The possibility of using biopreparations based on nanoparticles of biogenic metals in crop production and plant protection

D Churilov, S Polischuk, G Churilov, A Shemyakin, V Churilova, K Andreev, I Arapov and I Obidina

1Ryazan State Agrotechnological University named after P A Kostychev, 1 Kostychev Street, Ryazan 390044, Russian Federation
2Ryazan State Medical University Named after Academician I P Pavlov, 9 Vysokovoltnaya Street, Ryazan 390026, Russian Federation

1E-mail: churilov.dmitry@yandex.ru

Abstract. Modern agricultural production requires innovative technologies. For a labile technological process of grain growing the following things are necessary: precise (coordinate) farming, crops protection in the form of controlled coexistence with undesirable life forms and a single pre-treatment with biopreparations based on biogenic metal nanoparticles. Physical-chemical properties of nanoparticles contribute to biocompatibility and biological activity when interacting with wheat and other crops. The paper shows relationship between the size of metal nanoparticles and their effect on wheat seedlings at concentrations of 0.001-1.000 g/t. The activity of superoxide dismutase and catalase in the crops significantly decreased when maximum morpho-physiological parameters and increased sharply at concentrations above 100 g/t and 10 g/t, respectively, for nanoparticles sized 35-60 nm and up to 20 nm. For nanoparticles sized 35-60 nm the stability limit was the concentration of nanoparticles much higher than 100 g/t, and for those up to 20 nm it was 0.001-10 g/t. The content of hormone indole acetic acid increased under the action of nanoparticles, which increased energy efficiency of plant respiration, activity of enzymatic reactions and wheat yield and grain quality when strengthening the seedlings’ viability, reducing the effect of weeds to minimum.

1. Introduction

Precision or coordinate farming is a systematic approach to crop management, based on the use of computer and satellite technologies, taking into account various conditions in different parts of the field [1]. Knowing the condition of the soil, the seeds characteristics and the plan for the production of grain crops, it is possible to increase the gross yield, reduce costs, obtain high-quality products, and improve the physical and chemical properties of the soil by using nano biopreparations. Adjusting the concentrations of nanoparticles and soil analysis make possible to extend the seed treatment by order of producers in any conditions, on different soils, for different varieties and types of cereals and other crops. On the basis of Ryazan State Agrotechnological University Named after P A Kostychev (RSATU) and Ryazan State Medical University Named after I P Pavlov of the Ministry of Health of Russia (RSMU) a great reserve in the field of technologies for increasing the yield of agricultural plants has been created and their influence on the realization of the genetic potential of varieties and hybrids of agricultural crops has been proved [2-4]. So far, the results of the effect of 25 nanoparticles...
of various physical-chemical properties on 35 agricultural and 5 medicinal plants growing in different climatic regions and on various soils have been obtained.

The action of nanoparticles and the concentration range of their activity depends on the method of production, the size of the structure and the composition. Works [5,6] showed that metal oxides accumulate in plants and inhibit their growth and development, while metal nanoparticles at the same concentrations and size range improve the morpho-physiological and biochemical parameters of plants without accumulating or polluting the soil [7]. For biologically active metal nanoparticles, one of the characteristic properties of the effect of low doses is the oscillatory nature of the dose dependence of the magnitude of the observed parameters, as well as the presence of a "dead zone" (a dose interval between two peaks of biological effects in which biological activity does not occur [8], which is due to the wave character of the propagation of spatial rearrangements of the permeability of membranes and supramolecular structures under the influence of the energy effect of nanoparticles increasing the concentration of protons. Given that nanoparticles are an additional source of protons and information that cells receive from high-energy nanoparticles through membrane or cytoplasmic receptors, this should lead to a change in hormonal status in cells, stimulate or suppress the activity of certain endogenous phytohormones. And the direction of information depends on the size, composition and concentration of nanoparticles. Biologically active nanoparticles, acting on the level of phytohormones, can affect the state of bio-membranes and indirectly act as a regulator of protein synthesizing plant cells. In turn, changes in the structure of membranes can lead to a change in the functional state of the cell, and the presence of poly-modality in the response can be explained by a change in the mechanism of action of the substance depending on the structure, chemical composition and concentration.

It is assumed that nanocrystalline metals have great potential in the field of mineral nutrition and energy exposure and due to uncompensated bonds they easily form complex compounds with organic substances. As a result, they activate the synthesis of various enzymes affecting carbohydrate and nitrogen metabolism, amino acid synthesis, photosynthesis and cellular respiration [9-11], which allows the use of metal nanoparticles as trace elements and growth stimulants with a prolonged effect. At the same time, nanoparticles in contact with living systems for a long time can move between organisms of different trophic levels, undergoing decomposition and accumulation in the food chain, so the question of their effect on living systems remains relevant.

2. Experimental Part

2.1. Laboratory studies of testing the effect of nanoparticles on wheat seeds

Laboratory studies were conducted on the basis of the Center for Nanomaterials and Nanotechnologies for the Agro-Industrial Complex of the Russian Federation at RSATU. Certified white mustard and wheat seeds, one year of harvest, corresponding to class 1 and not treated with disinfectants were used in the experiment. The following morpho-physiological parameters of seed growth and development were determined: germination energy and laboratory germination on the 3rd day; the length and mass of aerial and underground sprouts on the 7th day according to [12,13]. When germinating seeds, the liquid cultivation medium was used as a substrate. The replication was four-fold. Each Petri dish contained 50 seeds.

Suspensions of nanoparticles of various concentrations were prepared based on distilled water. Weight samples were weighed using a Vibri HT analytical balance (Shinko Denshi, Japan, accuracy ± 0.0001 g), poured into a 1 liter pre-prepared container of water and mixed with a glass rod for 20 seconds. Then they were dispersed using ultrasound for 5 minutes at power of 300 W and frequency of 23.740 kHz according to the developed sample preparation technique. The resulting suspension was added to a Petri dish filled with 20 ml of non-solidified polysaccharide substrate and mixed thoroughly. To study the biological activity, the effect of nanoparticles in the concentration range of 0.001-1.000 g/t of wheat seeds was investigated.
2.2. The technique to evaluate the bio-availability of nanoparticles in sprouts using electron microscopy

The maximum resolution of electron microscopy is achieved during the study of metals and crystal lattices. In the case of biological objects, their low contrast becomes an obstacle for research [10]. An increase in the accelerating voltage leads to a rapid degradation of biological objects, since their macromolecules are rather unstable.

The following method was developed for preparing samples for electron microscopic studies. Nanoparticles of iron, copper and cobalt were added to the biologically active medium. In this environment, wheat seeds were germinated for 10 days. As a control, seeds were also germinated without any nanoparticles. Seedlings were taken on the 5th and 7th day and subjected to fixation for analysis by electron microscopy. Plant seedlings were washed in deionized water and then the samples were frozen with liquid nitrogen. The frozen samples were mechanically crushed in a mortar, and the choice of material for the mortar and pestle should be due to the absence of surface defects, which determines the minimum contamination of the sample. Ultrasonic treatment was used for the final grinding of the samples. To do this, the crushed biological material was placed in a 30 ml medium containing ethanol and dispersed using an ultrasonic homogenizer for 5 minutes at a power of 400 watts. The resulting suspension was selected using a 5 ml sterile injection syringe and applied to the copper lattice with a carbon conductive film, followed by electron microscopic studies using standard methods.

2.3. Methods to determine the activity of enzymes: peroxidase, superoxide dismutase, catalase and phytohormones in plant tissues of white mustard sprouts

a) The method for determining the activity of peroxidase is based on the formation of colored products during the oxidation of benzidine under the action of an enzyme contained in plants, till the formation of a blue-colored oxidation product (n, n’-diamine-diphenylquinone) [11,12].

b) Determination of superoxide dismutase activity (SOD) was performed by the method proposed by V A Kostyuk [13], with a modification for plants.

c) Catalase activity was determined by estimating the rate of decomposition of hydrogen peroxide by the catalase of the test sample [11]. Catalase activity (C) was determined in relative units per gram of dry weight.

After extraction of the plant homogenate, ethyl acetate extracts were mixed, evaporated and the resulting residue was left to determine abscisic (ABA), indolylacetic (IAA) and gibberellic (GA) acids. After extraction, a 20% potassium hydroxide solution was added to the aqueous layer to create a pH of 8, after which it was extracted several more times with butanol saturated with water for 40 min at 200 rpm. The resulting butanol fraction was evaporated to dryness and cytokinins were determined in the residue. For analysis, liquid chromatography was used. The retention time was 12 min and the flow rate was 0.5 ml/min. A 40% methanol solution was selected as the mobile phase.

2.4. Small plot field studies

The studies were carried out at the Agrotechnological Station of RSATU, Stenkino Village in Ryazan District of Ryazan Region. The farm land is located in the zone of dark gray forest soils. Experimental plots were located on gray forest soils, the most common in the southern non-chernozem region. The humus content in the topsoil was from 2.4 to 3.8%. The reaction of the soil solution was medium and slightly acid (pH – 4.8-5.4). The provision of mobile phosphorus was 13.5-15.1 mg/100 g of soil and that of potassium was 12.1-12.9 mg/100 g of soil. The density of the soil was 1.1-1.2 g/cm. The territory of the station was located in the temperate continental zone. The average annual air temperature was +4.3 °C. In general, the weather conditions of 2017 favored the growth and development of experimental crops. The object of the study was wheat.
3. Results and discussion

3.1. Laboratory studies

According to the results of laboratory and field studies of the growth and development of wheat seeds when treating with suspensions of nanoparticles of various sizes (20-100 nm), concentrations and physical-chemical properties, the advantages of the preparations were determined:
- chemical interaction of nanoparticles with a liquid medium is one of the determining factors in stimulating the development of plants, which makes it possible to use nanoparticles both as micronutrients and as stimulants for the growth and development of grain crops;
- due to the large specific surface area (Table 1), they easily form various compounds with organic substances, as a result of which various enzymes affecting carbohydrate and amino acid synthesis, photosynthesis and cell respiration are synthesized and activated. Then it causes:
  a) the yield increase by 15-20%;
  b) the implementation of the genetic orientation of various varieties and plant hybrids;
  c) enhancing the biosynthesis of biologically active compounds (vitamins, protein, polysaccharides) up to 20% without additional chemical effects (patent);
  d) improvement of the quality of crop production (the content of gluten in the grain of wheat increases by 8-10%).

It is known that the biological activity of metal nanoparticles is related to their physical-chemical characteristics. Such parameters as the particle size and shape, and the phase state affect the biological activity of metal nanoparticles.

The used NPs of Fe, Co and Cu are single-crystal structures of a round regular shape (Table 1). Metal nanoparticles have a complex structure, which is characterized by a metal core and an oxide film on the surface of the particles, which is formed as a result of the passivation of particles with air to reduce the nanoparticles pyroforma.

| NP  | Size, nm | Crystalline content, % | metal form | Oxide content, % | Specific surface, m²/g |
|-----|----------|------------------------|------------|------------------|------------------------|
| Fe  | 56.0±0.9 | 6                      | Fe₂O₃      | 94               | 45.7                   |
| Cu  | 65.0±1.2 | 58                     | Cu₂O, CuO  | 36.6             | 36.5                   |
| Co  | 51.0±2.1 | 12                     | Co₁O₄, Co₁O | 67.21            | 52.1                   |

When conducting laboratory studies, it was established that for nanoparticles of iron, copper and cobalt obtained by chemical means with particle sizes of 35-60 nm, the biological activity and the effect of "small doses" are most pronounced. Active concentrations for germination energy were 0.5 and 5.0 g/t (ton of seeds); those for the length of the root were 0.1 and 1.0 g/t, those for the mass of the 3-day sprout were 0.05 and 5.0 g/t; those for the mass of the 7-day sprout were 0.1 and 1.0 g/t and those for the mass of the root were 0.5 g/t. Moreover, the concentrations differed from each other most often in 10, less often in 100 times. At a concentration above 100 g/t, the dose-effect dependence ceased to appear, but all parameters: germination energy (figure 1a), root length (figure 2), sprout length (figure 4a) remained significantly above the control, that is, there was no depression even at a concentration of 500 g/t. When determining the effect of metal nanoparticles of Cu, Co, Fe, Zn on the growth and development of plants: vetch, mustard, cucumber, and wheat. It was proved that they show high biological activity in the concentration range of 0.01-100 g/t (ton of seeds), showing the same pattern. The amount of activity depends on the specific surface area and size.

Nanoparticles with a particle size of up to 20 nm demonstrated the effect of LD only at low concentrations of up to 10 g/t and at a concentration of 100 g/t all parameters: germination energy (figure 1b), root length (figure 3), sprout length (figure 4b) were below the control and some inhibition of plant development was observed (figures 1,3,4). Nanoparticles with a size below 20 nm are dangerous to be used in bio-preparations, which is associated with their high reactivity. Such nanoparticles exhibit high physical-chemical activity and at the same time lose their individuality.
Figure 1. The effect of nanoparticles on white mustard seeds.

Figure 2. The length of the wheat roots with nanoparticles size of 35-60 nm.

Figure 3. The length of the wheat roots with nanoparticles size of up to 20 nm.

Figure 4. The length of wheat sprouts.

During the research, the most important characteristics of metal nanoparticles (Table 2), affecting the bio-compatibility and, as a consequence, the biological activity of nanoparticles were identified. The physical-chemical activity of nanoparticles depends on the composition and morphology of the
particles, the thickness and composition of the oxide film on the surface, as well as the method of obtaining [13].

Table 2. Characteristics of nanoparticles.

|                      | Cu  | Co  | Fe  | Zn  |
|----------------------|-----|-----|-----|-----|
| Particle size in one  | 14-63| 10-46| 20-54| 18-66|
| dimension, nm        |     |     |     |     |
| Form factor (ratio of| <10 | <10 | <10 | <10 |
| maximum size to      |     |     |     |     |
| minimum one)        |     |     |     |     |
| Solubility in water, | Insoluble | Insoluble | Insoluble | Insoluble |
| wt. %                |     |     |     |     |
| Solubility in        | <1  | <1  | Insoluble | Insoluble |
| biological fluid     |     |     |     |     |
| (extraction buffer), |     |     |     |     |
| wt. %                |     |     |     |     |
| Charge               | +   | +   | +   | +   |
| Aggregation resistance | Low | Low | Low | Low |
| Hydrophobic nature   | detected by electron microscopic analysis | detected by electron microscopic analysis | detected by electron microscopic analysis | detected by electron microscopic analysis |
| Adhesion to plant surfaces | + | + | + | + |

Nanoparticles up to 20 nm in size have approximately the same, but higher solubility, increasing over time (Table 3).

Table 3. Characteristics of nanoparticles with a size of 10-20 nm: solubility, wt.%

| Solvent            | Time, h | Cu  | Fe  | Zn  | Co  |
|---------------------|---------|-----|-----|-----|-----|
| Water               | 5       | 1.5 | 2.6 | 2.0 | 2.3 |
|                     | 24      | 8.9 | 12.1| 10.7| 11.6|
| Extraction buffer   | 5       | 0.8 | 1.6 | 0.96| 1.4 |
| pH 7.8              | 24      | 9.7 | 12.8| 11.8| 12.2|

Electron-microscopic studies performed on a scanning electron microscope and analysis of the distribution of metals in tissues of experimental plants for sprout homogenate samples exposed to metal nanoparticles 35-60 nm in size made it possible to show peaks characteristic of metals on the diagrams. In the ratio of the content of the remaining elements, no deviations from the control values were recorded. Their accumulation on the surface of cells of the aerial and underground parts was not found. An additional elemental analysis has not shown any authentic accumulation of nanoparticles in plant tissues as compared with the control, for all metals with a size of 20–60 nm to a concentration of 1000 g/t. This is important for poorly soluble metal nanoparticles. Therefore, the possible negative effects can be associated only with the individual effects of nanoparticles, and not with the accumulation of heavy metals in plants and soil.

For nanoparticle suspensions, a change in pH was observed depending on the composition and size of the nanoparticles. We assume a direct relationship between the increase in the number of protons and the energy produced in the cells, which is used for seed germination. The activity of enzymes, the transport of substrates through membranes and the direction of enzymatic reactions depend on pH in the cell [14-17]. The relationship of hydrogen ions and energy produced in cells and used for germination is confirmed by recent research in the field of enzymes and ours in the framework of this project. The mechanisms of dissolution of the iron group metals are studied in detail from which it follows that the main role is played by adsorbed water molecules that can facilitate the ionization of the metal. In this case, a surface complex with charge transfer is formed [18]. Moreover, the lower the
concentration, the higher the availability of surface iron atoms for interaction with water. A similar dependence is observed for cobalt and copper nanoparticles, which can explain their high biological activity at low concentrations. A comprehensive study of the effect of different concentrations and sizes of nanoparticles on the activity of three antioxidant enzymes (SOD, catalase and peroxidase) made it possible to establish that the peroxidase activity at maximum morpho-physiological parameters was high and decreased in “dead zones” at both low and high concentrations of metal nanoparticles. The activity of SOD and catalase in the studied cultures significantly decreased under the conditions of maximum morpho-physiological parameters and sharply increased at concentrations greater than 100 g/t for nanoparticles 35-60 nm in size and above 10 g/t with a size of up to 20 nm. For nanoparticles with a size of 35-60 nm, the stability limit was the concentration of metal nanoparticles much higher than 100 g/t, for sizes up to 20 nm in the range of 10 g/t.

In the concentration range of 0.001-100.0 g/t, the content of the hormone of indolylacetic acid (IAA) increases under the action of nanoparticles, which leads to an increase in the energy efficiency of plant respiration [19-21] and to large changes in the activity of various enzymatic reactions. Further, the values of IAA decrease, approaching the control parameters. The increase in the content of gibberellinic and abscisic acid remained to a concentration of 100 g/t, but at higher concentrations of metal nanoparticles, the values remained at the control level.

On the basis of the research, the bio-compatibility of iron, copper and cobalt nanoparticles up to 60 nm in size has been determined, which can be explained by such factors as: high adhesion to the surface of plants; the size of 10-60 nm; pH shift of nanoparticle suspensions; increasing the number of protons, an additional source of energy for the cell; increasing the activity of enzymes and phytohormones.

3.2. Field studies.
Field tests confirm the bio-compatibility of metal nanoparticles with a size of 35-60 nm and their high biological activity. Moreover, they indirectly have some protective effect. During field tests, it was noted that a single pre-treatment of wheat seeds with suspensions of Fe, Co, and Cu nanoparticles at a concentration of 0.1 g/t, increasing the yield and viability of the first sprouts, minimized the effect of weeds (figure 4).

Such parameters as the density of planting and photosynthetic parameters increased. The use of nanoparticles contributed to the formation of a powerful and actively working leaf apparatus, making possible to increase the amount of accumulating energy, which was a good prerequisite for the growth of crop productivity. Moreover, if the maximum yield increase from the action of herbicides was obtained in wet and moderately hot years, then in 2009 (a hot dry year) their influence was insignificant, whereas nanoparticles on the contrary showed the maximum yield gain in different periods. The genetic potential of plants is also being realized, and the quality of wheat is increasing.

After harvesting, the mass fraction of raw gluten exceeded the control in 2016 in the variants with iron and copper nanoparticles (by 4.02% and by 4.43%, respectively), in 2018 the gluten content was higher than the control in all experimental variants: using iron nanoparticles – by 5.61%, copper nanoparticles – by 8.24% and cobalt nanoparticles – by 4.95%. Wheat was assigned to quality group II, for copper nanoparticles at a concentration of 1.0 g/ha and to quality group I for cobalt at 0.5 g/ha.

4. Conclusion
Under exacerbating environmental problems, domestic highly effective, biologically active, non-toxic growth stimulants of broad activity are offered. These are nanoparticles with the ability to protect seedlings, get high yields and high-quality seeds.

According to the results of the experiment, it can be concluded that the size of the nanoparticles has a very large effect both on their biological activity and on the effect of low doses. The chemical interaction of nanoparticles with a liquid medium is one of the determining factors of their biological activity. A change in the pH of the medium due to the high reducing ability of nanoparticles...
apparently increases the permeability of membranes, contributing to an increase in their bio-
compatibility.

Although the pore size is 5-20 nm, the cell throughput can vary [22-24], since new pores can form
under the influence of NPs. Therefore, nanoparticles up to 100 nm in size are biologically active and
have the effect of "low doses". The size of nanoparticles (not more than 100 nm) allows them to pass
through biological membranes, accumulate in the internal environment, and possibly affect the
mitochondrial genome (mtDNA) indirectly, which in turn triggers or slows down the mechanism of
biosynthesis of mitochondrial protein enzymes and, as a result, the activation of energy processes.

This ability depends on the information (those properties) that particles of different sizes,
composition and physical-chemical characteristics possess. Metal nanoparticles of Cu, Co, Fe, Zn
stimulate the growth and development of plants: vetch, mustard, cucumber, as well as wheat, and it
has been shown to exhibit the same regularity and high biological activity in the concentration range
of 0.01-100 g/t (ton of seeds). However, the oxides of the same metals are characterized by the
accumulation of nanoparticles in the plant structure and a decrease in plant growth and development at
a concentration of 0.1 g/t.

Studying the mechanism of action of nanoparticles on living systems, including plants, will help
solve food security problems and improve agricultural production by increasing the efficiency of input
resources and minimizing the corresponding waste. Nanomaterials as unique carriers facilitate targeted
controlled delivery of nutrients with enhanced plant protection.

5. Acknowledgments
The study was carried out with the financial support of the Russian Federal Property Fund in the
framework of the research project No. 18-33-00510.

References
[1] Andreev K P Danilenko Zh V Kostenko M Yu Nefedov B A Terentyev V V and Shemyakin A
V 2018 Determining the inequality of solid mineral fertilizers application. J. of Adv. Res. in
Dynamical and Control Systems 10(10) 2112
[2] Churilov G 2015 Bio-ecological consequences of crop seeds treatment with metal
nano-powders. IOP Conf. Ser.: Mater. Sci. Eng. 98 012035 DOI: 10.1088/1757-899X/98/1/012035
[3] Churilov G, Polishuk S, Kutskir M, Churilov D and Borychev S 2015 Activators of
Biochemical and Physiological Processes in Plants Based on Fine Humic Acids. IOP Conf.
Ser.: Mater. Sci. Eng. 98 012040 DOI: 10.1088/1757-899X/98/1/012040
[4] Raykova A P Panichkin L A and Raykova N N 2003 The use of ultrafine metal powders for
presowing treatment of seeds (Moscow) pp 81–84
[5] Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A 2013 Toxicity of Ag,
CuO and ZnO nanoparticles to selected environmentally relevant test organisms and
mammalian cells in vitro: a critical review. Arch. Toxicol 87 1181 DOI: 10.1007/s00204-
013-1079-4
[6] Tsitsuashvili V S, Minkina T M, Nevidomskaya D G et al 2017 Effect of copper nanoparticles
on plants and soil organisms. Bulletin of Agrarian Science of the Don 3(39) 93 [in Russian]
[7] Shang Y, Hasan M K, Ahammed G J , Li M, Yin H, Zhou J 2019 The use of nanotechnology in
crop production and plant protection: a review. Molecules 24(14) doi: 10.3390/molecules24142558
[8] Churilov GI, Churilov DG, Nazarova AA, Polischuk SD, Churilova VV, Borychev SN,
Byshov NV 2019 Dynamics of accumulation of pollutants and essential elements in the
process of plant growth and development. Int. J. of Nanotechnology 16(1/2/3) 42
DOI: 10.1504 / IJNT.2019.102391
[9] Folmanis G E and Kovalenko L V 1999 Ultradispersed metals in agricultural production,
(Moscow, IMETRAS)
[10] Vekilova G V Ivanov A N Yagodkin Yu D 2009 Diffraction and microscopic methods and devices for analysis of nanoparticles and nanomaterials (Moscow, MISiS)

[11] Ermakov A I Arasimovich V V Yarosh N P Peruanskiy Yu V Lukovnikova G A and Ikonnikova M I 1987 Methods of biochemical studies of plants (Leningrad, Agropromizdat) pp 43–44

[12] Averyanov A A 1991 Active forms of oxygen and plant immunity. Successes of Modern Biology 111(5) 722

[13] Kostyuk V A Potapovich A I and Kovaleva Zh V 1990 Simple and sensitive method for determining superoxide dismutase activity based on quercetin oxidation reaction. Matters of Med. Chem. 2 88

[14] Arsentyeva I P Dzidziguri E L Zakharov N D Pavlov G V Ushakov B K Folmanis G E and Arsentyev A A 2004 Regularities of the structure and biological activity of iron nanoparticles. Perspective Materials 4 64

[15] Skulachev V P 1989 Energy of biological membranes Biological and technical membranes, (Moscow, Science)

[16] Skulachev V P 1997 The laws of bioenergy. Soros Educational J. 1 9

[17] Romanovskiy Yu M and Tikhonov A N 2010 Molecular transducers of living cells. Proton ATP synthase - a rotating motor. Adv. of Physical Sci. 180(9) 932

[18] Podobaev A N 2008 Adsorption interaction of water with metals and its role in the processes of electrochemical corrosion (Moscow)

[19] Kurapov P B 1996 Hormonal balance. Methods for its study and regulation. Dissertation, Moscow

[20] Makronosov A T 1983 Integration of growth and photosynthesis functions. Plant Physiology 5(30) 868

[21] Kulayeva O N 1982 Hormonal regulation of physiological processes in plants at the level of RNA and protein (Moscow, Science)

[22] Nel A E, Mädler L D, Velegol, Xia T, Hoek M E V, Somasundaran P, Klaessig F, Castranova V, Thompson M 2009 Understanding of bio-physical-chemical interactions on nano bio interface. Natural Materials 8 543

[23] Auffan M, Rose J, Bottero J-Y, Lowry G V, Jolivet J-P, Wiesner M R 2009 On the determination of inorganic nanoparticles from the point of view of environment, health and safety. Nature Nanotechnology 4 634

[24] Oberdorster G, Stone V, Donaldson K 2007 Toxicology of nanoparticles: a historical perspective. Nanotoxicology 1 2 doi: 10.1080/17435390701314761