaterials can have ecotoxicological effects [5]. The toxicological behavior of nanomaterials in direct contact with cells depends on their chemical composition, amount, solubility, shape, area and charge. Effects may depend on how long the nanoparticles remain intact and how they can accumulate in the biosystem. Impurities formed during the production of nanomaterials also affect their toxicity.

Simultaneously, much like the initial stages of nanotechnology, the advancement and integration of novel materials outpaces the progress of biomedical research concerning the potential hazardous implications of nanomaterials on human health and environmental ecosystems.

Nanoparticles of titanium dioxide are used in the production of a wide range of products, which can ultimately create an additional burden on ecosystems. It is also known that skin exposed to ultraviolet light can be a significant target for photosensitized damage by commercial TiO_2 nanoproducts at concentrations of 0,2–3,0 mg/ml [5].

Our own experimental studies established that TiO_2 +Ag nanocomposite (mass fraction of Ag ~ 4 % by weight) and TiO_2 nanopowder in concentrations of 3 mg/ml initiate pathological changes in spermatozoa of cattle (bull), which are markers of oxidative stress (head, middle part and tail abnormalities, as well as the absence of acrosome, etc.), while the pathological effect of TiO_2 +Ag nanocomposite (4 %) is more pronounced [6].

Experimental studies in vitro showed that under the influence of TiO_2 at a concentration of 30 $\mu\text{g}/\text{ml}$ on peripheral blood mononuclear cells, we observed a statistically significant increase in IL-1 production. Instead, TiO_2+Ag in concentrations of 30 $\mu\text{g}/\text{ml}$ is able to increase the functional activity of peripheral blood mononuclear cells by the production of pro-inflammatory cytokines IL-1, IL-6, $\text{TNF-}\alpha$ and IL-4 production in volunteer donors, which indicates a potential effect on the formation of chronic inflammation and allergic reactions in the corresponding category of nanoproduction workers [7]. The results of the study of the effect of titanium-containing nanomaterials on beneficial entomofauna – honey bees (*Apis mellifera*) – are of interest, since it is believed that a person who used bee products (pollen, propolis, etc) can be affected by nanoparticles. The LC_{50} values evaluated for 96 hours were 5,865 mg/L for TiO_2 and 312,845 mg/L for TiO_2+Ag . The concentration group that had the greatest effect on mortality was 100 mg/L for TiO_2 , compared to 10 mg/L for TiO_2+Ag . The toxic effect of TiO_2 and TiO_2+Ag nanoparticles increased with increasing concentration and exposure time [8].

Obviously, today the potential impact of metal-containing nanoparticles on the biota of ecosystems requires detailed study.

The attention of scientists is focused on the need to use a complex approach in the issue of assessing the potential toxicity of nanoparticles in relation to higher plants, but such data are variable and fragmentary in nature. Determination of phytotoxicity of doped titanium-containing nanomaterials requires in-depth analysis, especially in the context of the mechanisms of their interaction with the plant organism. Due to their small size, nanoparticles may not be recognized by the body's defense systems, they are not subject to biotransformation

and are not excreted from the body. Therefore, there is a tendency to accumulate nanomaterials in both plant and animal organisms, as well as micro-organisms, transmission along the food chain, which increases the probability of their entry into the human body.

The purpose of research was to assess the potential toxic effect of additional doping of nanopowders with heavy metals (silver) on the plant test system *Secale cereale L.*

Materials and methods of research

The research used nanomaterials synthesized at the I. M. Frantsevich Institute of Problems of Materials Science of the National Academy of Sciences of Ukraine:

- nanopowder of titanium oxide (TiO_2), synthesized by the method of thermal decomposition;
- titanium oxide complex doped with silver (TiO_2+Ag nanocomposite, mass fraction of Ag ~ 4 % by mass);
- titanium dioxide complex doped with silver (TiO_2+Ag nanocomposite, mass fraction of Ag ~ 8 %).

According to the developers, the TiO_2 nanopowder has a mesoporous structure (mesopores – pores from 2 to 50 nm), contains soft conglomerates ranging in size from 50 nm to 500 nm. The average aerodynamic diameter of TiO_2+Ag (4 %) nanocomposite particles, which was determined by dynamic light scattering using the Analysette 12 DynaSizer (Fritsch, Germany), was (48.65 ± 1.08) nm, while the average aerodynamic diameter of TiO_2+Ag (8 %) was about 80,8 nm.

Secale cereale L. seeds were selected as a plant test system. *Secale cereale L.* seeds were exposed to the investigated nanomaterials in a glucose-citrate buffer at a ratio of 1:4 in a concentration of

3 mg/ml of distilled water. Control plants were grown on distilled water. On the 4th day (96 hours), a morphometric analysis of the seedlings of the experimental plants was carried out (40 plant seeds, the experiment was repeated three times).

The phytotoxic effect was determined by the criteria of changes in morphometric (biometric) indicators of the test culture (length of shoots and roots, mass of experimental plants).

The phytotoxic effect (PE) based on the change in the mass of seedlings of experimental and control plants was calculated according to the formula:

$$PE = M_0 - M_x/M_0 \cdot 100, \quad (1)$$

where M_0 is the average weight of seedlings in the control version;

M_x is the average weight of seedlings of the experimental variant.

The phytotoxic effect based on the change in the length of the shoots of experimental and control plants was calculated according to the formula:

$$PE = L_0 - L_x/L_0 \cdot 100, \quad (2)$$

where L_0 is the average length of the plant grown in the control version;

L_x is the average length of plants grown in the experimental version.

The phytotoxic effect based on the change in the length of the roots of experimental and control plants was calculated according to the formula:

$$PE = R_0 - R_x/R_0 \cdot 100, \quad (3)$$

where R_0 is the average length of plant roots in the control version;

R_x is the average length of plant roots in the experimental version.

Phytotoxic effect is considered as an indicator, which is analyzed as a manifestation of phytotoxicity (if $PE > 0$) or phytostimulation, if $PE < 0$. The

effect is considered proven if its value is more than 20.0 %, when fixing the indicator < 20 phytotoxicity is not manifested, 20–40 – weak phytotoxicity, 40–60 – medium phytotoxicity, > 60 – strong manifestation of phytotoxicity [9].

Based on the results of the experiment, the toxicity index of the factor (ITF) was calculated, which is estimated by the formula:

$$ITF = TF_e/TF_c, \quad (4)$$

where TF_e – the value of the registered test function in the experiment, TF_c – in the control [10].

Seeds of *Secale cereal L.* were exposed to the investigated nanomaterials at a concentration of 3 mg/ml of distilled water. On the 4th day (96 hours), the weight analysis of the seedlings of the experimental plants was carried out. For analysis, biological repetition was taken – 40 plant seeds, repetition in the experiment – three times.

Statistical analysis was carried out using the Statistica 8.0 program, data were calculated using MS Excel.

Results of the research and their discussion

The issue of the potential and versatile impact of nanomaterials on metabolic processes and the physiological state of the body as a whole remains key in fundamental and applied research on the interaction of nanoparticles with the biological environment. Information on the peculiarities of the interaction of doped metal-containing nanoparticles with higher plants (including the rhizosphere of grain crops) in the process of ontogenesis has fragmentary dynamics and needs to be expanded with modern, new knowledge.

For studies of phytotoxicity of nanomaterials, it is advisable to use nanopowders in physiological

solution, which can be applied during the first day. The use of a sterile solution of polysaccharides with sodium dinitrogen as a stabilizer of powdered titanium-containing nanomaterials is impractical due to rapid agglomeration of nanoparticles.

The results of the biometric analysis of test plants *Secale cereale* L. treated with the studied titanium-containing materials indicate that the growth processes of the studied plants were inhibited in almost all variants with nanomaterials (Table 1).

The seed similarity of *Secale cereale* L. under the action of titanium-containing compositions of nanomaterials did not significantly differ from the control variant (without treatment).

During the growth of *Secale cereale* L. treated with titanium dioxide, the length of the roots of the test plants decreased by 53.3 % relative to the control, and by 59.2 % in the version with TiO₂+Ag nanocomposite (4.0 %), respectively. The same regularity was observed in the length of the aerial mass (leaves) of plants. When growing seedlings after exposure to TiO₂+Ag (8.0 %), a decrease in morphometric parameters by 58,6 % (compared to the control) was recorded, with the exception of the root length indicator (close to the control – up to 10,5 mm).

The obtained experimental data made it possible to estimate the phytotoxic effect of the investigated titanium-containing materials, which ranged from weak to medium (Table 2).

Table 1

Biometric indicators of 4-day test plants *Secale cereale* L. under the action of titanium-containing nanomaterials (model laboratory experiment)

Options research	Seed similarity, %	Mass, g	% to the control	Length shoots, mm	% to the control	Length roots, mm	% to the control
Control	94.17 ± 5.20	2.57 ± 0.40	–	11.60 ± 2.73	–	10.03 ± 2.94	–
TiO ₂	92.50 ± 4.30	2.28 ± 0.20	88.7	8.21 ± 1.60	70.8	5.35 ± 1.80	53.3
TiO ₂ +Ag (4.0 %)	89.16 ± 1.40	2.10 ± 0.10	81.7	5.86 ± 2.24	50.5	5.94 ± 4.10	59.2
TiO ₂ +Ag (8.0%)	95.80 ± 2.90	2.27 ± 0.30	88.3	6.80 ± 0.50	58.6	10.53 ± 4.70	105.0

Table 2

Phytotoxic effect during pre-sowing treatment of *Secale cereale* L. seeds with titanium-containing nanomaterials (model experiment, 96 hours)

Options research	Phytotoxic effect (PE), %			Level of phytotoxicity
	by the length of the above-ground part	by the length of the root system	by the mass of seedlings	
Control (without treatment)	–	–	–	–
Nanopowder TiO ₂	29.2	46.7	11.3	Weak
Nanocomposite TiO ₂ +Ag (4.0 %)	49.5	40.1	18.3	Weak
Nanocomposite TiO ₂ +Ag (8.0 %)	41.2	-4.5	24.1	Medium

Phytotoxic effect during pre-sowing treatment of *Secale cereale* L. seeds with titanium-containing nanomaterials (model experiment, 96 hours)

The analysis of the works of scientists on the assessment of the toxicity of solutions of metal-containing nanomaterials and our studies of phytotoxicity through the determination of the factor toxicity index (by the effect of nanomaterials) using *Secale cereale* L., indicate the specific properties of nanopowders additionally doped with heavy metals and the corresponding changes in the value of test functions in a laboratory experiment. The calculated factor toxicity index on the morphometric indicators of *Secale cereale* L. is presented in the Table 3.

These data are consistent with the above calculations regarding the fluctuation of phytotoxicity of the studied nanomaterials from weak to medium level. Thus, the ITF for the mass of seedlings of *Secale cereale* L. for titanium dioxide was 0.9 (low toxicity, IV), for the length of the roots – 0.5 (medium, III), and for the length of the shoots – 0.7, which indicates low toxicity. In turn, ITF for the mass of seedlings of *Secale cereale* L. for TiO₂+Ag (4.0 %) was 0.8 for the mass of seedlings (low toxicity, IV), for the length of roots and seedlings – 0.6 and 0.5, respectively (medium toxicity, III).

The obtained results of a comparative analysis of the potential danger of doped titanium-containing nanomaterials using different test objects (bovine spermatozoa as a marker of oxidative stress) revealed a significant number of residual bodies

after exposure of bull spermatozoa to the TiO₂+Ag nanocomposite, which is the result of cell death. In addition, it was established that the specified changes, along with the detected abnormalities of spermatozoa, testify in favor of a more pronounced pathological effect of TiO₂+Ag nanocomposite compared to TiO₂ nanoparticles. In this sense, the comparison of ITF data (relationships of additional doping and biological action of titanium-containing nanomaterials), which are calculated both for the plant test system *Secale cereale* L. and for the animal test system – bovine spermatozoa, is of some interest in this sense (Table 4).

As a result of the research, the estimated value of ITF by induction with titanium dioxide nanopowder (due to the presence of morphological abnormalities of bull spermatozoa) was established, which was 0.76 (low toxicity, IV). ITF for TiO₂+Ag nanocomposite (4.0 %), this index did not exceed 0.5, which corresponds to the average value of toxicity.

Hence, it is imperative to conduct a comprehensive investigation into titanium-containing nanomaterials to enrich our understanding of their phytotoxicity and to mitigate potential risks and adverse repercussions associated with their utilization. The outcomes of the research have unveiled that both titanium dioxide and TiO₂+Ag nanocomposite (at 4.0 % concentration) have led to a reduction in the test function value, as compared to the control group, manifesting a spectrum ranging from low to medium impact when assessed with the

Table 3

Toxicity index of the factor under the conditions of action of nanomaterials (test system *Secale cereale* L.)

Nanomaterial	Toxicity index of the factor (by the mass of seedlings)	Toxicity index of the factor (by the length of the roots)	Toxicity index of the factor (by the length of the seedlings)
Nanopowder TiO ₂	0.9 (low, IV)	0.5 (medium, III)	0.7 (low, IV)
Nanocomposite TiO ₂ +Ag (4.0 %)	0.8 (low, IV)	0.6 (medium, III)	0.5 (medium, III)

Table 4

**Toxicity index of the factor under the conditions of action of nanomaterials
(test system – bovine spermatozoa)**

Nanomaterial	Toxicity index of the factor (according to the proportion of normal spermatozoa)	Toxicity index of the factor (by the transmission coefficient)
Nanopowder TiO ₂	0.76 (low toxicity, IV)	–
Nanocomposite TiO ₂ +Ag (4.0 %)	0.5 (medium, III)	0.6 (medium, III)

Secale cereale L. test system. Moreover, delving into prior studies concerning doped titanium-containing nanomaterials using an animal test system, specifically cattle spermatozoa, reveals discernible indications of the initiation of pathological changes. This becomes evident in terms of the increased occurrence of morphological abnormalities within spermatozoa subjected to these studied materials. Further, a decrease in the test function value within the experimental group, in comparison with the control, is observed, varying from low to medium in its magnitude.

Conclusions

1. Based on the determination of the morphometric (biometric) indicators of the test object rye seed (*Secale cereale L.*) in a model laboratory experiment, it was established that the growth processes of the studied plants were inhibited in almost all variants with titanium-containing nanomaterials.

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2. The similarity of rye seeds sown under the action of titanium-containing compositions of nanomaterials did not significantly differ from the control option (without treatment). During the growth of *Secale cereale L.* treated with titanium dioxide, the length of the roots of the test plants decreased by 53.3 % relative to the control, and by 59.2 % in the variant with TiO₂+Ag nanocomposite (4.0 %), respectively. The same regularity was observed in the length of the aerial mass (shoots) of plants. When growing seedlings exposed to TiO₂+Ag (8.0 %), morphometric parameters decreased by 58.6 % (compared to the control), with the exception of the root length indicator (close to the control – up to 10.5 mm).

3. The research findings revealed that when subjected to titanium dioxide and TiO₂+Ag nanocomposite (4.0 %), a decrease in the test function value was observed within the experimental setups involving both rye seedlings and cattle spermatozoa.

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