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NATURAL AND SYNTHETIC NANOMATERIALS IN MICROBIAL BIOTECHNOLOGIES FOR CROP PRODUCTION

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Nanoparticles of various materials (up to 100 nm in size) are characterized by a large surface area, which significantly increases their reactive properties. This makes promissing the studies of their possible application in different technologies, including those in the agricultural production sector. This review summarizes the literature on the distribution and properties of natural nanoparticles in the environment. The features of the interaction between various types of microorganisms, nanoparticles of natural minerals, oxides of metals and carbon nanoparticles are analyzed. The review also summarizes the data on the effect of nanoparticles of different origin on microorganisms, plant growth and development. It also presents the information on the effectiveness of the use of clay mineral nanoparticles in the production of complex bacterial preparations for plant growing and the prospects of using nanoparticles of metal oxides in this industry.

Keywords: nanoparticles of natural and synthetic origin, interaction of nanoparticles, microorganisms and plants.

The world population is projected to increase to about 8 billion by 2025 and to 9 billion by 2050 [1]. As such, global agricultural productivity must rise substantially to feed the world's rapidly growing population. It is constantly in the public eye because climate change, energy and resource constraints and the increasing population of the planet are putting unprecedented pressure on food and water resources [1]. The demand of the growing global population for wheat may grow by 60 % by 2050 [2].

Intensive use of chemical fertilizers and plant protection products is accompanied with a decrease in soil fertility, plant productivity, quality of the products obtained and an increase in environmental pollution [3]. The UN warns that if current trends in land use persist, significant areas of natural land, roughly the size of Brazil, could be degraded [4].

It seems promising to use nanotechnology in agriculture for the intensification growth and development of plants, their protecting against phytopathogens, phytophages and increasing of their productivity [5–8]. The term "nanotechnology" was proposed in 1974 by the Japanese physicist Norio Taniguchi for technologies based on the use of objects, which did not exceed 100 nm in size in at least one dimension [9] It should be noted that the unique properties of highly dispersed particles of clay minerals and other materials have attracted the attention of researchers for a long time and were used in many technologies [10].

However, in recent decades, the researchers have especially focused on nanomaterials due to special properties of their surface, which significantly increases with the decrease of particle size. The specific surface area of cubic particles can be determined from the ratio: $S = 6/d$, where d is the size of the particle [11]. Spherical particles of 3.5–5.0 nm have a surface area exceeding 900 m^2/g [12].

Nanotechnologies can be used in various spheres of life. They are promising for the industrial production of materials with highly specified characteristics, for transport and semiconductor industries, for pharmaceuticals and medicine, for agriculture to protect plants, monitor their growth, identify plant and animal diseases, increase food production, and improve quality [13–15]. Currently, nanoparticles of natural minerals are successfully used in biotechnology in manufacture of highly effective microbial preparations for crop production [6, 16].

The properties of nanomaterials, their reactivity, solubility, mechanical (elasticity, hardness, etc.), electronic (conductivity, redox behavior, etc.), and nuclear (magnetic) traits and, therefore, their effect on ecology and biological objects, often change depending on the size of the particles. These changes may be unforeseen [17].

Nanoparticles in the environment

There is an exceptionally wide variety of nanoparticles on the Earth, which are actually distributed both in the abiotic regions of our planet and in the biotic environment [18–19]. Nature itself creates a large number of nanoparticles. It provides us with a range of small particles, from inorganic ash, soot, sulfur and mineral particles found in the air or in wells, to nanoparticles of sulfur and selenium, produced by many bacteria and yeast [20]. Microbes interact with minerals, since minerals support the growth of microbial populations, and the latter change the solubility of minerals in the process of metabolism and the degree of oxidation of some of their components [21]. The most common nanoparticles are those from volcanoes and forest fires, sea salt aerosols, and oxides of iron and other transition metals in soils, rivers and oceans [17, 22–25]. They are always present even in interplanetary and interstellar space [22]. Thus, at the influence of number of factors, a significant amount of natural nanomaterials accumulate in the environment [26].

The clouds of volcanic ash contain a wide variety and amount of polydisperse micro- and nanoparticles range from 100 to 200 nm and mainly composed of silicate and iron compounds. They are easily suspended in the air and can cause serious breathing problems when inhalation. Indeed, although the particles in the lower micrometer range are deposited in the upper airways, nanoparticles penetrate and settle in the tracheobronchial and alveolar regions, where they can cause serious respiratory distress [27]. The soot “Carbon Nanotube” produced by burning Texas pine Pin̉on has been shown to contain multi-walled carbon nanotubes ranging in size from 15 to 70 nm. These carbon-based objects are easily air-borne and pose a serious health hazard to animals and humans [28].

Due to the desertification of many regions [29], the burning of biomass, engine exhaust, mining and other types of anthropogenic activities, the release of nanoparticles into the environment, inadvertently produced by humans, increases significantly [17]. The soil is the main habitat for microorganisms and contains a considerable amount of organic and inorganic particles [30–31]. Each gram contains billions of bacteria that belong to several thousand species [32], millions of actinomycetes, hundreds of thousands of fungi and algae [33], a significant number of protozoa, nematodes and representatives of the mesofauna [34].

Interaction between microorganisms and natural nanomaterials

It has been shown that many organisms can synthesize nanomaterials and are capable of transferring electrons and energy [35]. Most microorganisms in the soil are in the sorbed state. This process is largely determined by the size of soil particles, the charge of exchangeable cations, the concentration of the electrolyte, and other properties [31].

The results of microelectrophoresis methods that the surface of various types of bacteria also contains negatively and positively charged groups due to the presence of carboxyl, amino acid and other groups. When nanoparticles of silicon dioxide, montmorillonite, palygorskite, and other natural minerals are added to bacterial suspension, the cells come into contact with these particles, during which nanoparticles cover a large part of the cell surface, which significantly changes the charge of their surface [5, 36].

Minerals and microbes have coexisted for much of the Earth’s history. The close interaction between microbes and clay minerals, which has been occurring on a geological time scale, is a complex, simultaneously developing system [37]. They interact on a microscopic scale, but their effects are macroscopic. Minerals support the growth of microbes providing essential nutrients, and microbial activity changes the mineral solubility and oxidation state of some of the constituent elements of minerals [21].

Microbes play a key geoactive role in the biosphere, especially in biotransformation and biogeochemical cycles, the transformation of metals and minerals in soils and sediments. Geomicrobial processes are transformations of metals and minerals under the influence of microorganisms [38]. All types of microbes, including prokaryotes and eukaryotes, and their symbiotic associations can contribute to geological phenomena. It has been shown that many organisms can synthesize nanomaterials and transfer electrons and energy [35].

Certain types of bacteria are capable of fractionating sulfur isotopes, precipitating pyrite [39], promoting the accumulation of carbonate in shallow seas [39], and participating in the synthesis of various minerals [41–42], including deposits of clay minerals [43–46]. Microbes promote the transformation of minerals, release of phosphorus and other nutrients necessary for the growth of microbial populations [47]. Along with microorganisms, clays are among the most catalytic surfaces in sedimentary media, which are important for various biogeochemical cycles [48].

Microbes, especially bacteria, affect the kinetics and course of reactions including the formation of many minerals in the lithosphere and hydrosphere of the Earth [49–51]. On the other hand, minerals strongly affect the survival of microorganisms, their physiological and biochemical activity [52–53].

The interaction between various types of microorganisms and nanoparticles of natural minerals is accompanied with a noticeable increase in the resistance of cells to the effects of adverse environmental factors. The interaction between bacteria *Methylomonas rubra* 15s, *Azotobacter chroococcum* and other microorganisms, and nanoparticles of clay minerals montmorillonite, palygorskite, and bentonite increased their viability significantly during long-term storage [54–56].

It was shown that the addition of 10 g/L of palygorskite nanoparticles to the suspension of many bacterial species significantly increased the yield of viable cells upon spray drying [57]. It was found that when a suspension of *A. chroococcum* 20 was heated for 10 minutes at 45 ^{0}C, no more than 30 % of cells remained viable. However, the addition of 1 % of montmorillonite nanoparticles to the suspension of these bacteria, followed by exposure to the same temperature, increased the number of viable cells to 68 % [58]. The addition of 1 % of palygorskite had a similar effect on the survival of *Agrobacterium radiobacter* 204 when the suspension of these bacteria was heated [59]. A significant protective effect was exerted by nanoparticles of clay minerals on the survival of *Pseudomonas aureofaciens* UKMV-III during their long-term storage [60].

The interaction between minerals and microorganisms is important for the ecology of the soil and the environment [61]. This process is ubiquitous in natural conditions, but the general consequences of such interactions often remain unknown due to the lack of standard assessment methods [62].

Physiological and biochemical activity of microorganisms during their interaction with nanoparticles of natural minerals

Experimental studies have shown that pH, temperature, ionic strength, types of bacteria, and properties of minerals strongly affect the degree of bacterial adsorption on mineral surfaces [61, 63]. It has been demonstrated that the interaction between bacteria and clay minerals has a great effect on the physiological properties of microbial populations in aquatic systems [64]. It should be noted that as a rule, this interaction between microorganisms and particles of silicon dioxide, clay minerals, significantly stimulates the growth activity of microbial populations. For instance, in the presence of 20 mg/L of SiO_2 nanoparticles, the number of soil bacteria increased significantly [65].

When *A. radiobacter* 10 was cultivated in a nutrient medium containing 10 g/L of palygorskite or montmorillonite nanoparticles, the growth activity of the strain increased by 80 and 70 %, respectively, compared to the control [59]. A similar effect was observed when bacteria *A. chroococcum* 20 and *Azotobacter vinelandii* 56 were cultivated with these clay minerals [66].

It has been shown that the introduction of 0.2–1.0 % of clay minerals of montmorillonite or palygorskite into the nutrient medium significantly stimulates the growth of phosphate-mobilizing bacteria *Bacillus subtilis* in a medium containing hardly soluble calcium phosphate as the only source of phosphorus. Nanoparticles of these minerals had a more pronounced stimulating effect on the growth of these bacteria than particles of colloidal dispersion. An increase in the content of nanoparticles of these minerals in the medium up to 2 % was accompanied by a decrease in the stimulation of bacterial growth, which could be due to the sorption of glucose and phosphate on minerals [67]. A similar effect was exerted on the growth of *B. subtilis* IMV B-7023 by their cultivation in a medium containing 1 g/L of bentonite or saponite particles [68]. When these bacteria were cultivated in a medium containing 5 g/L of vermiculite particles, the number of bacilli increased by 49 % as compared with the control [69].

Silicon dioxide nanoparticles had a significant effect on the growth activity of many yeast species, *A. radiobacter* 204, bacteria of the genus *Azotobact*er and other species. Thus, upon cultivation of *Azotobacter chroococum* 20 in Ashby’s medium with sucrose, with the introduction of 0.05 % of these nanoparticles, the number of bacteria increased by 100 % as compared with the indicator without silicon dioxide [54].

One of the mechanisms of nanomaterials stimulating the growth activity of aerobic microorganism can be the effect of these particles on the mass transfer of oxygen into the culture medium. It was shown that upon stirring a liquid medium containing 1 % of palygorskite nanoparticles, the oxygen mass transfer increased by 17 % [70].

It should be noted that the cultivation of bacteria in media containing nanoparticles of clay minerals significantly affects the biochemical activity of populations. Thus, when *A. chroococcum* 20 was grown in Ashby's medium containing sucrose and 2 g/L of palygorskite nanoparticles, the synthesis of thiamine (vitamin B1) increased by 116 %, and when *A. vinelandii* 56 was cultivated under such conditions, the synthesis of pyridoxine increased 7 times as compared with the medium without this mineral [66].

The cultivation of *B. subtilis* IMV B-7023 in a medium, containing nanoparticles of SiO_2, had a significant effect on the antioxidant potential of these bacteria [71]. Their cultivation in a medium containing 0.05–0.5 g/L of SiO_2 nanoparticles or 1.5–2.5 g of vermiculite was accompanied with an increase in the extracellular peroxidase activity of bacilli [72]. At low concentrations, silicon dioxide nanoparticles have no toxic effect on the biota, and also reduce the toxicity of surfactant solutions [73].

It was found that the accumulation of amino acids in the culture medium of *A. vinelandii* IMV B-7023 increased 5–6 times in the nutrient medium containing 5 g/L of glauconite or saponite particles. After the introduction of SiO_2 nanoparticles into the nutrient medium of *B. subtilis* IMV B-7023 the content of zeatin in the culture increased by 85 % as compared with the control. A visible stimulating effect of these bacteria on the synthesis of phytohormones was observed in the case of vermiculite nanoparticles using. It was found, that during the cultivation of bacilli with 5 g/L of this minerals the content of zeatin in the culture increased by 17 %, that of zeatin riboside – by 20 %, and zeatin glucoside – by 144 % [69]. These nanoparticles had a significant stimulating effect on the synthesis of phytohormones by the bacteria *A. vinelandii* IMV B-7076 [74].

The interaction between these strains of bacteria and vermiculite nanoparticles significantly increased their dehydrogenase activity along with the activity of antioxidant enzymes. After the introduction of 0.5–10.0 g/L of this mineral into the nutrient medium with *A. vinelandii* IMV B-7076 there was an increased in the dehydrogenase activity by more than 40 %. However, the silica nanoparticles did not have a stimulating effect on the dehydrogenase activity of this strain. The vermiculite nanoparticle had a positive impact on the antioxidant enzyme activity of *B. subtilis* IMV B-7023. It was shown, that after the addition of 1.5–2.5 g/L of this nanomaterial into a nutrient medium the peroxidase activity of bacteria increased 3 times [69]. The addition of saponite and especially bentonite nanoparticles led to considerable stimulation of superoxide dismutase activity of these bacteria [68].

Prospects for the use of synthetic nanomaterials in agrobiotechnology

Nanotechnology provides opportunities for the development of new means and mechanisms delivering of agrochemical agents (synthetic nanomaterials) to increase crop yields and reduce the use of pesticides. They can be used to create nanobiosensors in plant protection, to detect residues of agrochemicals, diagnose plant diseases, and in other fields [75–78]. At the same time, there are concerns about the safety of using nanomaterials due to insufficient research on their possible negative impact on the environment, and unknown consequences [75, 79–80]. Different kinds of nanoparticles may have different effects on plants.

It was shown that the use of ZnO and TiO_2 nanoparticles in the doses of 100–1000 ppm did not affect the germination of *Cicer arientinum* seeds. The treatment of seeds with zinc oxide nanoparticles was accompanied with a higher level of chlorophyll accumulation in plants compared to the use of TiO_2 nanoparticles [81]. Foliar treatment of winter wheat plants grown under drought conditions (30 % out of full moisture capacity) with Avatar microelement complex containing nanoparticles obtained by chelation of several compounds (magnesium, copper, iron, zinc, molybdenum, and cobalt) with carboxylic acids increased the resistance of the photosynthetic apparatus of plants to soil drought, and contributed to a significant increase in their productivity. The stimulating effect of these microelements on grain yield was manifested to a greater extent in varieties less resistant to drought [82].

Research results indicate that when carbon nanoparticles and metal oxides are used in crop production, they can accumulate in the soil and plant tissues, exerting both a favorable and a negative effect on their growth and productivity [83–84]. It was shown that pretreatment of wheat seeds with silicon nanoparticles protected wheat seedlings from ultraviolet radiation, regulating oxidative stress by increasing the activity of the antioxidant defense system [85].

At the same time, nanoparticles of metals and metal oxides can be highly toxic to soil biota. This can have a negative effect on microbial communities of soils and their fertility [86]. In accordance with the data [87], the introduction of metal nanoparticles into the soil causes a decrease in the microbial biomass, enzymatic activity, and affects the composition of the microbial community, including bacteria, yeast, and fungi. Such nanoparticles can pose a hazard to human health [87].

It was shown that the introduction of silver nanoparticles into the soil was accompanied by a change in the number of dominant microorganisms of the phyla *Proteobacteria, Actinobacteria,* and *Femicutes* important for the agriculture by 25–45 %. Silver nanoparticles can disrupt the morphology of membranes, significantly increase their permeability, which can lead to uncontrolled transportation of compounds across the membrane and to the death of cells [88].

Cerium oxide (CeO_2) nanoparticles are a striking example of potential capabilities of metal oxide nanoparticles. They find their applications in industry and biomedicine. These nanoparticles are widely used as an abrasive in the production of semiconductors, as components of catalytic converters for automobile exhaust gases, as a fuel additive for accelerating combustion, and in other technologies [89–90]. Recently, it was demonstrated that CeO_2 nanoparticles have antioxidant activity at physiological pH values and therefore can be useful in biomedicine, protecting cells from oxidative stress or inflammation [91–92]. However, the use of nanoparticles of metal oxides is associated with their possible toxic effect on the environment [93]. It was found that cerium nanoparticles inhibited the growth of *Escherichia coli* and *Bacillus subtilis*, while they did not affect *Shewanella oneidensis* [62]. The study of the toxicity of CuO and TiO_2 nanoparticles indicated that TiO_2 nanoparticles did not have any toxic effect on *Saccharomyces cerevisiae* even at the concentration of 20 000 mg/L. At the same time, CuO nanoparticles showed high toxicity to this type of yeast [94].

Zinc oxide nanoparticles did not have a significant effect on the bacterial community of the soil [95], but caused a decrease in plant biomass and a change in the shape of roots [96]. It was shown that Zn and ZnO nanoparticles were more toxic than Al_2O_3, Fe_3O_4, and SiO_2 nanoparticles [97]. ZnO nanoparticles negatively affected the development of rice seedlings, inhibiting the development of roots and reducing their number. At the same time, TiO_2 nanoparticles did not affect these parameters of rice plants [98]. According to the results of other researchers, ZnO and TiO_2 nanoparticles in concentrations of 100–1000 ppm also had different effects on the germination of *Cicer arietinum* seeds. It was shown that ZnO nanoparticles did not affect this process, but reduced the weight of roots and shoots. However, the content of chlorophylls and carotenoids in the leaves increased. At the same time, when using TiO_2 nanoparticles, the opposite effects were observed – stimulation of seed germination and a decrease in the content of pigments in the leaves of these plants [81].

Studying the effect of palladium (Pd) nanoparticles on the growth of barley, it was shown [99] that nanoparticles accumulating in a given plant remain in plant tissues. It should be noted that different plants may differ in their interaction with metal nanoparticles. Thus, Zhu et al. [100] found that when using Fe_2O_3 nanoparticles in growing pumpkin and beans, this oxide accumulated in all pumpkin tissues, while it was not found in beans.

A study of the toxicity of fullerenes C70 showed that when they were used to treat plants, these nanoparticles entered the tissues and were passed on to the offspring through seeds [101], creating oxidative stress and leading to a decrease in the viability of rice cells [102]. Carbon nanotubes can penetrate the cell membrane [101]. Hydrophobic fullerenes are characterized by a higher penetrating ability, whereas their derivatives, hydrophilic nanoparticles, can only be adsorbed on the surface of cell membranes [103]. Nanomaterials can damage cell membranes by generating reactive oxygen species (ROS), which can oxidize double bonds on fatty acid tails of membrane phospholipids during lipid peroxidation. This process can affect membrane permeability, making the cell more susceptible to osmotic stress and unable to assimilate substrates [104]. In addition, peroxide fatty acids can transform free radicals that can damage DNA [105].

Conclusions

The creation of new types of nanoparticles, the study of their properties and the creation of new nanotechnologies, including those for agro-industrial applications, attract the attention of many scientists around the world. However, the information on the properties of many nanomaterials of metal oxides and others elements, the levels of their toxicity, is still insufficient. Therefore, the use of these materials is limited due to the lack of knowledge about the assessment of consequences for the environment and human health. The use of these technologies requires the development of a comprehensive database and signaling system, as well as international cooperation in the field of regulation and legislation [76].

At present, clay minerals nanoparticles appear to be more predictable for the use in biotechnology to produce highly effective complex microbial preparations for plant growing. The use of such preparations can significantly increase the availability of nitrogen and phosphorus for plants, improve their growth and development, limit the spread of phytopathogens and phytophages in the phytocenosis and increase productivity. Based on the interaction of highly efficient strains of nitrogen-fixing bacteria *A. vinelandii* IMV B-7076 and phosphate-mobilizing *B. subtilis* IMV B-7023 with bentonite particles, we have created various forms of a highly stable complex bacterial preparation Azogran (granular, free-flowing and nanocomposite), which significantly increases the growth of decorative plants, the development of floral and other types of plants, the yield of industrial crops, vegetables and cereals by 18–37 % [6, 16]. However, the prospects of widespread use of nanoparticles of different origin in human activity are beyond doubt.

ПРИРОДНІ ТА СИНТЕТИЧНІ НАНОМАТЕРІАЛИ В МІКРОБНИХ БІОТЕХНОЛОГІЯХ ДЛЯ РОСЛИННИЦТВА

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Наночастки різних матеріалів (що мають розміри до 100 нм) характеризуються значною поверхнею, що значно підвищує їх реакційні властивості. Це викликає інтерес дослідження особливостей наноматеріалів з метою їх застосування в різних технологіях, в тому числі в аграрному секторі виробництва. Даний огляд присв›ячений узагальненню літературних відомостей про поширення в навколишньому середовищі наночасток природного походження та їх властивостей. Аналізуються особливості взаємодії різних видів мікроорганізмів з наночастками природних мінералів, оксидів металів та наночасток вуглецю. Узагальнені відомості про вплив наночасток різної природи на мікроорганізми, ріст і розвиток рослин. Наведені відомості щодо ефективності застосування наночасток глинистих мінералів у створенні комплексних бактеріальних препаратів для рослинництва і перспективності використання в цій галузі наночасток оксидів металів.

Ключові слова: наночастки природного і синтетичного походження, взаємодія наночасток з мікроорганізмами і рослинами.

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