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ASSESSMENT OF THE HAZARD OF METAL NANOPARTICLES FOR THE SOIL-MICROBIOTA-PLANT SYSTEM.

166.01. ECOLOGY

Abstract of PhD thesis in environmental sciences

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The dissertation was performed in the Doctoral School of Natural Sciences, and in the Institute of Microbiology and Biotechnology of the Technical University of Moldova.

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Commision for public defence of PhD thesis:

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SECRIERU Silvia	Doctor in Biological Sciences, associate professor, Technical University of Moldova — ***referent***
COROPCEANU Eduard	Doctor in Chemical Sciences, professor, "Ion Creangă" State Pedagogical University — ***referent***

The defense will take place at 08 september, 2026, at 15:00 in the session of the Commission for public defense of the doctoral thesis within the Doctoral School of Natural Sciences at the address: Moldova State University (http://www.usm.md), str. M. Kogălniceanu 65 A, building 3, room 321, MD-2009, Chisinau, Moldova.

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CONTENTS

CONCEPTUAL LANDMARKS OF THE RESEARCH.

Background and importance of the studied topic.

The unique properties of metal nanoparticles (NPs), including their ability to provide controlled delivery of active agents, antimicrobial activity, and modulation of biochemical processes, make them promising for application in industry, medicine, electronics, environmental protection [1–3], as well as for the development of nanopesticides and nanofertilizers [4, 5]. It has been shown that silver nanoparticles (AgNPs) can increase crop yields by improving plant growth and seed germination, as well as by suppressing fungal growth [6, 7]. Low concentrations of AgNPs exert a stimulatory effect on seedlings, whereas higher concentrations cause an inhibitory effect [8].

The increasing production of NPs inevitably leads to their release into the environment, including plant tissues [9]. According to global estimates, from 63 to 91 % of produced NPs end up in landfills, 8–28 % accumulate in soils, and 0.4–7 % are released into water bodies. Moreover, approximately 95 % of copper released into the environment accumulates in soil and bottom sediments [10, 11]. The accumulation of NPs in soils poses risks of phytotoxicity and bioaccumulation in edible parts of plants. The degree of risk depends on NPs bioavailability, regulated by soil physicochemical properties, which in turn may affect biogeochemical cycles and microbial community structure. The effect of metal NPs on soil microorganisms varies depending on the microbial taxon and the type of particles applied [12, 13], while the phytotoxic effects of NPs depend on plant species, dose, and particle characteristics [14, 15]. For example, treatment of wheat seeds with AgNPs and copper nanoparticles (CuNPs) stimulated germination rate [16], while species-specific responses affecting plant mineral composition were reported for copper-containing NPs [17].

It has been established that NPs can be translocated from the rhizosphere to the aerial parts of plants, with subsequent accumulation in cellular structures, from where they may enter trophic chains into animal and human organisms. For a comprehensive assessment of environmental and toxicological risks, it is necessary to conduct studies covering the entire chain: "soil - soil microbiota - plant - consumer".

The aim of the thesis:

Assessment of the potential environmental hazard of Ag, Au and Cu nanoparticles for the soil-soil microbiota-plants system, based on the investigation of their accumulation and translocation, and analysis of their impact on the physiological and biochemical parameters of plants and the activity of soil microbiota.

Main objectives:

1. To investigate the processes of Ag, Au and Cu nanoparticles accumulation and distribution of in plant tissues as a function of concentration and application route.
2. To evaluate the effect of Ag, Au and Cu nanoparticles on the physiological and biochemical parameters of plants.
3. To evaluate changes in the activity of soil microbiota under the influence of Ag, Au and Cu nanoparticles
4. To estimate the translocation of Ag, Au and Cu nanoparticles into the edible parts of plants and assess the risks associated with the consumption of potentially contaminated plant products.
5. To assess the phytotoxicity of Ag, Au and Cu under conditions close to those of natural ecosystems.
6. To assess the sanitary and environmental risks caused by the contact of plants with Ag, Au and Cu nanoparticles.

The hypothesis of the research:

Upon short-term entry of Ag, Au, and Cu nanoparticles into the "soil - soil microbiota - plant" system, they accumulate in plant tissues occurs, leading to specific dose-dependent changes in plant biochemical parameters and impairment of the functional activity of soil microbiota, which collectively may pose a potential threat to the sustainability of terrestrial ecosystems and to human health.

The synthesis of the research methodology and justification of the research methods:

The following test objects were selected for the study: *Petroselinum crispum* Mill., *Mentha spicata* L., *Calendula officinalis* L., and *Vaccinium myrtillus* L., which are of interest for food use and medicinal value. The elemental composition of plants and soil, NPs parameters, plant biochemical parameters, and functional characteristics of soil microorganisms were studied using modern analytical techniques. The quality of sample preparation and measurements was controlled using certified reference materials. The morphology of Au-, Ag-, and CuNPs was characterized by transmission electron microscopy using a Thermo Scientific Talos F200i microscope. Copper content was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using a PlasmaQuant PQ 9000 Elite spectrometer. Quantitative analysis of silver and gold was performed using electrothermal atomic absorption spectrometry (ETAAS), as well as by proton-induced gamma emission (PIGE), inductively coupled plasma mass spectrometry (ICP-MS), and k_0-based neutron activation analysis (k_0-NAA).

Biochemical analyses of plant biomass were carried out by standard spectrophotometric methods using a T60 Visible Spectrophotometer. Soil microbial activity was assessed by the CO_2 emission rate using the closed dynamic chamber method with a Li-850 gas analyzer. To evaluate metal uptake and translocation in plants, bioconcentration and translocation factors were calculated. The risk of non-carcinogenic effects on human health was determined based on the daily intake of potentially contaminated products and the hazard quotient. The reliability of the results was confirmed by statistical analysis methods.

1. METAL NANOPARTICLES IN THE ENVIRONMENT: SOURCES, MIGRATION, AND ECOLOGICAL EFFECTS.

Chapter 1 presents a review of the global nanomaterials market, systematizes information on the applications of metal NPs, and discusses the regulatory framework for nanosafety, including international standards and national regulations. Synthesis methods, pathways of NPs entry into the environment, and their interactions with ecosystem components are described. Particular attention is paid to the phytoeffects of NPs, their impact on soil microbial communities, the features of their translocation in plant tissues, and their migration through trophic chains. The final part of the chapter formulates conclusions that justify the need for a comprehensive assessment of ecological and toxicological risks in the "soil - microbiota - plant - consumer" system.

2. MATERIALS AND METHODS.

Chapter 2 presents the test objects, materials, and methods used in the study. Laboratory and field experiments with plant objects were conducted in the Sector of Neutron Activation Analysis and Applied Research of the Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research (JINR), during the period 2021–2025, in collaboration with the Institute of Microbiology and Biotechnology of the Technical University of Moldova. The test objects were exposed to solutions of AuNPs, AgNPs, and CuNPs with PVP coating. A schematic diagram of the experimental design is shown in Fig. 2.1.

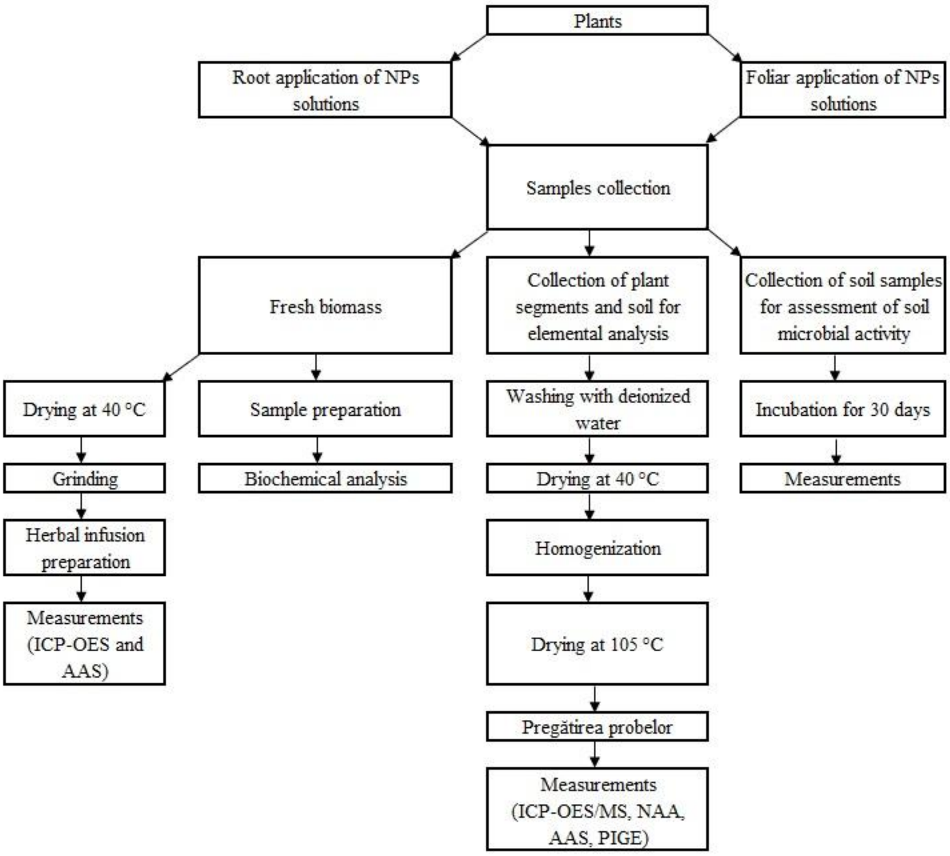

Figure 2.1. Experimental design diagram

This chapter describes the methods used for determining gold, silver, and copper content in soil and plant samples, as well as for assessment of plant biochemical parameters and NPs characterization. Formulas and standard procedures applied for the quantitative assessment of the effect of NPs solutions on soil microbial activity, the ability of plants to absorb and translocate metals, and the potential human health risks associated with the consumption of NPs-treated plants are presented.

3. EFFECT OF THE APPLICATION ROUTE ON GOLD AND COPPER NANOPARTICLES ACCUMULATION AND BIOCHEMICAL STATUS OF *PETROSELINUM CRISPUM* MILL. AS WELL AS FUNCTIONAL ACTIVITY OF SOIL MICROBIOTA.

To evaluate the effect of metal NPs on the soil-soil microbiota-plant system, solutions of AuNPs, AgNPs, and CuNPs stabilized with polyvinylpyrrolidone were used in the experiments. Transmission electron microscopy confirmed the spherical morphology of all studied NPs. It was found that gold nanoparticles (AuNPs) with a size of 1-5 nm were uniformly distributed in the solution, while AgNPs (1-2 nm) were distributed less homogeneously. CuNPs were characterized by a wide size range (15-70 nm) and exhibited a tendency to aggregate. Chapter 3 presents the results of the effect of AuNPs and CuNPs on *Petroselinum crispum* Mill. under foliar and root application. The biochemical parameters of the plants, metal accumulation and distribution in segments were analyzed, the effect of NPs on soil microbial activity was studied, and human health risks were assessed.

3.1. Application of nanoparticles by irrigation.

In the experiments studying of the effect of AuNPs and CuNPs on *Petroselinum crispum* Mill. under root application, it was found that the biochemical responses of the plants were dose-dependent and determined by the NPs type. Treatment with AuNPs solutions in the concentration range of 1-100 mg/L did not cause visible growth changes; however, irrigation with CuNPs solutions at concentrations of 50 and 100 mg/L led to the manifestation of phytotoxicity expressed in leaves wilting.

Treatment with 1 mg/L of AuNPs stimulated the photosynthetic apparatus: chlorophyll content increased by 25.9 % and of carotenoids by 12.7 % (Fig. 3.1). Increasing the NPs concentration above 10 mg/L led to a decrease in carotenoid and chlorophyll content. CuNPs exhibited toxic effects already at 1 mg/L, reducing pigment content by 10.2-37.4 %, with increasing concentration, the reduction in carotenoid content reached 44.4 %. Assessment of the antioxidant status using ABTS and DPPH assays revealed different effects (Fig. 3.1). Upon AuNPs application (1-100 mg/L), ABTS activity decreased by 32.7-41.9 %. In the DPPH assay, an increase in activity (up to 112 % at 5 mg/L) was observed at 1-10 mg/L, while a decrease of 68.4-69.0 % was observed at 50-100 mg/L. CuNPs in most cases reduced antioxidant activity: in the ABTS assay by 32.4-35.7 % (1-10 mg/L), in the DPPH assay by 35.5-71.0 % (1-100 mg/L), except at 100 mg/L where DPPH activity increased 1.5-fold.

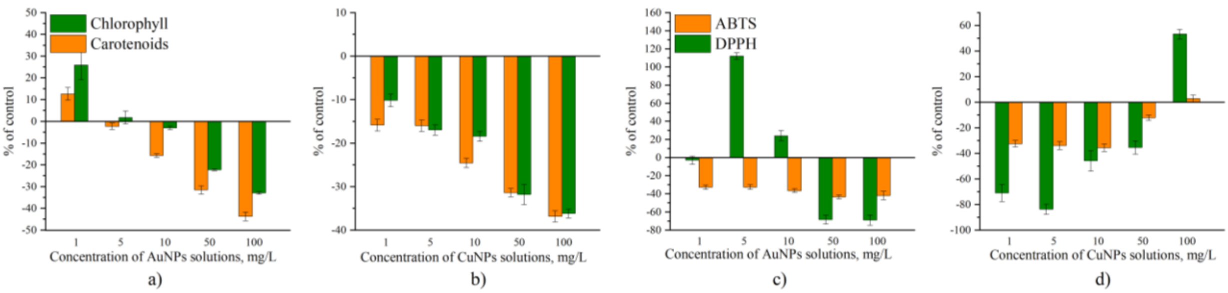

Figure 3.1. Effect of AuNPs and CuNPs on parsley biochemical parameters: (a, b) carotenoid and total chlorophyll content; (c, d) antioxidant activity of extracts.

Analysis of malondialdehyde (MDA) content as an oxidative stress marker showed a dose-dependent increase upon application of both NPs types. For AuNPs, the MDA increase ranged from 7.3 to 50.0 %, while for CuNPs from 16.3 to 56.6 % relative to the control. The reduction in photosynthetic pigment content and antioxidant activity accompanied by an increase in MDA forms a classical biochemical profile of oxidative stress, indicating pronounced phytotoxicity of the studied NPs when species-specific threshold concentrations are exceeded. Low doses of AuNPs (1 mg/L) exhibited a hormetic effect, stimulating the photosynthetic apparatus, whereas CuNPs even at minimal concentrations exerted an inhibitory effect.

When assessing soil microbial activity after irrigation, changes were observed that differed depending on the type of NPs and the duration of soil incubation (Fig. 3.2). In the control, microbial activity increased 2.3-fold by day 30 compared to day 7; in the treated soils, the increase was 82.0 %. On day 7, CuNPs caused a 19.0 % decrease in activity relative to the control, whereas AuNPs did not cause a significant decrease. By day 30, microbial activity under AuNPs was at the control level, while under CuNPs it was 37.0 % lower.

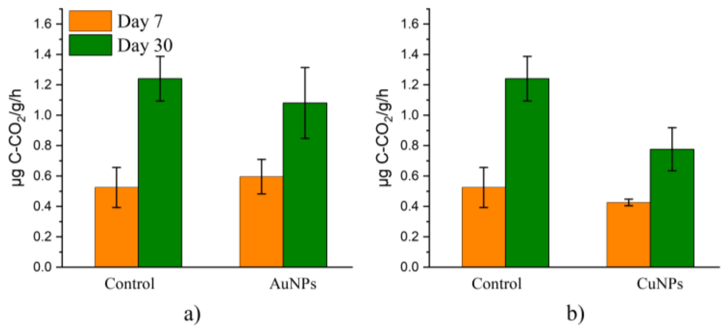

Figure 3.2. Change in microbial respiration rate in soil where parsley plants were grown, treated by irrigation with (a) AuNPs and (b) CuNPs.

At AuNPs concentrations of 1 and 5 mg/L, gold was predominantly accumulated in the root system, exceeding its content in leaves by 1.7-4.9-fold (Fig. 3.3). The bioconcentration factors (BCF) and translocation factors (TF) indicated active transport of gold to the aerial parts.

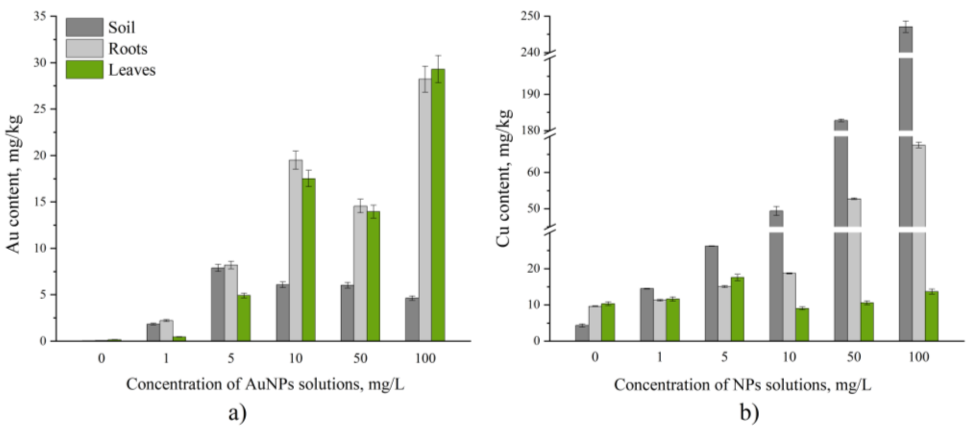

Figure 3.3. Gold and copper content in soil and parsley segments grown in soil treated with (a) AuNPs and (b) CuNPs.

Copper content in soil increased with increasing NPs concentration (at 1 mg/L 3.3-fold, at 100 mg/L 56.5-fold). Copper uptake by roots ranged from 11.3 to 67.6 mg/kg. The translocation efficiency of copper was inversely dependent on NPs concentration. BCF values for all concentrations were below 1.0, and TF exceeded 1.0 only at 1 and 5 mg/L, indicating weak transfer of copper to the aerial parts.

3.2. Application of nanoparticles by foliar spraying.

Foliar treatment with CuNPs had an inhibitory effect on parsley pigment content. Even at a concentration of 1 mg/L, carotenoid content decreased by 15.9 %, and chlorophyll by 18.2 % (Fig. 3.4); at 100 mg/L, the reduction in carotenoids and chlorophyll reached 44.4 % and 47.0 %, respectively. Antioxidant activity in the ABTS assay decreased by 32.4-35.7 % at the concentration range of 1-50 mg/L and by 66.0 % at 100 mg/L CuNPs. In the DPPH assay, at low concentrations activity was at the control level, while at 5–100 mg/L it decreased by 13-48 %. MDA content increased by 12.7-58.5 % at 5-100 mg/L.

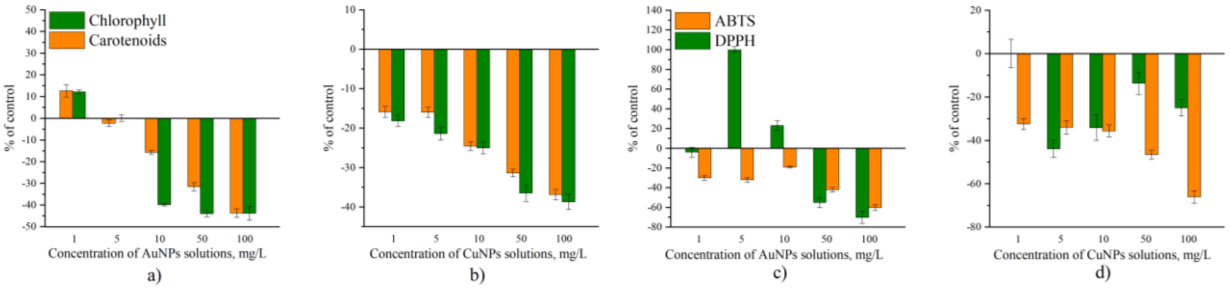

Figure 3.4. Effect of AuNPs and CuNPs on parsley biochemical parameters under foliar treatment: (a, b) carotenoid and total chlorophyll content; (c, d) antioxidant activity of extracts.

Foliar treatment with 1 mg/L of AuNPs stimulated pigments accumulation (carotenoids by 12.7 %, chlorophyll by 12.2 %); however, higher concentrations led to decrease in their levels (up to 43.8 %). In the ABTS assay, antioxidant activity decreased over the entire concentration range, reaching 60.0 % at 100 mg/L relative to the control. Thus, AuNPs at low concentrations (1-5 mg/L) exhibited a hormetic effect, whereas CuNPs even at the minimal concentration exerted a toxic effect.

Upon treatment with 10 mg/L of CuNPs, copper content in leaves corresponded to the control (Fig. 3.5); at 50 and 100 mg/L, it increased 3-fold and 2.5-fold, respectively. In roots, 2.2-6.0-fold decrease in copper content was observed (maximum reduction to 1.6 mg/kg at 10 mg/L). TF values for copper in all experimental variants were below 1.0, indicating weak transfer of copper from leaves to roots. Foliar treatment with AuNPs led to a dose-dependent gold accumulation: content in aerial parts increased from 6.2 to 18.5 mg/kg, and in roots from 0.88 to 2.9 mg/kg. TF values for gold in all variants were <1.0, indicating weak gold translocation from leaves to roots.

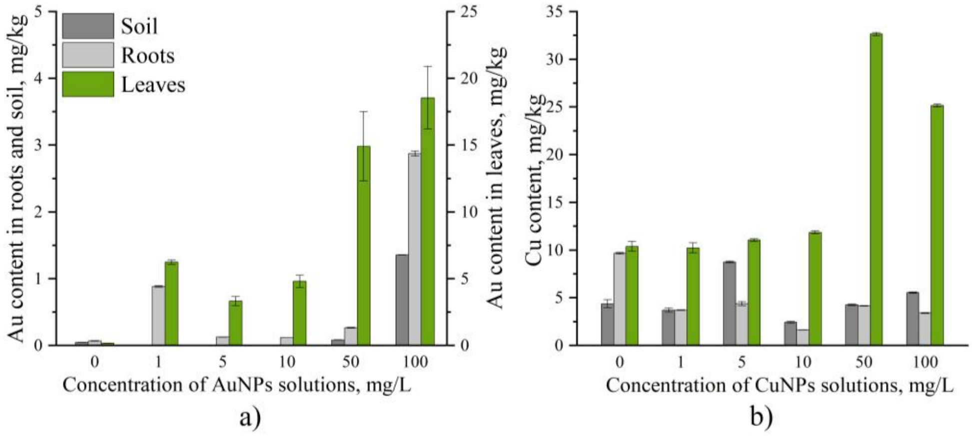

Figure 3.5. Gold and copper content in soil and parsley segments subjected to foliar treatment with (a) AuNPs and (b) CuNPs.

3.3. Assessment of the human health risk associated with the consumption of Petroselinum crispum Mill. treated with AuNPs and CuNPs

To calculate the estimated daily intake (EDI) and hazard quotient (HQ), a fresh herb consumption rate of 80 g/day was adopted in accordance with WHO recommendations. In all CuNPs treatment variants, as well as for AuNPs at concentrations of 1-10 mg/L (foliar application) and at concentrations of AuNPs 1-5 mg/L (irrigation), the HQ remained below 1.0, indicating no significant health risk. However, for parsley treated with AuNPs by foliar spraying at 50-100 mg/L, as well as by irrigation with AuNPs at 10 mg/L, the HQ values exceeded 1, indicating potential danger upon regular consumption of contaminated products [18].

4. EFFECT OF THE APPLICATION ROUTE OF METAL NANOPARTICLES ON THE BIOCHEMICAL PROFILE OF *CALENDULA OFFICINALIS* L., MICROBIAL COMMUNITIES, AND PLANT ACCUMULATION CAPACITY.

Chapter 4 presents the results of the effect of AuNPs, AgNPs, and CuNPs on *Calendula officinalis* Mill. under foliar treatment and root irrigation. The biochemical parameters of the plants, metal accumulation and distribution in segments were analyzed, the effect of NPs on soil microbial activity was investigated, and health risks associated with the consumption of contaminated products were assessed.

4.1. Application of nanoparticles by irrigation.

Root treatment with AuNPs, CuNPs, and AgNPs solutions (1-100 mg/L) affected the morphometric parameters of *C. officinalis* L., expressed in inhibition of flowering, twisting of stems and leaves, and reduction in plant height. The most pronounced negative effects were observed for AgNPs.

Total chlorophyll content in calendula leaves upon treatment with all NPs types exceeded control values by 22.1-82.4 % (Fig. 4.1). Upon exposure to AgNPs, an increase in chlorophyll content was observed over the entire concentrations range (by 34.4-80.8 % compared to the control). The maximum effect was noted upon irrigation with 50-100 mg/L AgNPs (80.8 and 80.2 %, respectively). When AuNPs and CuNPs were used, an increase in chlorophyll content was observed at 1-10 mg/L, whereas at 50 and 100 mg/L the stimulatory effect decreased. Carotenoid content upon treatment with AuNPs and CuNPs increased by 23.09-38.49 % only at 1-10 mg/L, while at 50 and 100 mg/L it was comparable to the control. For AgNPs, a stimulatory effect on carotenoid content was observed at all concentrations, with a maximum increase at 50 and 100 mg/L (45.7 % and 42.8 %, respectively). Upon treatment of calendula with AgNPs, antioxidant activity increased over the entire concentration range, whereas for AuNPs and CuNPs the greatest stimulatory effect was observed at low concentrations (1–5 mg/L) [19]. The decrease in MDA level accompanied by an increase in antioxidant activity and pigment content indicates an effective adaptive response of plants to NPs-induced stress.

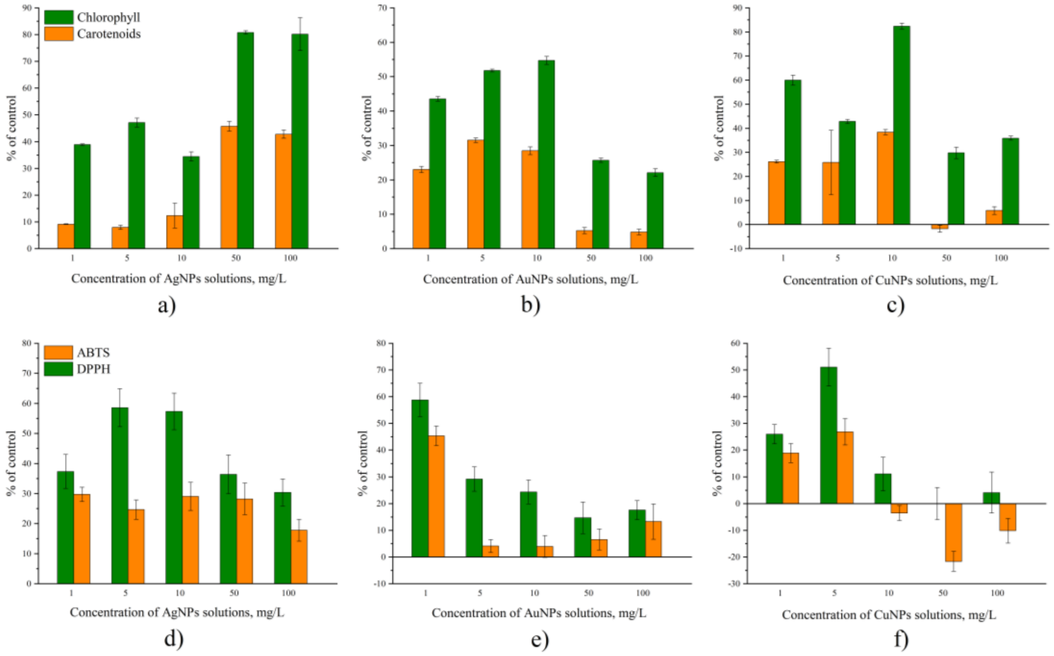

Figure 4.1. Effect of AgNPs, AuNPs, and CuNPs on calendula biochemical parameters under root treatment: (a, b, c) total chlorophyll and carotenoid content; (d, e, f) antioxidant activity of extracts.

Root application of NPs at 100 mg/L exerted different effects on soil microbial activity depending on NPs type and incubation time (Fig. 4.2). On day 7, AgNPs and CuNPs caused inhibition of soil respiration (decrease by 31.0 % and 24.0 %, respectively), whereas AuNPs stimulated activity (increase by 58.0 %). By day 30, an increase in respiration rate by 30.0 % was observed in the control, while all NPs treatments reduced it. The most pronounced inhibitory effect was observed for AgNPs (decrease by 73.0 %), while AuNPs and CuNPs caused a decrease by

30.0 % and 38.0 %, respectively. The observed inhibitory effects, especially in the case of AgNPs, indicate a significant environmental risk upon NPs accumulation in the soil environment.

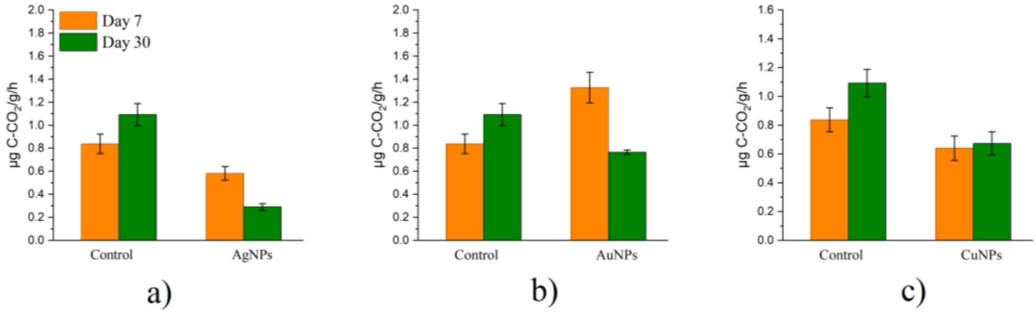

Figure 4.2. Change in soil microbial respiration rate in soil treated with (a) AgNPs, (b) AuNPs, and (c) CuNPs.

Root application of NPs solutions significantly increased metal content in the soil (Fig. 4.3). Gold accumulation in *C. officinalis* L. segments was dose-dependent; at 5 mg/L AuNPs, the maximum gold content was detected in stems (2.6 mg/kg); at 10 and 50 mg/L - in leaves (10.7 and 23.9 mg/kg). At 100 mg/L AuNPs, the order was the following: roots> stems> flowers> leaves. Translocation factors exceeded 1.0 only at concentrations 5 and 10 mg/L. Maximum copper accumulation was observed at 50 mg/L: in roots - 47.8 mg/kg, in leaves - 10.9 mg/kg, in flowers - 29.3 mg/kg. A 1.2- to 2.2-fold decrease in copper content was detected in the root system over the entire concentration range studied [19].

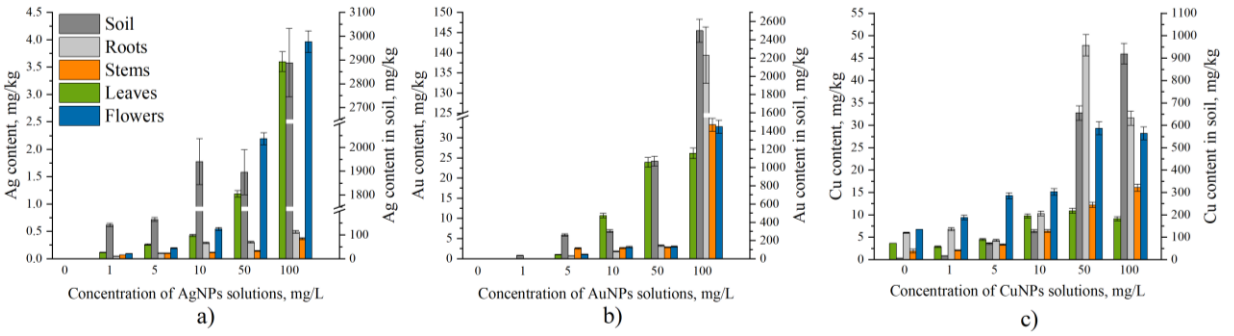

Figure 4.3. Silver, gold, and copper content in soil and calendula segments grown in soil treated by irrigation with (a) AgNPs, (b) AuNPs, and (c) CuNPs.

Upon irrigation with AgNPs, a direct correlation between NPs concentration and silver content in plant segments was found. The highest silver accumulation was observed in flowers (3.9 mg/kg) and leaves (3.6 mg/kg). BCF values for all NPs types were below 1.0, indicating low metal transfer from soil to plants. Selective translocation was established: leaves actively accumulated gold, while flowers - copper. The order of NPs accumulation in segments: in roots - Cu> Ag> Au; in stems, leaves, and flowers Cu> Au> Ag. Copper accumulation in the calendula root system was comparable to that in *P. crispum* roots; however, the transfer to aerial parts was different [20].

4.2. Application of nanoparticles by foliar spraying.

Foliar treatment with AuNPs, AgNPs, and CuNPs solutions at 1-100 mg/L exerted a dose-dependent effect on photosynthetic pigment content, antioxidant activity, and MDA level (Fig. 4.4). The response of *C. officinalis* L. to AgNPs and CuNPs application exhibited similar effects: low concentrations (1-10 mg/L) stimulated chlorophyll production or did not exert a significant effect, whereas higher concentrations (50-100 mg/L) caused a decrease [21].

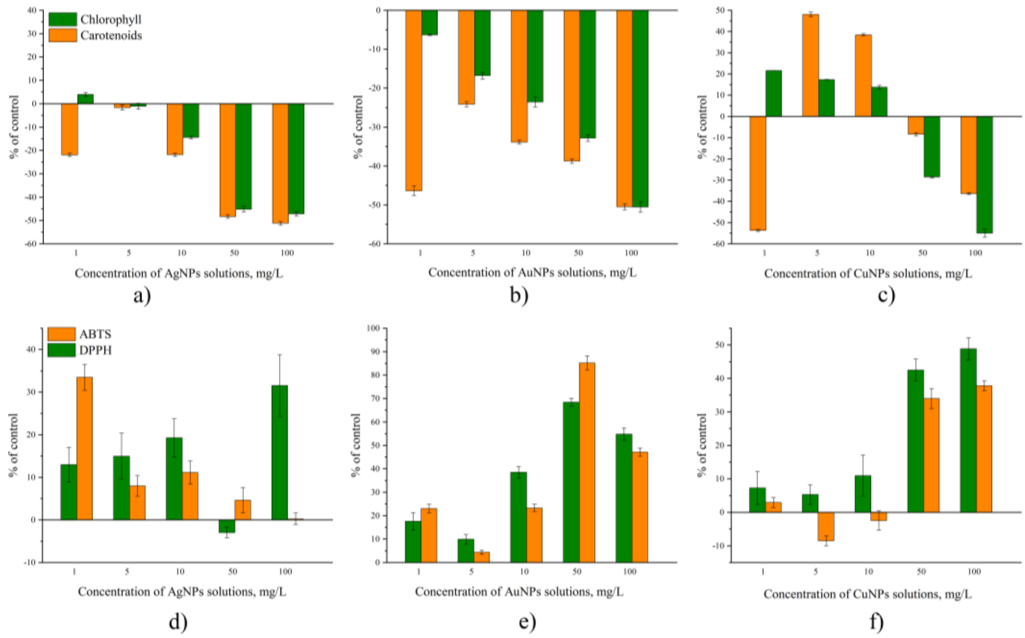

Figure 4.4. Effect of AgNPs, AuNPs, and CuNPs on calendula biochemical parameters under foliar treatment: (a, b, c) chlorophyll and carotenoid content; (d, e, f) antioxidant activity of extracts.

The maximum stimulatory effect was observed for 1 mg/L CuNPs (chlorophyll increase of 21.6 %). Upon AgNP treatment, an increase in chlorophyll content by 3.9 % was observed at 1 mg/L, while AuNPs caused a dose-dependent decrease by 6.3-50.5 %. Carotenoid synthesis under the action of AuNPs and AgNPs decreased over the entire concentration range: by 24.1-50.4 % and 1.7-51.2 %, respectively. CuNPs at 5–10 mg/L stimulated carotenoid synthesis, with a maximum increase by 48.0 % at 5 mg/L, while concentrations of 50-100 mg/L exerted an inhibitory effect. At 1 mg/L of CuNPs, the maximum decrease in carotenoid content (by 53.7 %) was noted.

Antioxidant activity increased most markedly upon treatment with AuNPs and AgNPs. Under the action of AuNPs, antioxidant activity in the ABTS and DPPH assays increased over the entire concentration range, reaching a maximum at 50 mg/L. AgNPs in most cases induced an increase in antioxidant activity. For CuNPs, a significant increase in antioxidant activity was observed at 50 and 100 mg/L (by 33.9 % and 37.8 %, respectively). Foliar treatment of *C. officinalis* L. with AgNPs and AuNPs contributed to an increase in MDA content by 15.0-37.1 %

and 7.6-17.5 %, respectively, relative to the control. The application of CuNPs at low concentrations did not affect MDA content, while at 50-100 mg/L an increase by 6.6-16.0 % was detected.

Foliar treatment with AgNPs, AuNPs, and CuNPs solutions led to the accumulation of silver, gold, and copper in soil and plant segments (Fig. 4.5). Upon exposure to AgNPs and AuNPs, the content of silver and gold in the soil ranged from 0.30 to 1.0 mg/kg and from 0.05 to 0.14 mg/kg, respectively, confirming their ability to translocate from leaves to roots and subsequently be release in the soil. For gold, the metal content in roots decreased in the concentration series from 1 to 10 mg/L, while at high concentrations (50-100 mg/L) active accumulation occurred. Upon CuNPs treatment, copper content in the soil decreased 2.7- to 7.7-fold compared to the control; a decrease in copper content in the root system was also observed with increasing NPs concentration. The maximum copper and silver content in leaves was 48.9 and 42.6 mg/kg, respectively, at 50 mg/L, and gold content reached 143 mg/kg at 100 mg/L.

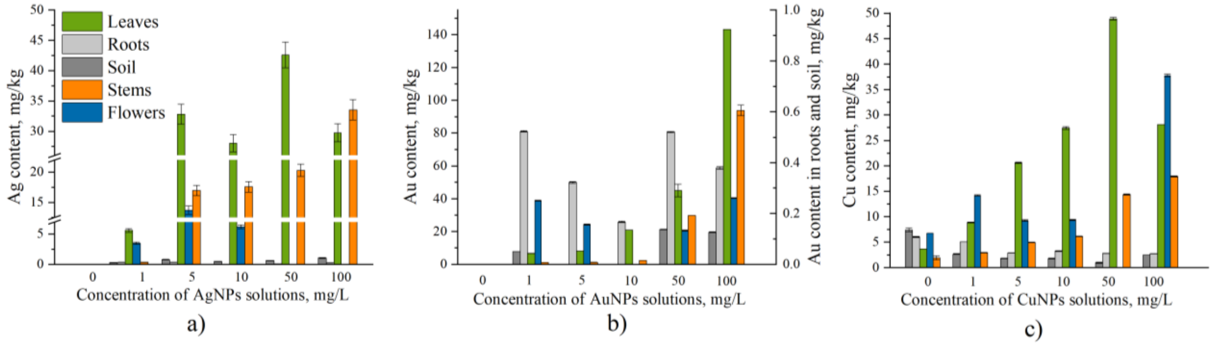

Figure 4.5. Silver, gold, and copper content in soil and calendula segments subjected to foliar treatment with (a) AgNPs, (b) AuNPs, and (c) CuNPs.

4.3. Assessment of the human health risk associated with consumption of herbal infusion from *Calendula officinalis* L. flowers treated with AuNPs, AgNPs, and CuNPs.

Since calendula inflorescences are widely used in phytotherapy, the risk of gold, silver, and copper transfer from plants treated with AuNPs, AgNPs, and CuNPs solutions was assessed. The EDI and HQ were calculated considering a standard single dose of raw material for infusion preparation (1.5 g/day), course intake (122 days per year), 30-year exposure period, and metal extraction coefficients upon brewing (copper 24.0 %, gold 35.0 %, silver 28.0 %). In all calendula treatment variants, the HQ values were below 1.0. Therefore, infusions from calendula inflorescences grown with NPs application at the studied concentrations are safe for health upon course oral administration.

5. EFFECT OF THE APPLICATION ROUTE OF METAL NANOPARTICLES ON THE BIOCHEMICAL PROFILE OF *MENTHA SPICATA* L., MICROBIAL COMMUNITIES, AND PLANT ACCUMULATION CAPACITY.

Chapter 5 presents the results of the effect of AuNPs, AgNPs, and CuNPs on *Mentha spicata* L. under foliar application and root irrigation. The biochemical parameters of the plants, gold, silver, and copper accumulation and distribution in segments were analyzed, the effect of NPs on soil microbial activity was studied, and health risks associated with the consumption of contaminated products were assessed.

5.1. Application of nanoparticles by irrigation.

Root treatment of spearmint with gold, silver, and copper NPs solutions exerted a dose-dependent effect on photosynthetic pigment content, antioxidant activity, and MDA level (Fig. 5.1). The greatest stimulatory effect on carotenoid content upon AuNPs treatment was observed at 1 and 100 mg/L (increase by 24.9 % and 29.9 %, respectively), while at 10 mg/L a decrease of 8.6 % was observed, and at 50 mg/L the most pronounced inhibition (decrease by 41.5 %). Chlorophyll content increased only at 1 mg/L (by 24.5 %); at 5 mg/L it was at the control level, while in the concentrations range of 10-100 mg/L it decreased by 14.2-33.5 %. Antioxidant activity in the ABTS assay increased maximally at 1 mg/L (increase by 52.9 %); at higher concentrations the increase was 12.6-19.4 %. In the DPPH assay, an increase in activity was observed only at 10 mg/L (by 16.9 %), while at 100 mg/L a decrease of 19.4 % was observed. MDA content at AuNPs concentrations of 1-10 mg/L decreased by 10-13 %, whereas at 50 and 100 mg/L it increased by 25.7 % and 29.0 %, respectively [22–24].

Upon irrigation with AgNPs, carotenoid content increased at 1 and 5 mg/L (by 33.7 % and 45 %, respectively). Chlorophyll content and antioxidant activity in the ABTS assay showed the greatest increase at 5 mg/L by 50.7 % and 41.6 %, respectively. In the DPPH assay, most treatment variants led to a decrease in activity, except at 50 mg/L where an increase of 8.1 % was observed. MDA content upon treatment with 5 mg/L AgNPs decreased slightly; in the other variants it increased by 2.8-9.6 % [24].

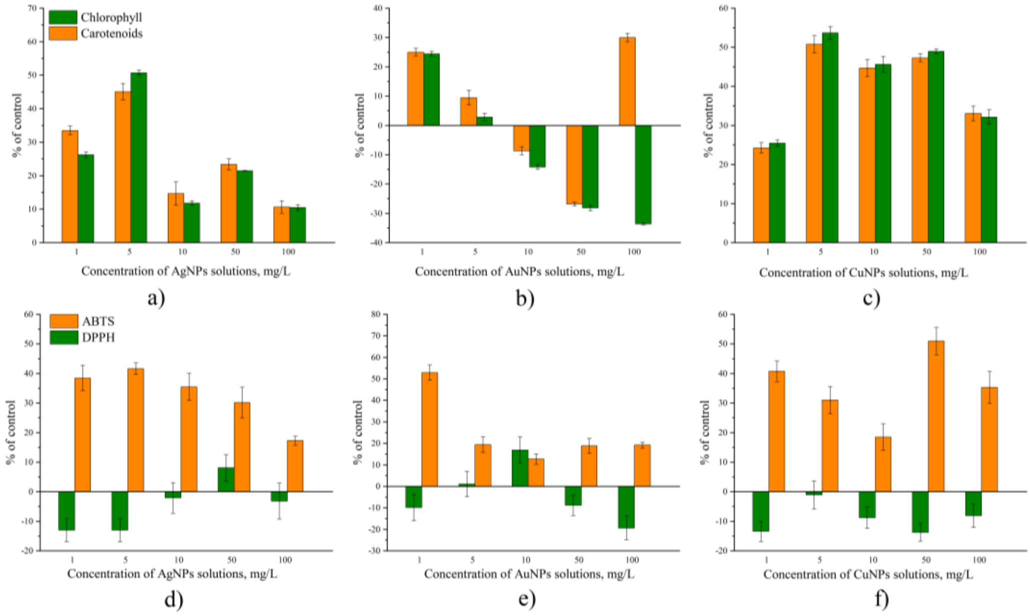

Figure 5.1. Effect of AgNPs, AuNPs, and CuNPs on biochemical parameters of spearmint aerial parts under root treatment: (a, b, c) carotenoid and total chlorophyll content; (d, e, f) antioxidant activity of extracts.

Irrigation with 1-100 mg/L of CuNPs led to an increase in carotenoid and chlorophyll content from 24.0 % to 51.0 %, with a maximum stimulatory effect (up to 50.0 %) in the range of 5-50 mg/L. Antioxidant activity in the ABTS assay increased by 18.0-51.0 % over the entire concentration range, while activity in the DPPH assay was at the control level or decreased. MDA content decreased by 14.4–20.4 % compared to the control in all experimental variants [22].

AuNPs exhibited a stimulatory effect on photosynthetic pigments mainly at low (1 mg/L) and high (100 mg/L) concentrations, whereas AgNPs and CuNPs demonstrated a consistent stimulatory effect on chlorophyll and carotenoid accumulation over a wide concentrations range. The decrease in MDA level accompanied by an increase in pigment content and antioxidant activity upon CuNPs treatment, as well as at low AuNPs concentrations, indicates an effective adaptive response of plants. In contrast, the increase in MDA level at high AuNPs concentrations (50-100 mg/L) and upon AgNPs treatment indicates the development of oxidative stress, which may reduce the therapeutic value of plant biomass.

Upon irrigation with 100 mg/L AuNPs, the soil respiration rate on day 7 increased by 86 %; however, by day 30 a decrease by 28 % was observed compared to the control; from day 7 to day 30, microbial activity decreased by 52.0 % (Fig. 5.2). The inhibitory effect of AgNPs was more pronounced. In the control, the respiration rate increased by 25.0 % from day 7 to day 30, whereas after AgNPs treatment, by day 30 the respiration rate was by 29.0 % lower than the initial level, and on days 7 and 30 it was 2.6- and 4.5-fold lower than the control, respectively. When CuNPs were used, an inhibitory effect was observed throughout the period: by 27.0 % and 65.0 % on days 7 and 30, respectively. Between these time points, respiration in CuNPs-treated samples decreased by 41 %, whereas in the control it increased by 25.0 %.

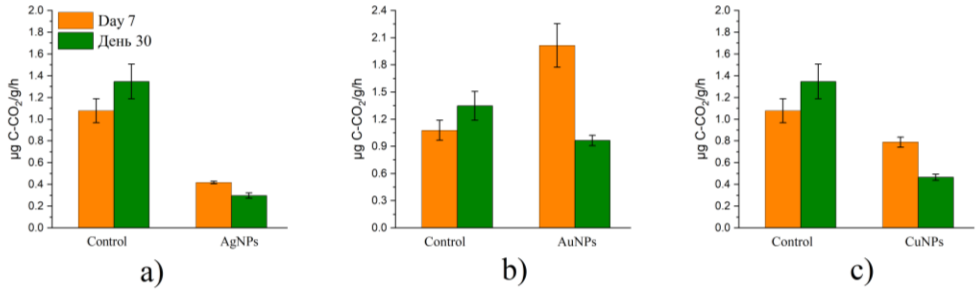

Figure 5.2. Change in soil microbial respiration rate in soil treated with (a) AgNPs, (b) AuNPs, and (c) CuNPs.

Upon irrigation of spearmint with AuNPs solutions (1-100 mg/L), a direct correlation was found between their concentration and gold content in the soil, roots, and leaves (Fig. 5.3). The highest gold accumulation was observed in the root system (3.0-454 mg/kg) compared to leaves (0.12-5.5 mg/kg) and stems (0.05-0.52 mg/kg) (Fig. 5.3). BCF values were below 1.0 for all soil-plant systems, and TF exceeded 1.0 only for the leaves-stems system [23].

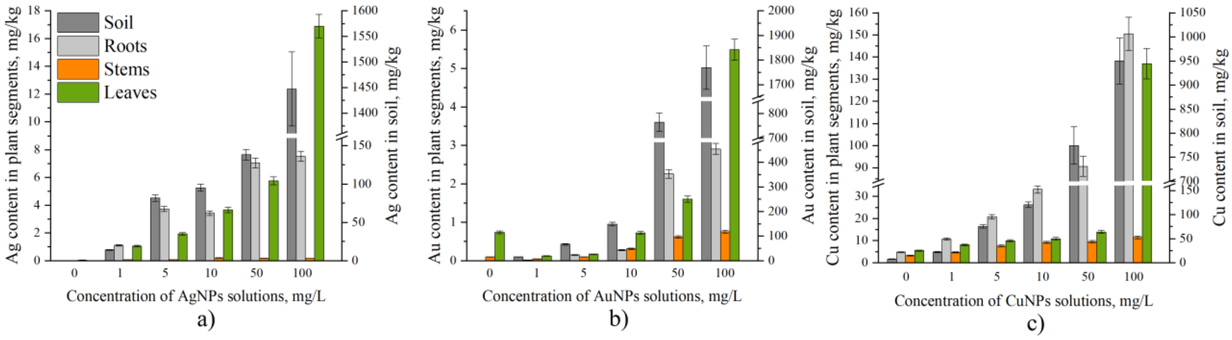

Figure 5.3. Silver, gold, and copper content in soil and spearmint segments grown in soil treated with (a) AgNPs, (b) AuNPs, and (c) CuNPs.

Root application of AgNPs led to a slowdown in plant growth, changes in stem shape, and leaves curling. Silver accumulation in the soil correlated with its concentration in the solution; at 1 mg/L silver content in the soil reached 14.0 mg/kg, and at 100 mg/L - 1447 mg/kg. A direct correlation was found between silver content in spearmint segments and NPs concentration in the solution; in roots, the content ranged from 1.1 to 7.5 mg/kg, in leaves from 1.1 to 16.9 mg/kg. At AgNPs concentrations of 5 and 50 mg/L, accumulation in the system followed the order: soil> roots> leaves> stems; at 1, 10, and 100 mg/L soil> leaves> roots> stems. BCF values were below 1.0 for all segments, and TF exceeded 1.0 for the stem-leaf system [24].

Upon root application of CuNPs, the main copper accumulation was observed in the soil (up to 950 mg/kg) and roots (up to 150 mg/kg). Copper content in the soil increased 3- to 128-fold, and in roots 2- to 31-fold compared to the control. TF values decreased from 0.44 to 0.08 for the root-stem system and from 1.7 to 1.2 for the stem-leaf system in the range of 1-100 mg/L. However, at 50 and 100 mg/L, the TF for the stem-leaf system increased to 1.5 and 11.9, respectively. Copper content in leaves exceeded the control by 1.4- to 2.5-fold at 1-50 mg/L, and at 100 mg/L reached 137 mg/kg, which was 25-fold higher than the control [22].

5.2. Application of nanoparticles by foliar spraying.

Foliar treatment of spearmint with AuNPs caused an increase in chlorophyll content over the entire concentration range by 16.5-41.5 % compared to the control. The maximum stimulatory effect was observed at 1 and 5 mg/L: chlorophyll content increased by 41.5 % and 37.7 %, and of carotenoids by 48.0 % and 43.5 %, respectively. Foliar application of CuNPs also stimulated chlorophyll and carotenoid production. Chlorophyll content increased by 24.2-33.4 % upon treatment with 1-50 mg/L CuNPs, whereas 100 mg/L had no significant effect (Fig. 5.4).

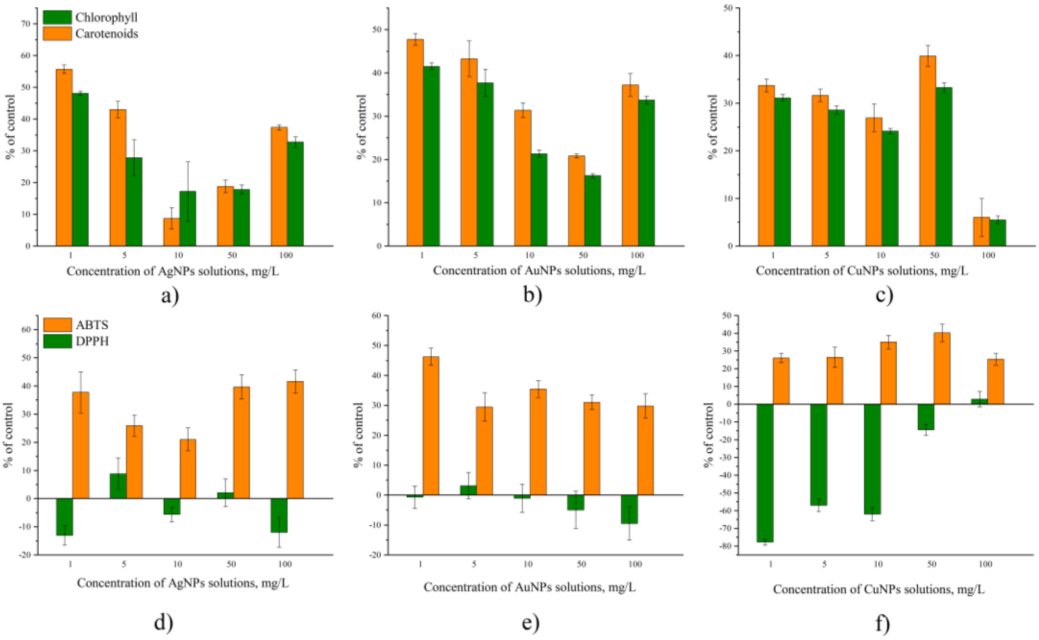

Figure 5.4. Effect of AgNPs, AuNPs, and CuNPs on spearmint biochemical parameters under foliar treatment: (a, b, c) carotenoid and total chlorophyll content; (d, e, f) antioxidant activity of extracts.

Antioxidant activity in the ABTS assay upon treatment with 1 mg/L AuNPs increased by 46.2 %; and at 5-100 mg/L by 39.4-35.4 %. MDA level decreased by up to 33.2 % relative to the control, indicating the absence of oxidative stress. Upon AgNPs treatment, ABTS assay antioxidant activity increased by up to 41.6 % (maximum at 100 mg/L), while DPPH activity decreased by no more than 13.0 %. The maximum decrease in MDA relative to the control was 11.2 %. Upon spraying with 5-50 mg/L CuNPs, ABTS assay antioxidant activity increased by 26.1-40.2 % relative to the control; and at 100 mg/L by 25.4 %. MDA content was at the control level or showed a slight decrease (maximum 14.8 %).

Spraying of *M. spicata* with AuNPs solutions caused a dose-dependent increase in gold content in the aerial parts: in leaves from 3.2 to 552 mg/kg (4- to 746-fold higher than the control), in stems from 0.57 to 18.4 mg/kg (6- to 188-fold higher) (Fig. 5.5). In all treatment variants, TF<1. AgNPs treatment also led to a dose-dependent accumulation of silver in the aerial parts: the maximum content (140 mg/kg) was detected in leaves at 100 mg/L. Calculated TF values indicated weak translocation of silver between segments (TF<1) in all variants. Silver accumulation was observed in the soil (1.1-2.4 mg/kg); at 1 mg/L, the silver content in the soil exceeded the control by 62-fold.

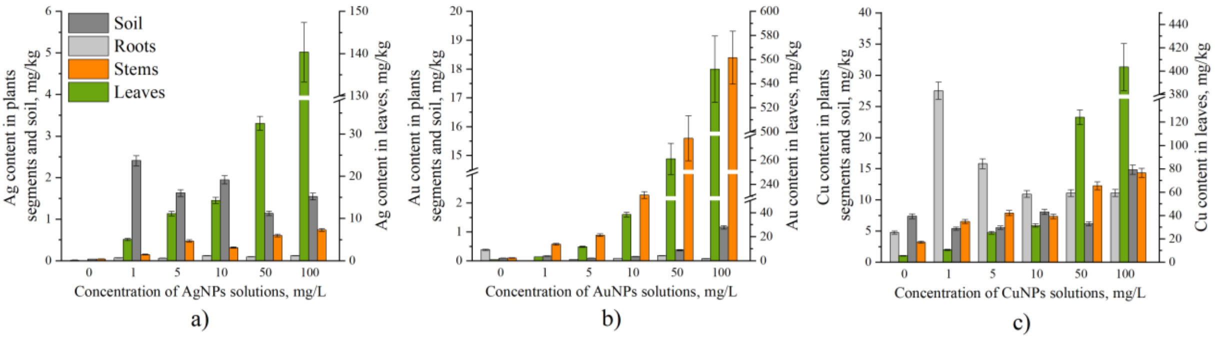

Figure 5.5. Silver, gold, and copper content in soil and spearmint segments subjected to foliar treatment with (a) AgNPs, (b) AuNPs, and (c) CuNPs.

Spraying with CuNPs led to a dose-dependent increase in copper content in stems and leaves, whereas an inverse relationship was observed in roots. The highest TF values for the stem-root system (4.2 and 2.0) was observed at 1 and 5 mg/L. Copper uptake into roots was maximal (27.5 mg/kg) at 1 mg/L, whereas at 10-100 mg/L it exceeded the control by only 2.3-fold. The maximum copper content in leaves (124 and 403 mg/kg) was achieved at 50 and 100 mg/L.

5.3. Assessment of the human health risk associated with consumption of herbal infusion from *Mentha spicata* L. treated with AgNPs, AuNPs, and CuNPs

To assess the risk of metal transfer from *M. spicata* treated with AuNPs, AgNPs, and CuNPs, infusions were prepared from dried leaves. The degree of extraction depended on the NPs type and application method: for gold 2-45 % (root) and 2-90 % (foliar); for silver 0.16-0.64 % (root) and up to 98 % (foliar); for copper 3-55 % (both methods). HQ values for spearmint infusions treated with AuNPs and CuNPs were below 1.0 regardless of the application method, indicating no toxicological risk. For AgNPs, under root treatment HQ also did not exceed 1.0; however, under foliar application at 10-100 mg/L, HQ values ranged from 1.2 to 21.5, indicating a potential health risk upon regular consumption. The traditional EDI and HQ indicators, based on the assessment of soluble element forms, do not fully reflect the specific properties of NPs. The obtained data confirm the need for a comprehensive approach to the safety assessment of plant products grown with NPs application [22–24].

6. EFFECT OF SINGLE APPLICATION OF METAL NANOPARTICLES ON THE BIOCHEMICAL PROFILE AND PLANT ACCUMULATION CAPACITY UNDER FIELD CONDITIONS.

6.1. Accumulation of gold, silver, and copper in soil and plant segments and their effect on the biochemical composition of *Mentha spicata* L. upon treatment with AgNPs, AuNPs, and CuNPs.

The main goal of the field experiments was to evaluate the response of *M. spicata* to a single root application of NPs under field conditions, followed by establishing correlations between NPs concentration, their accumulation level in soil, the intensity of translocation into plant tissues, and changes in the plant biochemical profile. It was found that the total metal content in soil and their accumulation in *M. spicata* decreased in the order Cu> Ag> Au. In roots and leaves, the accumulation order followed this trend, while in stems, silver accumulated less intensively than gold (Fig. 6.1).

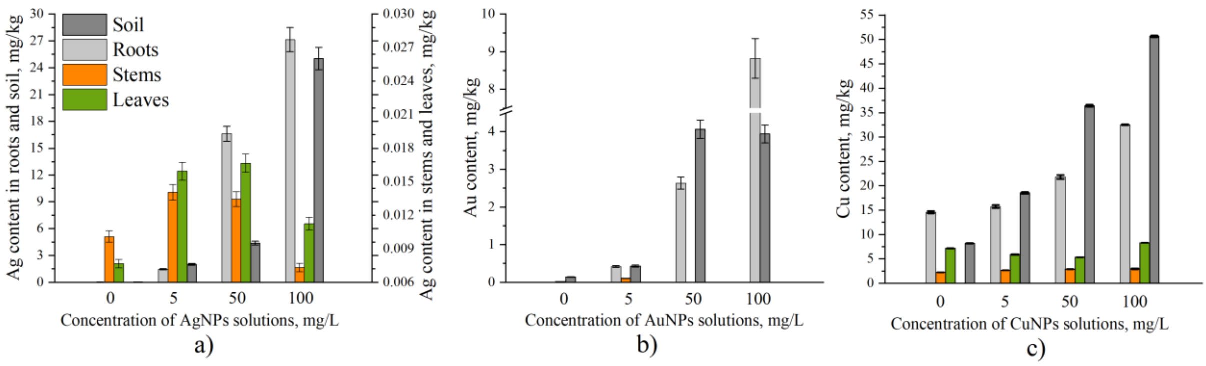

Figure 6.1. Silver, gold, and copper content in soil and spearmint segments subjected to root treatment with (a) AgNPs, (b) AuNPs, and (c) CuNPs under field conditions.

Gold accumulation occurred mainly in roots and soil. The maximum content in roots (8.8 mg/kg) was observed at 100 mg/L (BCF 2.2). Translocation to aerial parts was negligible: gold was detected only in stems at 5 mg/L (0.10 mg/kg) and 50 mg/L (0.002 mg/kg).

The application of AgNPs to the soil led to active accumulation of silver in the *M. spicata* root system. The high bioavailability and uptake capacity of the roots were confirmed by a bioconcentration factor (BCF) of 3.8. At the same time, the low TF indicates limited metal transfer from roots to aerial parts, suggesting that the root system acts as an effective barrier limiting silver transport to shoots [25].

Under the influence of CuNPs, copper content in the soil increased 2- to 6-fold (up to 50.6 mg/kg). In roots, the metal content increased 1.5- and 2.2-fold at 50 and 100 mg/L; however, BCF <1 indicated weak transfer from soil to roots. In stems, copper content increased by 18.0-32.0 %, whereas in leaves it decreased by 18.0 % and 25.0 % at 5 and 50 mg/L. Low TF values in the root-shoot system confirmed limited copper transfer to the aerial part, while high TF values for the leaf-stem system (1.8-3.2) indicated redistribution of copper within the shoots with accumulation in leaves.

The effect of AuNPs on pigments was characterized by an increase in chlorophyll content and a decrease in carotenoids (Fig. 6.2). Chlorophyll content increased maximally by 30 % at 5 mg/L, and at 50 and 100 mg/L its content increased by 20 %. Ascorbic acid (AA) level increased 2.1- to 2.9-fold (maximum at 50 mg/L). Glutathione (GSH) content at 50 mg/L decreased by 42.0 %, while at 5 and 100 mg/L its accumulation increased by 25.0 % and 16.0 %.

Upon root treatment with 5 and 100 mg/L AgNPs, carotenoid content did not differ from the control, while at 50 mg/L an increase of 22.0 % was observed (Fig. 6.2). Chlorophyll content increased by 22.0 % at 5 and 100 mg/L, with a maximum increase (54.0 %) at 50 mg/L. At 5 and 50 mg/L, AA level also increased 2.1-fold, and GSH content increased by 17.0 % and 18.0 %. Whereas at 100 mg/L, AA content corresponded to the control, and GSH level decreased 2.2-fold.

The effect of CuNPs on *M. spicata* was dose-dependent: a low concentration (5 mg/L) stimulated the synthesis of photosynthetic pigments (carotenoids by 16.0 %, chlorophyll by 30.0 %), while a high concentration (100 mg/L) caused their inhibition. AA level increased 1.5- to 2-fold (maximum at 100 mg/L), and glutathione content increased by 15.7-26.0 % over the entire concentration range. The stimulation of the antioxidant system indicates its important role in compensating for CuNPs-induced oxidative stress, even at concentrations toxic to the photosynthetic apparatus.

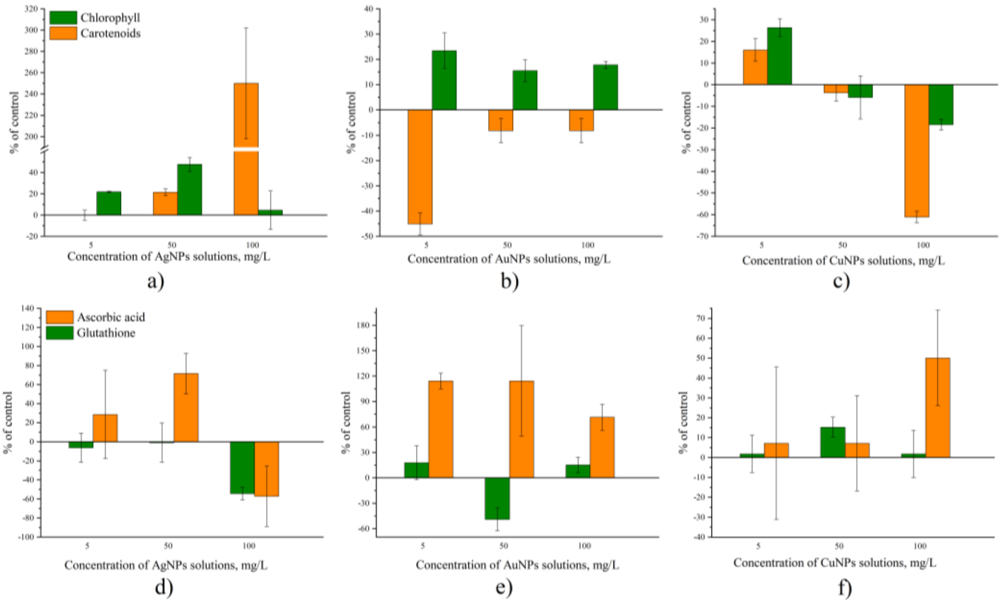

Figure 6.2. Effect of AgNPs, AuNPs, and CuNPs on spearmint biochemical parameters under field conditions: (a, b, c) carotenoid and total chlorophyll content; (d, e, f) ascorbic acid and glutathione content.

6.2. Accumulation of silver and copper in soil and plant segments and their effect on the biochemical composition of *Vaccinium myrtillus* L. upon treatment with AgNPs and CuNPs.

Under field conditions, *V. myrtillus* plants at the flowering stage were subjected to a single treatment with AgNPs and CuNPs solutions at 50, 100, and 200 mg/L. Soil and plant samples (roots, stems, leaves, and berries) were collected 60 days after NPs application. Irrigation with CuNPs led to a dose-dependent increase in copper content in the soil (up to 59.9 mg/kg at 200 mg/L) (Fig. 6.3). In roots, copper content increased from 8.9 to 14.1 mg/kg, exceeding the control by 1.6- to 2.5-fold, while in the aerial parts of the experimental plants, copper content was lower than the control. BCF <1 and TF <1 indicated low uptake and limited translocation of copper. However, berries at 200 mg/L contained 34 % more copper than the control [26]. Upon exposure to 200 mg/L AgNPs, silver content in the soil was 191 mg/kg, while in the control soil and soil treated with 50 and 100 mg/L, silver was not detected. In roots, silver content increased to 6.3 mg/kg (200 mg/L). A dose-dependent increase in silver was observed in berries, reaching 14.7 mg/kg upon AgNP treatment at the maximum concentration (TF>1 confirms active transport of silver from roots to berries).

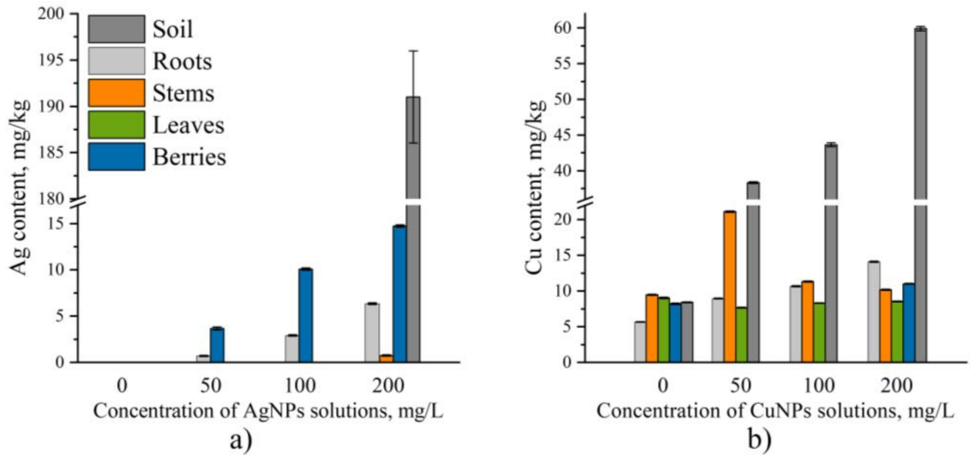

Figure 6.3. Silver and copper content in soil and bilberry segments subjected to treatment with (a) AgNPs and (b) CuNPs under field conditions.

Both NPs types caused an increase in carotenoid content, while chlorophyll level either increased or remained at the control level. The greatest stimulatory effect on pigment content was observed at 50 mg/L CuNPs, where total chlorophyll content exceeded the control level by 13.1 %, and carotenoid content by 27.7 %. AgNPs treatment in all cases led to a statistically significant increase in pigment content, with the greatest stimulatory effect exerted by 100 mg/L AgNPs.

The radical-scavenging capacity of leaf extracts treated with CuNPs and AgNPs in the DPPH and ABTS assays was significantly higher than the control. MDA content, both upon AgNPs (27.9-62.8 %) and CuNPs (16.8-39.8 %) treatment, increased in a dose-dependent manner with increasing solution concentration compared to the control, indicating the presence of oxidative stress in the treated plants. In all treatment variants, HQ was below 1, except for berries treated with 100 and 200 mg/L AgNPs (HQ>1), indicating potential danger upon regular consumption of silver-containing berries.

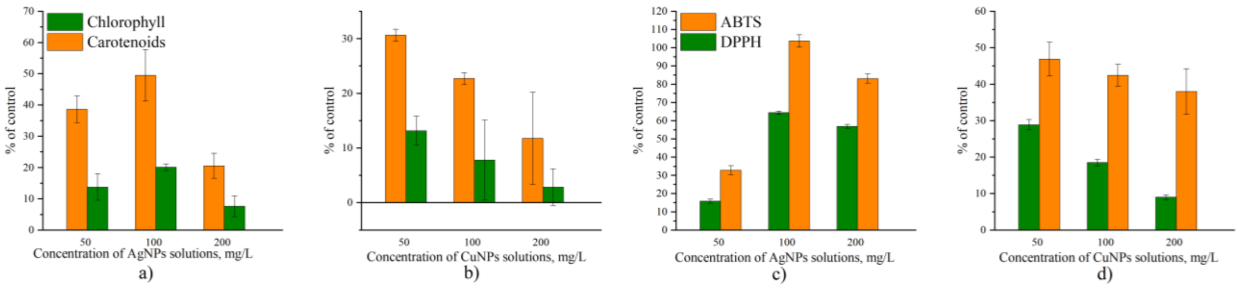

Figure 6.4. Effect of AgNPs and CuNPs on biochemical parameters of bilberry leaves under field conditions: (a, b) chlorophyll and carotenoid content; (c, d) antioxidant activity of extracts.

CONCLUSIONS AND RECOMMENDATIONS

1. The results obtained in this thesis confirmed the scientific hypothesis and showed that the pattern of Ag, Au, and Cu accumulation and translocation in the soil-plant system is determined by the NPs type, application route, concentration, and plant species-specific characteristics. Under root irrigation, the root system acts as an effective barrier: the accumulation of Ag, Au, and Cu occurred mainly in the soil and roots. However, selective translocation was observed: Ag was actively accumulated in the leaves of *Mentha spicata* L. and berries of *Vaccinium myrtillus* L., Au in leaves, and Cu in flowers of *Calendula officinalis* L. Foliar treatment with AgNPs, AuNPs, and CuNPs induced a reverse translocation process: Ag, Au, and Cu were not only accumulate in the aerial biomass but also translocated to the root system with subsequent release into the rhizosphere soil, creating a risk of secondary contamination of agroecosystems.

2. The effect of AgNPs, AuNPs, and CuNPs on the photosynthetic apparatus and antioxidant system was dose-dependent and species-specific. Low NPs concentrations (1-10 mg/L), as a rule, stimulated the synthesis of photosynthetic pigments and contributed to an increase in antioxidant activity, inducing plant defense mechanisms. Higher NPs concentrations (50-100 mg/L) inhibited these processes. At the same time, for AgNPs, despite stimulatory effects on the photosynthetic system and antioxidant production, morphological disturbances (inhibition of flowering, deformation of leaves and stems) were observed.

3. Long-term exposure to metal NPs led to inhibition of soil microbial community activity. The greatest inhibitory effect was exerted by AgNPs and CuNPs, causing a sustained reduction in soil respiration rate (up to 73 % relative to the control), which may disrupt biogeochemical cycles. The effect of AuNPs on soil microbiota varied from short-term stimulation

to subsequent inhibition of soil respiration, which requires further study of microbial community composition.

4. The ability of NPs to translocate into edible plant parts has been confirmed for all studied plants. The greatest danger was posed by AgNPs: the Ag content in bilberry berries reached 14.7 mg/kg, and in spearmint leaves 140 mg/kg. Regular consumption of products with Ag content above 10.0 mg/kg is associated with the risk of adverse effects. Foliar treatment leads to significantly higher metal extraction into herbal infusions compared to root treatment, which should be taken into account when developing regulations. The necessity of establishing separate reference doses and maximum permissible levels for nano-sized forms of metals in food products is substantiated.

5. Root treatment with AgNPs, AuNPs, and CuNPs solutions under field conditions confirmed the barrier function of the root system limited metal translocation. For *Mentha spicata* L., low mobility of Ag, Au, and Cu in the soil-plant system under single application is characteristic. However, for *Vaccinium myrtillus* L., species-specificity was revealed: silver was actively translocated into berries, indicating a higher risk for consumers. Moderate concentrations of nanoparticles under field conditions induced adaptive responses, increasing plant resistance and nutritional value of products.

6. The obtained data indicate the dual nature of of metal NPs impact on agroecosystems. Low concentrations (1-10 mg/L) can be considered as promising agrotechnological agents that improve the physiological state of plants. However, the presence of NPs in the system is associated with sanitary-ecological risks: toxic effects on soil microbiota, potential secondary soil contamination, and the possibility of transfer into trophic networks. Strict control of applied doses and consideration of crop species-specific characteristics are mandatory conditions for the prevention of negative consequences.

The scientific task of assessing the environmental consequences of the use of AgNPs, AuNPs, and CuNPs in agriculture has been achieved by establishing species-specific patterns of metal accumulation and translocation in the soil-plant system, depending on NPs type, concentration, and application method. Concentration ranges (1-10 mg/L) and application methods that stimulate the physiological and biochemical parameters of the studied plant species, as well as threshold levels at which negative effects for plants, soil microbiota, and human health risks occur, have been experimentally determined.

From a practical point of view, the identified concentration ranges and NPs application methods can be used in the development of environmentally safe agrotechnology and biostimulants. The established threshold exposure levels allow minimizing the risks of contamination of agricultural products and soil microbiota, and also substantiate the need for the development of regulations for nano-sized forms of metals in food products.

Practical recommendations:

1. For use as biostimulants, it is advisable to apply AuNPs, AgNPs, and CuNPs at concentrations not exceeding 10 mg/L. The choice of application route and NPs type should be made taking into account the intended use of the plant product and potential environmental risks.

2. To ensure the safety of raw food materials and food products, it is recommended to monitor the content of metal nanoparticles in edible plant parts.

3. To prevent degradation of soil micropinocytosis when using NPs in agroecosystems, the use of AgNPs and CuNPs, which exhibit the most pronounced inhibitory effect on soil microbiota, should be limited.

4. It is necessary to develop and establish separate regulations and reference doses for nano-sized forms of metals in food products, taking into account their increased bioavailability.

Suggestions for future research:

1. Studies of the mechanisms of metal NPs with different types of surface coatings translocation in plant tissues are required, as well as the identification of key factors determining the intensity and selectivity of these processes.

2. Research on the combined effect of mixtures of metal NPs on plants and soil microbiota is needed, including the assessment of synergistic and antagonistic effects under conditions as close to real agrotechnological scenarios.

3. A detailed study of the structural and functional changes of soil microbial communities under long-term exposure to metal nanoparticles is required to predict disturbances in soil processes.

4. Extended toxicological studies on laboratory animals are necessary to establish reference doses for chronic intake of nano-sized forms of metals with food of plant origin .

5. Research on the patterns of translocation of metal nanoparticles through trophic chains, taking into account the diversity of agricultural crops, soil types, and higher-level consumers, is required to assess environmental risks.

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22. PESHKOVA, A., ZINICOVSCAIA, I., CEPOI, L., RUDI, L., et al. Features of Translocation of Copper Nanoparticles in *Mentha Spicata* L. and Extraction into Infusion. In: *Plants*. 2025. vol. 14, nr. 21, p. 3318. Electronic ISSN: 2223-7747. Print ISSN: 2223-7747. DOI: 10.3390/plants14213318.
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LIST OF PUBLICATIONS ON THE SUBJECT OF THE THESIS

2. Articles in scientific journals

2.1. in journals indexed in Web of Science and SCOPUS

1. **PESHKOVA, A.,** ZINICOVSCAIA, I., GORELOVA, S., CEPOI L. Effects of Copper, Gold and Silver Nanoparticles on Mentha spicata L. Under Field Conditions. BioNanoSci. 16, 358 (2026). https://doi.org/10.1007/s12668-026-02608-3
2. **PESHKOVA, A.A.**, ZINICOVSCAIA, I., RUDI, L., CHIRIAC, T., CORCIMARU, S., YUSHIN, N., CEPOI, L. Evaluation of the Response of *Mentha spicata* L. and Soil Microbiota to Silver Nanoparticles and the Associated Risk of Herbal Tea Contamination. In: *Phys. Part. Nuclei Lett.* **23**, 57–64 (2026). https://doi.org/10.1134/S1547477125701948
3. **PESHKOVA, A.,** ZINICOVSCAIA, I., CEPOI, L., RUDI, L., CHIRIAC, T., CORCIMARU, S., YUSHIN, N., & LE ROUX, R. Features of Translocation of Copper Nanoparticles in *Mentha spicata* L. and Extraction into Infusion. *Plants*, 2025, *14*(21), 3318. ISSN 2223-7747. https://doi.org/10.3390/plants14213318
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6. **PESHKOVA, A.;** ZINICOVSCAIA, I.; CEPOI, L.; RUDI, L.; CHIRIAC, T.; YUSHIN, N.; GANEA, L. Evaluation of the Effects of High Silver and Copper Nanoparticle Concentrations on *Vaccinium myrtillus* L. under Field Conditions. In: *Nanomaterials* 2024, *14*, 1545. ISSN: 2079-4991. https://doi.org/10.3390/nano14191545
7. **PESHKOVA, A.**; ZINICOVSCAIA, I.; CEPOI, L.; RUDI, L.; CHIRIAC, T.; YUSHIN, N.; ANH, T.T.; MANH DUNG, H.; CORCIMARU, S. Effects of Gold Nanoparticles on *Mentha spicata* L., Soil Microbiota, and Human Health Risks: Impact of Exposure Routes. In: *Nanomaterials* 2024, *14*, 955. ISSN: 2079-4991. https://doi.org/10.3390/nano14110955
8. **PESHKOVA, A.,** ZINICOVSCAIA, I., CEPOI, L., RUDI, L., CHIRIAC, T., YUSHIN, N.; SOHATSKY, A. Features of Copper and Gold Nanoparticle Translocation in *Petroselinum crispum* Segments. In: *Nanomaterials.* 2023, nr. 13, 1754. ISSN: 2079-4991. https://doi.org/10.3390/nano13111754.

3. Articles in conference proceedings and other scientific events

3.1. in conference proceedings indexed by Web of Science and SCOPUS

1. CEPOI, L., **PESHKOVA, A.,** ZINICOVSCAIA, I., RUDI, L., CHIRIAC, T., YUSHIN, N. Translocation of Nano-Gold, -Silver and -Copper in *Calendula officinalis* L. Tissues Under Foliar Exposure. In: *IFMBE Proceedings: 7th International Conference on Nanotechnologies*

and Biomedical Engineering, ICNBME 2025, Ed. 6, 7-10 octombrie 2025, Chişinău. Berlin: Springer Science and Business Media Deutschland GmbH, 2025, Ediția 7, Vol. 134, pp. 436-445. ISBN 978-303206493-6. ISSN 16800737, https://doi.org/10.1007/978-3-032-06494-3_44.

3.2. in conference proceedings included in the national register

1. **PESHKOVA, A**. Распределение наночастиц золота в сегментах *Petroselinum crispum* в условиях прикорневого воздействия. В: *Technical scientific conference of undergraduate, master and PHD students*, Chisinau, april 5-7, 2023, Vol.IV. p.77-81. ISBN 978-9975-45-960-0 (PDF). https://ibn.idsi.md/vizualizare_articol/198437

3a. Abstracts in conference proceedings

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1. CEPOI, L., **PESHKOVA, A**., ZINICOVSCAIA, I., RUDI, L., CHIRIAC, T., IUSHIN, N. Translocation of Nano-Gold, -Silver and -Copper in *Calendula officinalis* L. Tissues under Foliar Exposure. In: *Nanotechnologies and Biomedical Engineering*. Ediția 7, 7-10 octombrie 2025, Chişinău. Chişinău: Service-Eurotipar, 2025, p. 86. ISBN 978-5-86654-533-9. https://ibn.idsi.md/vizualizare_articol/238132

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1. **PESHKOVA, Alexandra**, ZINICOVSCAIA, Inga, CEPOI, Liliana. Foliar application of nanoparticles: effects on plants and consumers. In: *Natural sciences in the dialogue of generations*. Ediția 8, 18-19 septembrie 2025, Chişinău. Chişinău: Centrul Editorial-Poligrafic al Universităţii de Stat din Moldova, 2025, p. 109. ISBN 978-9975-62-898-3. https://ibn.idsi.md/vizualizare_articol/243182
2. **PESHKOVA, A**.; ZINICOVSCAIA, I.; CEPOI, L.. Investigation of metal nanoparticles uptake in *Mentha spicata* L. and their transfer in herbal infusion. In: *International Conference Natural sciences in the dialogue of generations*, Ed. 7, 12-13 septembrie 2024, Chişinău. Chişinău: Centrul Editorial-Poligrafic al USM, 2024, p. 94. ISBN 978-9975-62-756-6, https://ibn.idsi.md/ro/vizualizare_articol/215871
3. **PESHKOVA, A.,** ZINICOVSCAIA, I., CEPOI, L. Influence of the technique of copper nanoparticles administration on *Mentha spicata*. In: National Conference with international participation *Natural sciences in the dialogue of generations*, Ed. 6th, Chişinău, September, 14-14, 2023, p. 100, ISBN 978-9975-3430-9-1, https://ibn.idsi.md/sites/default/files/imag_file/100_19.pdf

АННОТАЦИЯ

Пешкова Александра, Оценка опасности некоторых металлических наночастиц для системы почва-почвенная микробиота-растения. Диссертация на соискание степени кандидата экологических наук, Кишинев, 2026.

Структура диссертации: Аннотация (на русском, румынском и английском языках), введение, 6 глав, выводы и рекомендации, библиография из 341 источников, 41 рисунка, 20 таблиц и 137 страниц основного текста. Результаты диссертации отражены в 14 научных публикациях.

Ключевые слова: аккумуляция; транслокация; биоконцентрация; оценка рисков; наночастицы; орошение; опрыскивание; биохимический состав; активность микробиоты.

Цель работы: Оценка потенциальной экологической опасности наночастиц Ag, Au и Cu для системы «почва–почвенная микробиота–растения» на основе изучения процессов их аккумуляции и транслокации, анализа влияния на физиолого-биохимические показатели растений и активность почвенной микробиоты.

Задачи исследования: исследовать процессы аккумуляции и распределения наночастиц Ag, Au и Cu в тканях растений в зависимости от способа внесения и концентрации; оценить влияние наночастиц Ag, Au и Cu на физиолого-биохимические параметры растений; оценить изменения активности почвенной микробиоты при воздействии наночастиц Ag, Au и Cu; исследовать транслокацию наночастиц Ag, Au и Cu в съедобные части растений и провести оценку рисков, связанных с употреблением потенциально загрязненной растительной продукции; провести оценку фитотоксичности Ag, Au и Cu в условиях, приближенных к естественным экосистемам; оценить санитарно-экологические риски, обусловленные контактом растений с наночастицами Ag, Au и Cu.

Научная новизна и оригинальность исследования: впервые выполнена комплексная экотоксикологическая оценка взаимодействия наночастиц Ag, Au и Cu с системой «почва–почвенная микробиота–растение». Установлены особенности их аккумуляции и транслокации в растениях при различных концентрациях и способах внесения, а также выявлено влияние на физиолого-биохимические показатели растений и функциональную активность почвенной микробиоты. Впервые в полевых условиях исследовано воздействие высоких концентраций наночастиц металлов на *Vaccinium myrtillus* L. и *Mentha spicata* L. и проведена оценка рисков их переноса в растительную продукцию.

Результат, который способствует решению научной проблемы: экспериментально установлены особенности аккумуляции и транслокации наночастиц Ag, Au и Cu в системе «почва–почвенная микробиота–растение», а также их влияние на физиолого-биохимические показатели растений и функциональную активность почвенной микробиоты, что позволило выявить зависимости «доза–эффект» и оценить риски трофического переноса металлических наночастиц к человеку.

Теоретическая значимость работы: полученные результаты расширяют представления о закономерностях взаимодействия металлических наночастиц Ag, Au и Cu с системой «почва–почвенная микробиота–растение», выявляя дозозависимые эффекты их воздействия на физиолого-биохимические показатели растений и функциональную активность почвенной микробиоты. Установлены концентрационные интервалы стимулирующего действия и пороговые уровни, вызывающие фитотоксические эффекты и потенциальные риски трофического переноса наночастиц.

Практическая значимость работы: полученные результаты могут быть использованы для обоснования экологически безопасного применения наночастиц Ag, Au и Cu в агротехнологиях. Установленные концентрационные интервалы стимулирующего действия и пороговые уровни фитотоксичности позволяют минимизировать риски загрязнения растительной продукции и подавления активности почвенной микробиоты.

ADNOTARE

Peshkova Alexandra, Evaluarea pericolului unor nanoparticule metalice pentru sistemul sol-microbiota edafică-plante. Teză de doctorat în științe ale mediului, Chișinău, 2026.

Structura tezei: rezumat (în limbile rusă, română și engleză), introducerea, 6 capitole, concluzii și recomandări, bibliografie din 341 de surse, 41 de figuri, 20 de tabele și 137 de pagini de text principal. Rezultatele disertației sunt reflectate în 14 publicații științifice.

Cuvinte-cheie: acumularea; translocație; bioconcentrarea; evaluarea riscurilor; nanoparticule; irigarea; pulverizare; compoziția biochimică; activitatea microbiotei.

Scopul tezei: evaluarea pericol potențial de mediu al nanoparticulelor de Ag, Au și Cu pentru sistemul sol-microbiotă de sol-plante, bazată pe studiul proceselor de acumulare și translocare a acestora, analiza impactului asupra parametrilor fiziologici și biochimici plantelor și activitatii microbiotei solului.

Obiectivele tezei: studierea proceselor de acumulare și distribuție a nanoparticulelor de Ag, Au și Cu în țesuturile vegetale în funcție de concentrație și metoda de aplicare ; evaluarea efectului nanoparticulelor de Ag, Au și Cu asupra parametrilor fiziologici și biochimici a plantelor; evaluarea modificărilor activității microbiotei solului sub influența nanoparticulelor de Ag, Au și Cu; studierea translocării nanoparticulelor de Ag, Au și Cu în părțile comestibile ale plantelor și evaluarea riscurilor asociate cu consumul produselor vegetale potențial contaminate; evaluarea fitotoxicității a Ag, Au și Cu în condiții apropiate de ecosistemele naturale; evaluarea riscurilor sanitare și de mediu cauzate de contactul plantelor cu nanoparticulele de Ag, Au și Cu.

Noutatea și originalitatea științifică: Pentru prima dată a fost realizată o evaluare ecotoxicologică complexă a interacțiunii nanoparticulelor de Ag, Au și Cu cu sistemul «sol–microbiotă edafică–plantă». Au fost stabilite particularitățile acumulării și translocării acestora în plante la diferite concentrații și în funcție de modalitățile de aplicare, precum și a fost evidențiat impactul asupra indicatorilor fiziologico-biochimici ai plantelor și asupra activității funcționale a microbiotei edafice. Pentru prima dată, în condiții de câmp, a fost investigat efectul concentrațiilor ridicate de nanoparticule metalice asupra speciilor *Vaccinium myrtillus* L. și *Mentha spicata* L. și a fost realizată evaluarea riscurilor de transfer al acestora în producția vegetală.

Rezultatul obținut care contribuie la soluționarea unei probleme științifice importante: Au fost stabilite experimental particularitățile acumulării și translocării nanoparticulelor de Ag, Au și Cu în sistemul «sol–microbiotă edafică–plantă», precum și influența acestora asupra indicatorilor fiziologico-biochimici ai plantelor și asupra activității funcționale a microbiotei edafice, ceea ce a permis evidențierea relațiilor de tip «doză–efect» și evaluarea riscurilor transferului trofic al nanoparticulelor metalice către om.

Semnificația teoretică a tezei: Rezultatele obținute extind cunoștințele privind legitățile interacțiunii nanoparticulelor metalice de Ag, Au și Cu cu sistemul «sol–microbiotă edafică–plantă», evidențiind efectele dependente de doză ale acestora asupra indicatorilor fiziologico-biochimici ai plantelor și asupra activității funcționale a microbiotei edafice. Au fost stabilite intervalele concentraționale cu efect stimulator și nivelurile-prag care determină efecte fitotoxice și riscuri potențiale de transfer trofic al nanoparticulelor.

Valoarea aplicativă a tezei: rezultatele obținute pot fi utilizate pentru a justifica utilizarea sigură din punct de vedere ecologic a nanoparticulelor de Ag, Au și Cu în tehnologiile agricole. Intervalele de concentrație stabilite pentru efecte stimulatoare și nivelurile prag de fitotoxicitate permit minimizarea riscurilor de contaminare a produselor vegetale și de suprimare a activității microbiotei din sol.

ANNOTATION

Peshkova Alexandra, Assessment of the hazard of metal nanoparticles for the soil-microbiota-plant system. PhD thesis in environmental sciences, Chisinau, 2026.

Structure of the thesis: abstract (in Russian, Romanian and English), Introduction, 6 Chapters, Conclusions and recommendations, Bibliography containing 341 sources, 41 figures, 20 tables and 137 pages of main text. The results of the dissertation are reflected in 14 scientific publications.

Keywords: accumulation; translocation; bioconcentration; risk assessment; nanoparticles; irrigation; spraying; biochemical composition; microbiota activity.

The aim of the thesis: assessment of the potential environmental hazard of Ag, Au and Cu nanoparticles for the soil-soil microbiota-plants system, based on the investigation of their accumulation and translocation, and analysis of their impact on the physiological and biochemical parameters of plants and the activity of soil microbiota.

The objectives of the thesis: to investigate the processes of accumulation and distribution of Ag, Au and Cu nanoparticles in plant tissues as a function of concentration and application route ; to evaluate the effect of Ag, Au and Cu nanoparticles on the physiological and biochemical parameters of plants; to evaluate changes in the activity of soil microbiota under the influence of Ag, Au and Cu nanoparticles; to estimate the translocation of Ag, Au and Cu nanoparticles into the edible parts of plants and assess the risks associated with the consumption of potentially contaminated plant products; to assess the phytotoxicity of Ag, Au and Cu under conditions close to those of natural ecosystems; to assess the sanitary and environmental risks caused by the contact of plants with Ag, Au and Cu nanoparticles.

The novelty and scientific originality: for the first time, a comprehensive ecotoxicological assessment of the interaction of Ag, Au, and Cu nanoparticles with the soil–microbiota–plant system was conducted. The characteristics of their accumulation and translocation in plants were determined for various concentrations and application routs and their impact on the physiological and biochemical parameters of plants, and the functional activity of the soil microbiota were evaluated. For the first time, the effects of high concentrations of metal nanoparticles on *Vaccinium myrtillus* L. and *Mentha spicata* L. were studied under field conditions, and the risks of their transfer to plant products were assessed.

A result that contributes to the solution of a scientific problem: The patterns of accumulation and translocation of Ag, Au, and Cu nanoparticles within the soil–soil microbiota–plant system were experimentally established, as well as their effects on the physiological and biochemical parameters of plants and the functional activity of soil microbiota, which made it possible to identify dose–effect relationships and assess the risks of trophic transfer of metallic nanoparticles to humans.

The theoretical importance of the work: the obtained results expand our understanding about interactions between Ag, Au, and Cu nanoparticles and the soil–microbiota–plant system, revealing dose-dependent effects on plant physiological and biochemical parameters, and the functional activity of soil microbiota. Concentration ranges for stimulating effects and threshold levels for phytotoxic effects and potential risks of trophic transfer of nanoparticles have been established.

The applied value of the work: the obtained results can be used to justify the environmentally safe use of Ag, Au, and Cu nanoparticles in agricultural technologies. The established concentration ranges for stimulating effects and threshold levels of phytotoxicity help to minimize the risks of plant product contamination and suppression of soil microbiota activity.

UNIVERSITATEA DE STAT DIN MOLDOVA

Cu titlul de manuscript:
C.Z.U. 504.054:620.3:631.46:581.5

PESHKOVA ALEXANDRA

EVALUAREA PERICOLULUI UNOR NANOPARTICULE METALICE PENTRU SISTEMUL SOL-MICROBIOTA EDAFICĂ-PLANTE

166.01. ECOLOGIE

Teză de doctorat în științe ale mediului

CHIȘINĂU, 2026

PESHKOVA ALEXANDRA

ASSESSMENT OF THE HAZARD OF METAL NANOPARTICLES FOR THE SOIL-MICROBIOTA-PLANT SYSTEM.

166.01. ECOLOGY

PhD thesis in environmental sciences

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