БІОТЕХНОЛОГІЇ ТА БІОІНЖЕНЕРІЯ

UDK 636.5.084.52:547.1'123:577.12

Properties of silver and copper nanoparticles obtained by the methods of «green» chemistry

Tsekhmistrenko O. [iD]

Bila Tserkva national agrarian university

✉ E-mail: tsekhmistrenko-oksana@ukr.net

OPEN ACCESS

Significant challenges for the modern international community are population growth and climate change, and the latest technologies are helping to solve them. These include nanotechnologies that are capable of positively influencing the quality of agricultural products, minimising the negative impact of pesticides on the environment and human health, and increasing production productivity and food safety. Nanotechnology helps to minimise waste by producing more efficient products, leading to the development of precision agriculture and efficient resource management. Minimising the production of hazardous substances, reducing energy consumption in green nanotechnology and almost no toxic chemicals make green nanomaterials widely used and desirable in various fields of human activity.

Traditional and new methods for the formation of nanosized materials are considered, with an emphasis on their advantages and disadvantages. Extra- and intracellular ways of nanoparticle formation, as well as ascending and descending methods are described. The aim of the **work** was to establish the possibility of green synthesis of CuO nanoparticles using peppermint and lemon balm extracts.

The plant extracts were prepared by ethanol extraction from the leaves in an extractor. The extract was separated from the plant material by filtering through filter paper. Copper nanoparticles were synthesised by the reduction of copper sulphate in the presence of peppermint or lemon balm extract, which acted as both a reducing agent and a nanoparticle stabiliser.

The paper shows the possibility of synthesising copper oxide nanoparticles by the method using peppermint (*Mentha piperita*) and lemon (*Melissa officinalis L.*) balm leaf extracts. The TEM morphological study of ^{Cu0} metal nanoparticles in the volume of polymeric nanocomposites and those created by the green synthesis method showed that the average size of copper (^{Cu0}) nanoparticles is 3.5 and 12.2 nm, respectively. It was found that the thermo-chemical method of creating nanocomposites is cheap and accessible, but causes the formation of small metal nanoparticles, in contrast to the polymeric nanocomposite created by the green synthesis method, which is environmentally friendly and occurs at low temperatures.

Key words: nanobiotechnology, nanoparticles, environmentally friendly products, nanoparticles, copper oxide, silver particles

Problem statement and analysis of recent research. Population growth and climate change are significant challenges for the modern international community, which can be solved by the latest technologies. These include nanotechnologies that can positively affect the quality of agricultural products, minimise the negative impact of pesticides on the environment and human health, and increase production productivity and food safety. The unique properties of nanoscale materials contribute to their use in agriculture and related industries, such as nanoparticle-based fertilisers or plant growth promotion. Additionally, the use of fertilisers and pesticides is reduced with the use

of nanoparticle-based carriers and compounds without reducing productivity [6]. Nanotechnology helps to minimise waste by producing more efficient products, leading to the development of precision agriculture and efficient resource management [7]. The goal of developing "green" nanotechnologies, which use biological pathways to synthesise nanomaterials, is to minimise the production of hazardous substances. At the same time, the amount of energy consumed in green nanotechnologies is much lower than in other technologies; almost no toxic chemicals are produced during the synthesis, and their environmental friendliness is very high. Thus, the produced "green" nanomaterials can be widely used in various industries [2].

Depending on the purpose of use, different types of nanomaterials are used, often nanoparticles are used as carriers that gradually release the active ingredient to reduce their overall consumption. The nanomaterials used are selected for their biocompatibility, lack of negative health effects and ability to increase the shelf life of food products. Highly sensitive nanosensors with plasmonic properties (silver or gold nanoparticles) are used to measure environmental conditions and report changes in a timely manner and to intelligently control plant needs [14]. At present, the range of applications of nanotechnology includes nanosensors, tracking devices, targeted delivery of necessary components, food safety, and smart packaging [4; 23].

Several methods are currently available to improve the precise dilution and control of the nanoscale green synthesis process. Nanotechnologies can increase the resistance of materials to degradation, reduce the amount of chemicals used, which leads to a reduction in emissions and related environmental problems. Today, carriers have been developed to increase the connection between plant roots and the surrounding soil structure [5; 28]. Modified nanoparticles added to conventional fertilisers improve the ability of fertilisers to store nitrogen, reduce losses of the element, and improve nutrition to improve product composition. Nanoemulsions have been developed to increase the biological compatibility of herbicides and pesticides [20].

Microorganisms are important nanofactories capable of accumulating and detoxifying heavy metals due to the presence of various reductase enzymes that reduce metal salts to MtNPs [26]. Studies have shown that *Lactobacillus, Bacillus, Pseudomonas, Streptomyces, Klebsiella, Enterobacter, Escherichia, Aeromonas*, etc. are able to synthesise metal nanoparticles [26], and the produced nanoparticles have very good dispersion, stability, and significant lethal activity against pathogens [9]. Bacteria, fungi, yeasts, and microalgae produce MtNPs intra- and extracellularly, producing organic matter inside and transporting it outside their cells [15]. Microorganisms as nanofactories are environmentally friendly, inexpensive and non-toxic, while, compared to physicochemical methods, they do not require much energy for the synthesis of MtNPs. At the moment, the mechanisms of extracellular green synthesis of MtNPs are of great interest, since the extracellular location of the material eliminates the need for expensive and complex subsequent processing steps to recover intracellular nanoparticles [26]. The «green» synthesis of MtNPs using microorganisms, compared to traditional physicochemical methods, offers a faster, more cost-effective, non-toxic and environmentally friendly method for the synthesis of MtNPs with a wide range of sizes, shapes, composition and physicochemical properties [29]. The disadvantages of the synthesis of MtNPs by microorganisms are the complexity of microbial sampling, isolation, cultivation and storage, as well as the subsequent recovery of the obtained nanoparticles [26].

Physical, chemical and biological methods are used for the synthesis of MtNPs. The disadvantages of the first two methods are the use of expensive equipment, high heat and energy consumption, low yield [8; 27], and the use of toxic chemicals [27]. The need for an environmentally friendly option for the synthesis of MtNPs is currently focused on the green synthesis of MtNPs by microorganisms, plants, using microbial enzymes, polysaccharides, and degradable polymers [28; 29], and green synthesis methods are more advantageous than traditional physical and chemical methods due to their simplicity, cost-effectiveness, and the absence of toxic and harmful chemicals.

The applications of MtNPs are innovative and diverse [8, 13]. The synthesis of MtNPs is usually performed using top-down and bottom-up approaches. In bottom-up approaches, bulk materials are broken into nano-sized particles [8, 22], the disadvantage of which is obtaining nanoparticles with an imperfect surface structure, high cost and labor intensity, which is not favourable for large-scale production [22]. In bottom-up approaches, nanoparticles are formed by self-assembly of structures at the atomic and molecular levels, which leads to a more precise size, shape, and molecular composition [8].

The «green» synthesis of MtNPs mediated by microorganisms is characterized by a high growth rate of microbiota, ease of cultivation, the ability to grow in inherent environmental condi-

tions [1], the ability to synthesize metal nanoparticles with a defined shape, size, composition and monodispersity of particles [8; 13]. The mechanism of biosynthesis is carried out by capturing target metal ions from the environment and enzymatically transforming them into elementary form by the mechanism of reduction. The MtNPs production requires special metabolic pathways and cellular enzymes and depends on the ability of microorganisms to transport heavy metals by mimicking the natural process of biomineralization [19]. The intracellular mechanism of MtNPs biosynthesis includes transport systems of microorganisms, in which the cell wall plays an important role due to its negative charge: positively charged metal ions are deposited in negatively charged cell walls through electrostatic interaction. After being transported into the cells of the microorganism, the ions are restored using metabolic reactions mediated by nitrate reductase. The MtNPs formed and accumulated in the periplasmic space can then pass through the cell wall [10].

Extracellular MtNP biosynthesis is also mediated by nitrate reductase synthesis, in which synthesis enzymes are located in the cell wall or secreted from the cell into the growth medium. In this process, nitrate reductase reduces metal ions to metallic forms [19; 10].

MtNP recovery processes by microorganisms depend on the presence of enzymes, proteins, and NADH-dependent enzymes in them [19]. The reduction mechanisms start with electron transfer from NADH by means of NADH-dependent reductases [3], and microbial proteins can act as a stabilizing agent, provide colloidal stability and prevent MtNP agglomeration [19].

For intracellular synthetic approaches, microorganisms are cultivated in a suitable growth medium with favorable pH and temperature conditions [22]. Biomass is harvested after the optimal incubation period, thoroughly washed with sterile water to eliminate potentially unwanted effects of the culture medium, and subsequently incubated with a metal salt solution. An alternative to the intracellular synthesis of MtNPs is the use of cell-free (CF) approaches using culture supernatant or cell-free extracts (CFE) [13]. To do this, the mixture containing the culture medium and biomass is centrifuged, and the supernatant is collected and incubated with an aqueous solution of a metal salt for the synthesis of MtNPs. At the same time, the compounds of the culture medium are used for the synthesis of MtNPs and act as reducing agents and blocking agents. Microorganisms and culture media are removed by repeated washings, and only biomolecules released by cells due to autoly-sis or starvation conditions mediate the synthesis of MtNPs [1, 13]. In all cell-free processes, the color change of the reaction mixture is often the first sign of nanoparticle synthesis, and the color change depends on the exact nature of the formed MtNPs [1].

The microbial source, reaction temperature, pH, pressure, incubation time, and metal salt concentration affect the synthesis of various MtNPs [3, 11]. Nanoparticles synthesized in the cell are treated with ultrasound or reaction with appropriate detergents to release MtNPs after the destruction of the cell wall [27].

In agriculture, «green-synthesized» MtNPs are used to increase the productivity of agricultural sectors and create nanoscale pesticides, fungicides, biosensors, fertilizers, animal growth and development regulators, as a harmless alternative to antibiotics [3, 30].

The use of biosensors allows to increase the production of high-quality agricultural products due to the ability to quickly identify pathogens and their monitoring and analytical capabilities [18]. Pesticides are used to protect plants from pathogens and insects, to increase yield [17], but the problem is their negative impact on products and human health through the food chain [30].

Nanopesticides are new development for encapsulation of pesticides for controlled release and improvement of selectivity and stability of pesticides [30]. Their advantages are the increased efficiency, durability and reduced amount of the active ingredient required for their formulation [25]. Nanocomposition of pesticides with MtNPs showed a stronger effect against phytopathogens, insects and fungi [17], provide targeted drug delivery, greater bioavailability due to higher solubility and permeability, less doses and controlled release.

The use of probiotic microorganisms for MT-NPs production is an environmentally friendly and commercial attractive approach. The non-pathogenicity of probiotics and their ability to grow rapidly, regulating gene expression for the production of various proteins and enzymes involved in MtNPs production are useful in many ways. Lactobacillus and Bifidobacterium are the most popular probiotics used in the production of a wide range of products [3]. Green synthesis of MtNPs, nanoparticles of metal oxide (MONPs) and non-MtNPs probiotics is investigated. The useful effect of probiotics includes a direct effect on living cells and an indirect effect on a number of metabolites. Probiotics also have a negative electric kinetic potential, which is freely attracted by cations as a starting point for the process of nanoparticles biosynthesis.

Lactobacillus tendency to grow even in the presence of oxygen makes them metabolically very viable. Redox potential (rH_2) of bacteria is reduced by adding reducing agents [3], which in the cultural environment to create the necessary conditions can be created by reducing rH_2 for anaerobic conditions in the presence of oxygen or by increasing the pH of the environment for the creation of aerobic conditions in anaerobic environment. Thus, changing different culture medium conditions plays an important role in the biosynthesis of metal nanoparticles and their oxides. Energy efficiency, glucose (which controls rH_2), average ionic value, pH and overall oxidative capacity (rH_2), play an important role in *Lactobacillus strains*. Despite the relatively weak system of detoxification of metals lactobacilli, a slightly acidic pH and the decrease in rH_2 activate membranous oxyidoredctase and the metabolic MtONPs.

Copper is an important trace element that is combined with many proteins and metal enzymes, playing a significant role in metabolism and nutrition. CuNPs also have higher productivity than voluminous copper particles, thanks to the small size and high ratio of the surface to volume, showing antifungal and antibacterial activity against grampositive and gramnegative bacteria and pathogenic fungi [12], including *Fusarium oxyspor. Graminearum* and *Phytophhora Infestans*, act as sprouts and plant growth stimulants at concentrations below 100 ppm. It was reported [28] about the prospect of using *Lactobacillus Casei* of different shapes and size.

Extracellular biosynthesis of spherical CuNPs is reported with size of 5-50 nm in *Streptomyces Griseus* and 3.6–59 nm in endophytic actinomycetes [21].

The study [24] shows the significant antifungal and antibacterial activity of CuNPs, biosynthesized by *Actinomycetes sp.* and isolated from *Convolvulus arvensis*.

CuNPs reduced the formation of ROS by increasing the activity of superoxidimutase and catalase enzymes. In addition, the content of vitamin C, lycopene and phenol increased in the presence of CuNPs, as did the strength of tomato fruits. CuNPs biosynthesized with *Streptomyces Griseus* are effectively used as fungicides [16; 21].

Studies have been conducted on the effect of MtNP size on their toxicity. Although factors as the size, concentration and Zeta-potential of MtNP show different effects on different plants, there is a significant dependence between the size of MtNPs and the degree of toxicity created for the plant: the larger size MtNP is less toxic to plants than smaller ones. At the same time, the concentration of nanoparticles significantly affects their toxicity, in particular, the concentration of more than 0.2 mg/ml CuNPs impairs the growth and physiology of plants [17].

For the synthesis of nanoparticles, different chemical and physical methods are used, of which biosynthetic or green methods play an important role. The synthesis of nanoparticles of copper oxide CuO was not widely investigated, compared to other metals due to the easily oxidized nature of copper, which is enhanced in nanopreparations. Nowadays, a number of scientific works are known, which investigated the synthesis of nanoparticles with the help of extracts of leaves of different plants. **The aim of the research** was to establish the possibility of green synthesis of CuO nanoparticles using *Mentha piperita* and *Melissa officinalis L.* extracts.

Material and methods of research. Among the various nanoparticles of metal oxides, copper oxide CuO nanoparticles are widely used in electronics, air and liquid filtration, the production of ceramics, preservation of wood, fabrics, the creation of biologically active coatings, leather products, films, oils, etc. In addition, nanopowders are successfully used as mineral fertilizers and pesticides.

Dry peppermint (*Menta Piperita*) and *Melissa Officialis L.* leaves were used as vegetable raw materials. Vegetable extracts were prepared by extraction of ethanol from leaves in Soxlet extractor (C. Gerhardt GmbH & Co. KG, Germany). The extract was separated from the vegetable material by filtration through Whatman # 1 filter paper. The filtrate was stored at -18ºC until use in further experiments.

Copper nanoparticles were synthesized with the reaction of restoring copper sulfate in the presence of menta piperita extract or melissa officialis L. extract, which acted as both a reducing agent and a nanoparticle stabiliser.

In a typical synthesis, up to 20 ml of 1% solution of copper sulfate heated to 60ºC, 20 ml of vegetable extract was added to drops with continuous stirring for 2 hours. The color of the solution changed from green to brown. After 2 hours of mixing, a second portion (20 ml) of vegetable extract was added to the drops and heated for another 2 hours. When the colour of the mixture changed to dark brown, the solution was cooled to room temperature and investigated the process of copper nanoparticles by the UV spectroscopy by the SHIMADZU UV-1800 spectrophotometer.

Research results and discussion. Comparing the absorption spectra of copper oxide obtained by ethanol extracts of *Menta Piperita* and *Melissa Officialis L.* with the spectra of the reaction mixture, the appearance of a wide peak in the range

of 285-320 nm, as a manifestation of the phenomenon of surface plasma resonance. Probably because of relatively high polydispersity (variety of shapes and sizes), peak is blurred with maxima at almost the same wavelengths (*Menta Piperita* – 296 nm, *Melissa Officialis L.* – 297 nm). These wavelengths are consistent with the values obtained in experiments with extracts of other plants - 262-310 nm, but differ significantly from the wavelengths characteristic of Cu0 nanoparticles - 536-550 nm, which suggests that CuO nanoparticles are formed as a result of the use of plant extracts and the direct interaction of extracts with a solution of copper salts.

Plant extracts obtained from *Menta Piperita* and *Melissa Officialis L.* successfully played the role of both the reducing agent and the CuO nanoparticles agent. The extracts of the plant provided a simple, less time-consuming and cheap method of synthesis of nanoparticles.

Preparations obtained from vegetable raw materials with pre-obtained nanopreparations based on chitosan-chloride and anionic Na-phosphate starch were compared. The existence in the spectrum of functionalized starch was established when the spectra of the specimens of native and Na-phosphate starch of wax corn, in particular two intense stripes (doublet) for ν = 1305 and 1287 cm^{-1}, characterizing the fluctuations ν = 1101 and 1025 cm^{-1}, indicating the valence vibrations of the P=O groups and the strip (ν = 753 cm - 1) identifies the valence vibrations of the group C–O–P. The indicated absorption strips in the FTIR spectrum of Na-phosphate of starch identify the starch of wax corn, functionalized by Na-tripolyphosphate.

In conducting the analysis of X-ray diffractograms of the initial samples of strong cation (chitosan-chloride) and anionic (Na-phosphate starch) polyelectrolytes found that both samples have an amorphous crystalline structure of different levels of its crystalline. The calculations of this structural parameter showed that the level of crystalline of strong cationic polyelectrolyte is 55%, and strong anion polyelectrolyte – 21%, with a relative error of measurement of the area of diffraction maxima did not exceed 3%.

When comparing X-ray diffractograms of chitosan and chitosan-chloride, it is clear that the protonization of amino groups of this polysaccharide with hydrochloric acid causes a significant change in its amorphous crystalline structure due to the existence of far-speaking (compared to hydrogen) intermolecular Coulomb forces between ionic groups. The calculations found that the relative level of crystalline structure of chitosan and chitosan-chloride is almost the same (X$_{кр}$≈55 %), whereas the size of crystallites L (in the direction perpendicular to the registration plane), determined by the Sherryr method, is slightly larger in the volume of strong cationic polyelectrolyte as a result of Coulomb interactions between ionic groups in chitosan-chloride. The calculation of chitosan crystallites was performed at the main discrete diffraction maximum with an angular position equal to 20.0°, with the value L$_{20.0°}$≈4,5 nm. To calculate the size of crystallites in the volume of chitosan-chloride used two intense discrete diffraction maxima at $2\theta_m$=11.0 and 25.0 ° on the X-ray diffractogram of this strong cationic polyelectrolyte. The calculations showed that L$_{11.0°}$=5.0 nm, and L25.0 ° = 7.4 nm, with the average value

The amorphous crystalline structure of chitosan-chloride differs significantly from the amorphous crystalline structure of chitosan. The crystalline structure of chitosan is characterized by the manifestation of the multiplical diffraction maximum with the vertex at $2\theta_m$≈10,0° and unrequited two intense discrete maxima at $2\theta_m$=20,0 and ~21,4°, which are on the background of the imaginary diffuse diffraction $2\theta_m$≈20,3°. The crystalline structure of chitosan-chloride is represented by two intense discrete diffraction maxima with angular position ($2\theta_m$) 11.0 and 25.0° and two low-intensive maximums at 18.2 and 37.6°, with diffraction maxima at $2\theta_m$=18.2 and 25.0° are manifested on the background of the imaginary diffuse diffraction maximum (amorphous halo) at $2\theta_m$~24,0°.

The crystalline structure of Na-phosphate of starch characterizes the manifestation on the X-ray diffractogram of this strong anionic polyelectrolyte (on the background of diffuse diffraction maximum-an amorphous halo with the vertex at $2\theta_m$≈21,4°) of two discrete diffraction at $2\theta_m$=16,2 and 17,8°. The size of the crystallitis size in the volume of Na-phosphate of starch found that L≈7.5 nm. In determining the size of the crystallites of Na-phosphate, starch found that X$_{кр}$≈21 %. Analysis of the X-ray diffractogram of the sample of the stechiometric FEC showed that both strong cationic and anionic polyelectrolytes, and created on their basis, FECs have an amorphous crystalline structure. This is indicated by the manifestation on the X -ray diffractogram of the sample of the FEC of one discrete diffractional maximum at $2\theta_m$=25.1° against the background of the diffuse diffraction maximum (amorphous halo), the vertex of which is 2θ ≈19,4°. Determining the relative level of crystalline of the sample of FECs showed that X$_{кр}$≈14 %. In turn, the calculation of the size of crystallites (L) showed that crystallites in the volume of FECs have a rather small value (3.0 nm).

When comparing X-ray diffractograms of PMC samples containing Ag^+ cations (and NO^- anions) and Cu^{2+} (and SO^{2-} anions) in the volume of cations, both polymer-metal complexes have an amorphous structure, unlike PMC sample. The absence of a crystalline phase in the volume of polymer-metallic complexes is a consequence of the formation of metals of the chelate-macroligand of metals. This is indicated by the manifestation on X-ray diffractograms of PMC samples of two diffuse diffraction maxima (amorphous halo) of varying intensity. In this case, the manifestation of less intense halo at $2\theta_m \approx 10,2°$ and $2\theta_m \approx 10.8°$ on the diffractograms of PMK with Ag^+ cations and with cations Cu^{2+}, accordingly, identifies the presence of polymer-metal complexes. A slightly higher intensity of amorphous halo, identifying the existence of chelate-macroligand type complexes in volume of PMK with Cu^{2+} cations compared to the intensity of a similar halo on the Dipractogram of PMK with Ag^+ cations, indicates more cordy-core cots. The main intensity of amorphous halo ($2\theta_m \approx 19.4°$), which is manifested on X-ray diffractograms of samples of polymer-metallic complexes, characterizes the amorphous structure of the polymer base, i.e. a stechiometric FEC.

When conducting the analysis of X-ray diffractograms of nanocomposites of the type of polymer-metal based on FECs and metal nanoparticles of silver (Ag^0) and copper Cu^0) obtained by the method of thermo-chemical recovery reduction of Ag^+ and Cu^{2+} cations, as well as reduced Cu^{2+} cations by the green synthesis method it has been found that the polymer matrix (FEC) of nanocomposites has an amorphous structure, while metal nanopar-ticles of silver and copper are characterized by a similar crystalline structure with the structure of metal and copper metals. This is indicated by the manifestation on X-ray diffracters of nanocomposites containing metal nanoparticles Ag^0 and Cu^0, intense discrete maximum at $2\theta_m = 38.0$ and $42.6°$ respectively with angular semiwidth ($\beta = 0,027$ and $0,041$ for AgNPts and CuNPts accordinely). In particular, the calculation of the size of the crystals of metal nanoparticles Ag^0 and Cu^0 in the volume of polymer nanocomposites created by thermal chemical method found that it is 4.1 and 2.8 nm, respectively. However, the size of crystals in the volume of metal nanoparticles Cu^0 polymer nanocomosis created by the method of green synthesis is much larger, as evidenced Diffractogram of this nanocomposition. As the calculation of the size of the crystals in the volume of "green" nanoparticles Cu^0, carried out by Sherrer, the value of $L \approx 7.2$ nm.

The research was carried out by the topics of TEM morphology of Cu^0 metal nanoparticles (Fig. 1) in the volume of polymer nanocomposites such as polymer-metal, created by the restoration of cations Cu^{2+} by thermo-chemical method and green synthesis method. It is shown that the average size of copper nanoparticles (Cu^0) is 3.5 and 12.2 nm, respectively.

It was found that the thermal chemical method of restoring metal cations and creating nanoenzymes is cheap and affordable, but the high temperature of recovery of these cations to a metal state causes the formation of small-sized metal nanoparticles, unlike polymer nanocomposition created by the method of green synthesis and occurs at low temperatures.
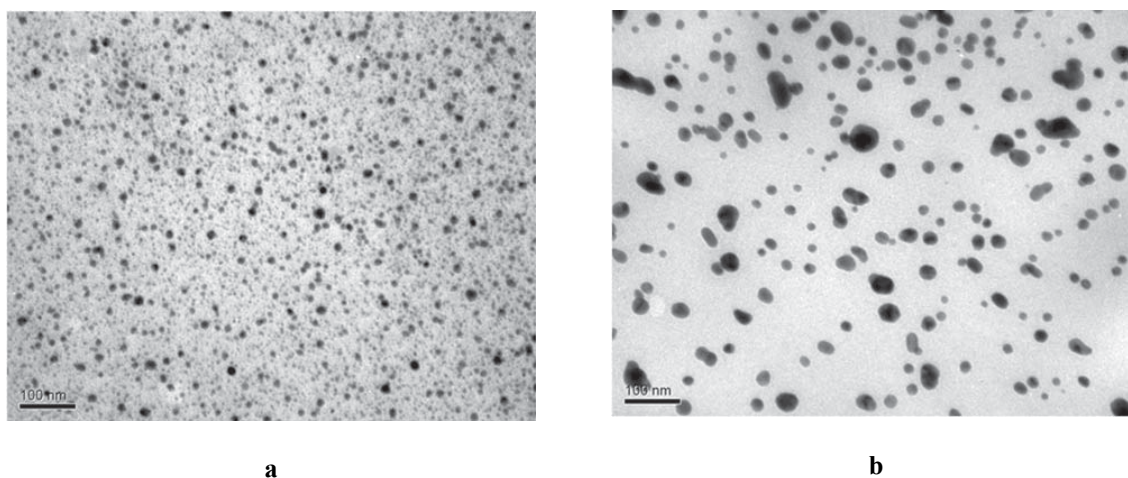

a   b

Fig. 1. **TEM microphotographies of Nanocomposites based on FECs and Cu^0 metal nanoparticles created by the restoration of Cu^{2+} thermo-chemical method (A) and the green synthesis method (b).**

Green synthesis of MtNPs using microorganisms is a promising and environmentally friendly approach to agricultural applications such as nanofertilizers, nanopesticides and nanbiosensers [28]. Despite the favorable physical and chemical properties of MtNPs, the complexity of soil crop ecosystems means that the environmental behavior of these nanoparticles is not yet fully predictable after use [29]. Therefore, before its full potential can be exploited, it is necessary to assess the effects and interactions with living systems. At this stage, screening of nanomaterials is important for assessing their potential toxicity and understanding their mechanisms of action to prevent their adverse effects in the future.

MtNPs nano sizes determine many of their beneficial properties, and can also increase their potential adverse effects [28]. MtNPs toxicity is influenced by various factors: solubility and their specificity of binding to biological objects and their individual parts, so there is a need to obtain biocompatible nanomaterials without adverse health and environmental effects.

AgNPs are produced primarily for antiseptic applications and have potential antimicrobial activity against many pathogens, while they are toxic not only for bacteria but also for brain cells, liver and stem cells, which can lead to severe damage [3; 7; 13]. MtNPs are caused by toxicity due to important cellular processes: increasing the level of ROS, reducing the level of intracellular glutathione and reducing the potential of the mitochondrial membrane. AgNPs can adversely affect the cells and embryos of freshwater fish, inhibiting the activity of lactate dehydrogenase and antioxidant enzymes in the liver, causing glutathione depletion and lipid peroxidation in the liver and gills with varying degrees of histological damage in the tissues.

MtNPs can have an adverse effect on agroecosystems [1], in which MtNPs can be included in both direct and indirect pathways. Agricultural MtNPs can reach the soil and atmosphere through leaching, precipitation, air flow and trophic transfer. The interaction between MtNPs and plants leads to numerous physiological, morphological and genotoxic changes that need to be fully understood in order to ensure the effective use of nanotechnology in agriculture. The effect of MtNPs on plants depends on the stage of plant growth, the time of exposure to nanoparticles, the method of adsorption, as well as various physical and chemical properties of the plants themselves. However, some MtNPs has a positive effect on the plant system and can improve seed germination and stimulate growth parameters.

At the cellular level, MtNPs can enter various organelles and interfere with mitochondrial and chloroplast chains. In these cases, they can activate metabolic pathways associated with oxidative stress, which is associated with increased concentrations of reactive oxygen and leads to cyto- and genotoxic effects: damage to the membrane, chlorophyll degradation, vacuole shrinkage, DNA damage and chromosomal aberrations. Excessive MtNPs receipt can cause oxidative outbreaks by preventing electron transfer circuit and disrupting ROS detoxification, which leads to genotoxic effects. As a result, the production of secondary metabolites and phytohormones is damaged and growth retardation occurs [1]. The phytotoxic and side effects of MtNPs include impaired water transfer, reduced photosynthetic rate, reduced growth hormone production, metabolic disorders, increased oxidative stress, chromosomal disorders, reduced growth, transcriptional changes in genes, and hypersensitivity to natural toxins. MTNP can also affect useful plant germs in the surrounding soil when used to control phytopathogens. Microbes are associated with epiphytically and endofitically with plants in the rhizosphere and soils near the root of the plant and can significantly contribute to the growth of plants by fixing nitrogen and phosphate salulator [1]. MtNPs used for plant and soil can have a toxic effect on these useful germs as they have on plants. Impact on soil microbiota can be evaluated by measuring breathing and enzymatic activity in the soil [1].

Thus, thorough experimental studies on studying the long-term effects of MtNPs on plant organisms, MtNPs migration in the food chain and the study of changes caused by nanoparticles, in animal and human organisms are necessary.

Conclusions. Green synthesis technology offers a potentially simple, efficient, pure, non-toxic and environmentally friendly method for MtNPs synthesis and has received much attention in recent years because of its economic prospects. Various microorganisms and plant extracts can be used for effective MtNP biosynthesis. Although MTNP synthesis using plant extracts is simpler than the use of microorganisms, the use of microorganisms to produce MtNP is more economical.

The study shows that copper oxide nanoparticles can be synthesised by a simple and environmentally friendly method using peppermint (*Menta piperita*) and lemon balm (*Melissa officialis L.*) leaf extracts.

The research was carried out by the TEM morphology of Cu^0 metal nanoparticles in the volume of polymer nanocomposites such as polymer-metal, created by the restoration of cations Cu^{2+} thermal chemical method and green synthesis method.

It is shown that the average size of copper nanoparticles (Cu^0) is 3.5 and 12.2 nm, respectively.

It was found that the thermochemical method of restoring metal cations and creating nanocomposites is cheap and affordable, but the high temperature of reduction of these cations to the metallic state causes the formation of small metal nanoparticles, in contrast to the polymeric nanocomposite created by the «green» synthesis method, which is environmentally friendly and occurs at low temperatures.

REFERENCES

1. Ali, M. A., Ahmed, T., Wu, W., Hossain, A., Hafeez, R., Islam Masum, M. M., Li, B. (2020). Advancements in plant and microbe-based synthesis of metallic nanoparticles and their antimicrobial activity against plant pathogens. Nanomaterials, 10 (6), 1146 p.

2. Bartolucci, C., Antonacci, A., Arduini, F., Moscone, D., Fraceto, L., Campos, E., Scognamiglio, V. (2020). Green nanomaterials fostering agrifood sustainability. TrAC Trends in Analytical Chemistry, 125, 115840 p.

3. Bityutsky, V. S., Tsekhmistrenko, S. I., Tsekhmistrenko, O. S., Tymoshok, N. O., Spivak, M. Y. (2020). Regulation of redox processes in biological systems with the participation of the Keap1/Nrf2/ARE signaling pathway, biogenic selenium nanoparticles as Nrf2 activators. Regulatory Mechanisms in Biosystems, 11 (4), pp. 483–493.

4. Dasgupta, N., Ranjan, S., Mundekkad, D., Ramalingam, C., Shanker, R., Kumar, A. (2015). Nanotechnology in agro-food: from field to plate. Food Research International, 69, pp. 381–400.

5. Dhillon, N. K., Mukhopadhyay, S. S. (2015). Nanotechnology and allelopathy: synergism in action. J Crop Weed, 11 (2), pp. 187–191.

6. Duhan, J. S., Kumar, R., Kumar, N., Kaur, P., Nehra, K., Duhan, S. (2017). Nanotechnology: The new perspective in precision agriculture. Biotechnology Reports, 15, pp. 11–23.

7. Fraceto, L. F., Grillo, R., de Medeiros, G. A., Scognamiglio, V., Rea, G., Bartolucci, C. (2016). Nanotechnology in agriculture: which innovation potential does it have?. Frontiers in Environmental Science, 4, 20 p.

8. Gahlawat, G., Choudhury, A. R. (2019). A review on the biosynthesis of metal and metal salt nanoparticles by microbes. RSC Adv. 9, pp. 12944–12967.

9. Golinska, P., Wypij, M., Ingle, A. P., Gupta, I., Dahm, H., Rai, M. (2014). Biogenic synthesis of metal nanoparticles from actinomycetes: biomedical applications and cytotoxicity. Applied microbiology and biotechnology, 98, pp. 8083–8097.

10. Hulkoti, N. I., Taranath, T. C. (2014). Biosynthesis of nanoparticles using microbes—a review. Colloids and surfaces B: Biointerfaces, 121, pp. 474–483.

11. Jeyaraj, M., Gurunathan, S., Qasim, M., Kang, M. H., Kim, J. H. (2019). A comprehensive review on the synthesis, characterization, and biomedical application of platinum nanoparticles. Nanomaterials, 9 (12), 1719 p.

12. Kasana, R. C., Panwar, N. R., Kaul, R. K., Kumar, P. (2016). Copper nanoparticles in agriculture: biological synthesis and antimicrobial activity. Nanoscience in Food and Agriculture, 3, pp. 129–143.

13. Kato, Y., Suzuki, M. (2020). Synthesis of metal nanoparticles by microorganisms. Crystals, 10 (7), 589 p.

14. Kingsley, J. D., Ranjan, S., Dasgupta, N., Saha, P. (2013). Nanotechnology for tissue engineering: need, techniques and applications. Journal of pharmacy research, 7 (2), pp. 200–204.

15. Mandal, D., Bolander, M. E., Mukhopadhyay, D., Sarkar, G., Mukherjee, P. (2006). The use of microorganisms for the formation of metal nanoparticles and their application. Applied microbiology and biotechnology, 69, pp. 485–492.

16. Mishra, V. K., Kumar, A. (2009). Impact of metal nanoparticles on the plant growth promoting rhizobacteria. Dig J Nanomater Biostruct, 4 (3), pp. 587–592.

17. Mittal, D., Kaur, G., Singh, P., Yadav, K., Ali, S. A. (2020). Nanoparticle-based sustainable agriculture and food science: Recent advances and future outlook. Frontiers in Nanotechnology, 2, 579954 p.

18. Mocan, T., Matea, C. T., Pop, T., Mosteanu, O., Buzoianu, A. D., Puia, C., Mocan, L. (2017). Development of nanoparticle-based optical sensors for pathogenic bacterial detection. Journal of nanobiotechnology, 15, pp. 1–14.

19. Mohd Yusof, H., Mohamad, R., Zaidan, U. H., Abdul Rahman, N. A. (2019). Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. Journal of animal science and biotechnology, 10, pp. 1–22.

20. Pant, M., Dubey, S., Patanjali, P. K., Naik, S. N., Sharma, S. (2014). Insecticidal activity of eucalyptus oil nanoemulsion with karanja and jatropha aqueous filtrates. International Biodeterioration & Biodegradation, 91, pp. 119–127.

21. Ponmurugan, P., Manjukarunambika, K., Elango, V., Gnanamangai, B. M. (2016). Antifungal activity of biosynthesised copper nanoparticles evaluated against red root-rot disease in tea plants. Journal of Experimental Nanoscience, 11 (13), pp. 1019–1031.

22. Prasad, R. (2019). *Microbial nanobionics*. Springer International Publishing.

23. Rizwan, M. D., Singh, M., Mitra, C. K., Morve, R. K. (2014). Ecofriendly application of nanomaterials: nanobioremediation. Journal of Nanoparticles, pp. 1–7.

24. Saad, E. L., Salem, S. S., Fouda, A., Awad, M. A., El-Gamal, M. S., Abdo, A. M. (2018). New approach for antimicrobial activity and bio-control of various pathogens by biosynthesized copper nanoparticles using endophytic actinomycetes. Journal of Radiation Research and Applied Sciences, 11 (3), pp. 262–270.

25. Shang, Y., Hasan, M. K., Ahammed, G. J., Li, M., Yin, H., Zhou, J. (2019). Applications of nanotechnology in plant growth and crop protection: a review. Molecules, 24 (14), 2558 p.

26. Singh, P., Kim, Y. J., Zhang, D., Yang, D. C. (2016). Biological synthesis of nanoparticles from

plants and microorganisms. Trends in biotechnology, 34 (7), pp. 588–599.

27. Soni, M., Mehta, P., Soni, A., Goswami, G. K. (2018). Green nanoparticles: Synthesis and applications. IOSR J. Biotechnol. Biochem, 4 (3), pp. 78–83.

28. Tsekhmistrenko, O. S., Bityutskyy, V. S., Tsekhmistrenko, S. I., Kharchishin, V. M., Melnichenko, O. M., Rozputnyy, O. I., Onyshchenko, L. S. (2020). Nanotechnologies and environment: A review of pros and cons. Ukrainian journal of ecology, 10 (3), pp. 162–172.

29. Tsekhmistrenko, S. I., Bityutskyy, V. S., Tsekhmistrenko, O. S., Horalskyi, L. P., Tymoshok, N. O., Spivak, M. Y. (2020). Bacterial synthesis of nanoparticles: A green approach. Biosystems Diversity, 28 (1), pp. 9–17.

30. Usman, M., Farooq, M., Wakeel, A., Nawaz, A., Cheema, S. A., ur Rehman, H., Sanaullah, M. (2020). Nanotechnology in agriculture: Current status, challenges and future opportunities. Science of the Total Environment, 721, 137778 p.

Властивості наночастинок срібла та міді, отриманих методами «зеленої» хімії

Цехмістренко О. С.

Суттєвими викликами для сучасного міжнародного співтовариства є зростання населення та зміни клімату, вирішити які допомагають новітні технології. До таких відносять нанотехнології, здатні позитивно впливати на якість агропродукції, мінімізувати негативний вплив пестицидів на довкілля та здоров'я людини, підвищувати продуктивність виробництва та харчову безпеку. Нанотехнології допомагають мінімізувати відходи, виготовляти ефективніші продукти, сприяють розвитку точного землеробства та ефективного управління ресурсами. Мінімізація виробництва небезпечних речовин, зниження витрат енергії в «зелених» нанотехнологіях та майже відсутність утворення токсичних хімікатів робить «зелені» наноматеріали широко вживаними та бажаними у різних галузях людської діяльності.

Розглянуто традиційні та нові методи утворення нанорозмірних матеріалів, акцентовано увагу на їх перевагах та недоліках. Описано поза- та внутрішньоклітинні шляхи утворення наночастинок, висхідні та нисхідні методи. Безпосередньо метою роботи було встановити можливість «зеленого» синтезу наночастинок CuO за допомогою екстрактів м'яти перцевої та меліси лікарської.

Рослинні екстракти готували шляхом екстракції етанолом з листя в екстракторі. Екстракт відділяли від рослинного матеріалу фільтруванням через фільтрувальний папір. Мідні наночастинки синтезували за допомогою реакції відновлення сульфату міді у присутності екстракту перцевої м'яти або меліси лікарської, який відігравав роль як відновника, так і стабілізатора наночастинок.

У роботі показана можливість синтезу наночастинок оксиду міді за допомогою методу з використанням екстрактів листя м'яти перцевої та меліси лікарської. ТЕМ-морфологічне дослідження металевих наночастинок Cu^0 в об'ємі полімерних нанокомпозитів, створених методом «зеленого» синтезу показало, що середній розмір наночастинок міді (Cu^0) становить 3,5 і 12,2 нм, відповідно. Виявлено, що термохімічний метод створення нанокомпозитів дешевий і доступний, однак спричинює утворення малих за розміром металевих наночастинок, на відміну від полімерного нанокомпозиту, створеного методом «зеленого» синтезу, який є екологічним, і відбувається за низьких температур.

Ключові слова: нанобіотехнології, наночастинки, екологічно чиста продукція, наночастинки, оксид міді, частинки срібла.

ORCID iD:
Tsekhmistrenko O.   https://orcid.org/0000-0003-0509-4627