BIO Web of Conferences **108**, 14009 (2024) https://doi.org/10.1051/bioconf/202410814009
IDSISA 2024

Ecotoxicity of AgNPs according to the state of biota

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Abstract. The work objective is to evaluate the ecotoxicity of silver nanoparticles (AgNPs) according to the state of the biota of Haplic Chernozems Calcic. Contamination of Haplic Chernozems Calcic with 10 nm AgNPs in concentrations of 0.1, 0.5, 1, 5, 10, 50, and 100 mg/kg was simulated in laboratory conditions. In this study, biodiagnostical methods were used to assess the ecotoxic effect of AgNPs on soil biota: the activity of catalase, dehydrogenases, invertase, phosphatase, urease, the total number of bacteria, the Azotobacter sp. abundance, germination, and length of radish roots. The response of soil biota to exposure to AgNPs was assessed by the difference between the values in contaminated and uncontaminated soil. It was found that the more silver nanoparticles were introduced into the soil, the stronger the ecotoxic effect on the biota. The highest sensitivity degree to contamination of Haplic Chernozems Calcic with AgNPs was recorded for the total number of bacteria. The critical value of the AgNPs content in Haplic Chernozems Calcic, which is 0.4 mg/kg has been developed.

1 Introduction

As a result of human activity, including the synthesis, processing, and disposal of products containing silver nanoparticles (AgNPs), silver intake into the environment, including into the soil, increases [1, 2]. The diagnostic limits of silver detection in soils located near mining, ash dumps from 8 to 126 mg/kg [3]. The consequences of soil contamination by AgNPs are manifested both directly (interaction of particles with living organisms) and indirectly (reduced bioavailability of nutrients for representatives of soil biota) [4]. The ecotoxicity of AgNPs has been noted for most representatives of soil biota and is manifested in the inhibition of bacterial abundance [5, 6], the activity of soil enzymes [5, 7, 8], decreased germination and length of plant roots and shoots [9-11]. The number of soil animals decreased under the influence of AgNPs [12]. Despite the existing studies devoted to the study of AgNPs influence on representatives of soil biota, there is still insufficient information to predict the consequences of soil pollution with Ag. Silver content in the soil is not normalized in any of the countries of the world. However, to effectively manage the condition and quality of soil functioning in case of Ag contamination, it is necessary to normalize the content and develop maximum permissible concentrations. Changes

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occurring in the soil caused by anthropogenic load can be diagnosed using the state of soil biota [13]. Thus, the change in the state of soil biota caused by AgNPs can serve as an indicator of any adverse impact that AgNPs have on soil quality and fertility. Biological indicators are often used in monitoring soil contamination with silver [14, 15]. There are studies in the literature in which a wide range of doses of AgNPs has been studied for individual biological indicators; however, such a comprehensive study devoted to studying the effects of different concentrations (0.1, 0.5, 1, 5, 10, 50, and 100 mg/kg) of AgNPs on a wide range of biological indicators will be conducted for the first time. It is relevant to assess the ecotoxicity of AgNPs according to the state of soil biota, depending on the concentration, to identify the most sensitive and informative indicators for AgNPs contamination, as well as establishing the critical value of the AgNPs content in the soil.
The developed critical value of the AgNPs content in the soil can be further used in the normalization of soils. This study will contribute to increasing the understanding of the risks to the state of soil biota from AgNPs contamination.
The work objective is to assess the ecotoxicity of AgNPs according to the state of biota.

2 Materials and Methods

Haplic Chernozems Calcic were chosen as the object of research [16]. This type of soil plays a huge role in the production of agricultural products, and a decrease in their fertility because of pollution is highly undesirable. Soil samples for simulation laboratory studies were selected from the surface layer (A_p — 0–20 cm) of the soil on the arable land of the experimental site of the Botanical Garden of the Southern Federal University (Rostov-on-Don, Russia, 47°14'17.54" N, 39°38'33.22" E). The soil used in the study has a clay loam granulometric composition, C_{org} = 4.4%, and a neutral reaction of the medium (pH = 7.8). In this study, pH was determined in an aqueous extract. The study used nanoparticles of metallic silver (CAS 7440-22-4) with a size of 10 nm in the form of a powder, with a spherical shape, produced by Alfa Aesar by Thermo Fisher Scientific (Germany). The chemical purity of AgNPs according to the manufacturer is about 99.99%. Contamination of Haplic Chernozems Calcic with AgNPs in concentrations of 0.1, 0.5, 1, 5, 10, 50, and 100 mg/kg of soil was simulated in laboratory conditions. The duration of soil incubation at 22-25°C and 45% humidity is 30 days.

To assess the biological properties of chernozem and the ecotoxicity of AgNPs, biological indicators: total number of bacteria (by luminescent microscopy), Azotobacter sp. Abundance, germination, length of roots, activity of catalase, dehydrogenase, urease, invertase, phosphatase.

Statistical processing of the data was carried out using variance followed by least significant difference (LSD) determination in Statistics 12.0.

3 Results and Discussion

The results of the influence of AgNPs on representatives of the biological indicators of soil are presented in Figure 1. Already at a dose of 0.1 mg/kg AgNPs in Haplic Chernozems Calcic, the total number of bacteria was inhibited by 28% relative to the control values. When the dose of AgNPs was increased to 0.5-100 mg/kg, a decrease in the total number of bacteria was observed to 41-87%, respectively, relative to the control. When applying to Haplic Chernozems Calcic 50 and 100 mg/kg of AgNPs, the inhibition of *Azotobacter* sp. abundance was noted to 91 and 81% compared with the values in uncontaminated soil.

Concentrations of AgNPs of 0.1, 0.5, 1, 5, 10, 50, and 100 mg/kg inhibited the catalase activity of Haplic Chernozems Calcic to 86, 84, 83, 81, 75, 70, and 34% relative to the

 https://doi.org/10.1051/bioconf/202410814009

values in uncontaminated soil.

When 0.5, 1, 5, 10, 50, and 100 mg/kg of AgNPs were applied to the soil, the activity of dehydrogenases of Haplic Chernozems Calcic was inhibited up to 92, 92, 89, 85, 82, and 69% compared to the values in uncontaminated soil.

Under the influence of 0.5, 1, 5, 10, 50, and 100 mg/kg of AgNPs, a decrease in invertase activity was observed to 83, 79, 67, 64, 57, and 51% compared with the results obtained in uncontaminated soil. Inhibition of phosphatase activity was recorded only at concentrations of AgNPs 50 and 100 mg/kg up to 90 and 87%, respectively, compared with the control. Doses of AgNPs of 0.5, 1, 5, 10, 50, and 100 mg/kg inhibited urease activity compared to the control to 93, 86, 85, 82, 80, and 67% relative to values in uncontaminated soil.

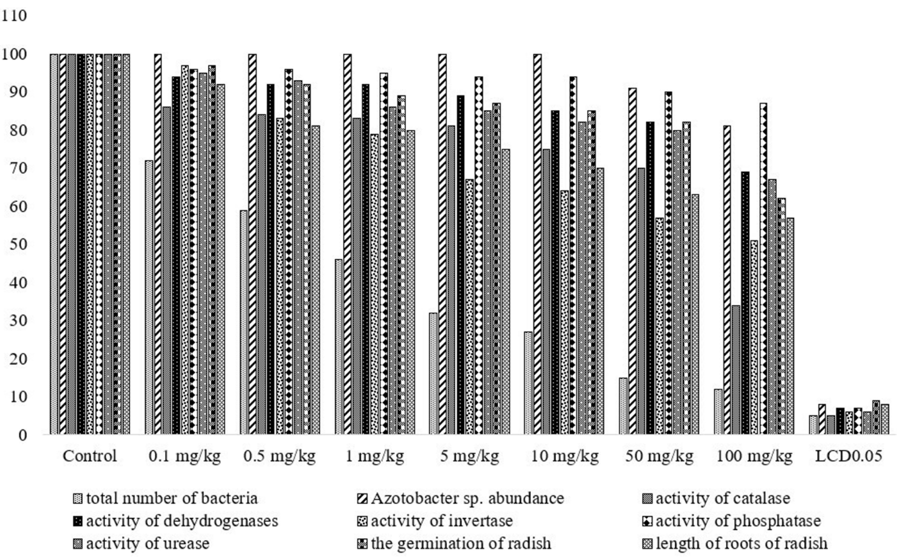

Fig. 1. Effect of AgNPs pollution on biological indicators, of control

The content of AgNPs of 1, 5, 10, 50, and 100 mg/kg in Haplic Chernozems Calcic caused a decrease in the germination of radish to 89, 87, 85, 82, and 62% compared with the values in uncontaminated soil. Doses of AgNPs 0.5-10 mg/kg inhibited the length of radish roots grown on Haplic Chernozems Calcic chernozems by 19-30% compared to the control values. When doses of 50 and 100 mg/kg AgNPs were reached, inhibition of radish root length by 37 and 43% was recorded compared to the control. The content of 0.1 mg/kg of AgNPs in Haplic Chernozems Calcic was observed to decrease the IIBS by up to 92% compared with the values obtained in uncontaminated soil. Doses of 0.5, 1, 5, and 10 mg/kg of AgNPs reduced the IIBS to 77, 83, 79, and 76% compared to the uncontaminated Haplic Chernozems Calcic. Under the influence of 50 and 100 mg/kg of AgNPs, a decrease in the IIBS was observed to 70 and 68% compared to the values obtained in uncontaminated soil.

Silver nanoparticles have a more expressed ability to penetrate the biological barriers of the body, accumulate in cells, and release silver ions under the action of oxidants [17]. In this study, the impact of AgNPs on the biota of Haplic Chernozems Calcic was evaluated. With an increase in the dose of AgNPs in the soil, the ecotoxic effect on the state of the biota increased (Table 1). A similar pattern is typical for microbial activity [18] and biomass [5]. Concentrations of AgNPs of 0.01, 0.1, and 1 mg/kg contributed to the reduction of microbial biomass [5]. Doses of AgNPs of 60, 145, 347, 833, and 2,000 mg/kg changed the structure of bacterial communities while the degree of change depended on the dose [19]. Antimicrobial activity of AgNPs is due to the release of silver cations from the nanostructured surface [20].

Silver nanoparticles smaller than 10 nm can penetrate the cytoplasm, disrupt cellular metabolism, and have an inhibitory effect on biochemical processes [21, 22]. Silver bioavailability is determined by the size of AgNPs. The smaller the size of AgNPs, the more effective the contact with the cell and the higher the bioavailability of silver [23].

Urease activity was inhibited under the influence of AgNPs 0.001, 0.01, 0.1 mg/kg [3]. For urease activity, a decrease in the indicator was recorded starting from a dose of 1 mg/kg by 14%, and for phosphatase activity, a decrease in the indicator was noted only at doses of 50 and 100 mg/kg AgNPs by 10 and 13% relative to the control. Doses of AgNPs of 0.01, 0.1, and 1 mg/kg inhibited aminopeptidase activity [5].

There are results showing the greatest ecotoxicity of both smaller-sized AgNPs [24] and larger-sized AgNPs [9]. Nanoparticles of many metals (Ag, Au, CeO_2, CuO, Fe_2O_3, Fe_3O_4, TiO_2, ZnO, etc.) negatively affect the photosynthetic apparatus of plants, which entails a change in morphometric parameters [25, 26]. In a study by Susana Pereira and co-authors (2018), it was found that AgNPs affected the incidence of chlorosis of duckweed leaves (*Lemna minor* L.) and enzyme activity [27]. The course of chlorosis is affected by the period of nanoparticle contamination: it was after 14 days that an increase in chlorosis was found in plants exposed to AgNPs and a decrease in the number of affected plants exposed to AgNPs compared to 7 days. Silver nanoparticles induce oxidative stress in cells, which led to the inhibition of enzymatic activity as a self-defense mechanism.

The photochemical efficiency of the photosystem of bean leaves (*Vicia faba* L.) was evaluated when exposed to AgNPs with diameters of 20, 51 and 73 nm [28]. Silver nanoparticles affect stomatal conductivity and CO_2 assimilation. In addition, AgNPs induced overproduction of reactive oxygen species in bean leaves. Silver nanoparticles can negatively affect the photosynthesis process when accumulated in leaves, since a slight release of Ag^+ from nanoparticles has been detected. The effect of AgNPs with a size of 18 nm on the photosynthetic apparatus of plants has also been established on algae (*Chlorella vulgaris Beijer* L.) and oat seeds (*Avena sativa* L.) [29]. The values of EC_{50} for oats according to morphometric indicators (germination energy, weight of roots and seedlings) ranged from 7 to 20 mg/l, respectively. At the same time, the most sensitive indicator to the action of AgNPs and its ions (Ag^+) was the optical density of the algae suspension, and the most sensitive organ of oats to the effects of AgNPs was the root.

In ecotoxicology, there are cases of stimulating action of various chemical compounds that enter living organisms or the soil. They are called the "light dose effect" or hormesis. In the present study, no such effect was recorded. Previously, the stimulating effect of the influence of AgNPs on the root length of beans and corn was noted [30]. Previously, in a study conducted by Rahmatpour et al. (2017), it was noted that AgNPs in concentrations of 0.01, 0.1, 0.5, 1, 5, and 10 mg/kg had a stimulating effect on the activity of urease and phosphatase [31].

Among the factors affecting silver mobility in the soil, such parameters as organic matter content, the acidity of the soil medium, granulometric composition, etc. were noted [32]. These parameters determine the characteristics of soils, which indicates that the mobility of silver is determined by soil type. Silver ecotoxicity for representatives of soil biota depends on the amount of organic matter in the soil [33] since silver can bind to organic matter and form complex compounds [34]. Silver bioavailability is influenced by soil pH, and accordingly, in acidic soils, ecotoxicity for biota is higher than in slightly acidic ones [35]. Silver ecotoxicity in clay loam soils is lower than in soils with a light granulometric composition [31, 35].

According to the results of the study, it was determined that biological indicators showed sensitivity and informativeness about contamination with AgNPs (Table 1).

Therefore, it is advisable to use them when rationing soils contaminated with AgNPs. It is known that the ecosystem functions of the soil are disrupted by contamination with various pollutants, including heavy metals [37].

Table 1. Determination of the critical value of AgNPs in Haplic Chernozems Calcic by the degree of disturbance of ecosystem functions

Degree of violation of ecological functions	Degree Decline IIBS of the soil[1], %	Disturbed ecosystem functions [2]	Concentration of AgNPs in Haplic Chernozems Calcic	Methods of rehabilitatio
Not polluted	< 5	–	< 0,2	Not required
Slightly polluted	5 – 10	Information	0.2 – 0.4	Phytoremediat ion
Medium-polluted	10 – 25	Chemical, physico-chemical, biochemical; holistic	0.4 – 8.4	Chemical reclamation
Heavily-Polluted	> 25	Physical	> 8.4	Technical reclamation

Note:
[1] Determination of the IIBS of soils by S.I. Kolesnikov et al. (2019) [37]
[2] Classification of ecosystem functions of soil according to G.V. Dobrovolsky and E.D. Nikitin (2006) [38]

The number of heavy metals in the soil affects the intensity of disturbance of ecosystem functions. The degree of reduction of the IIBS determines the order of violation of the ecosystem functions of the soil. The optimal state of environmental functions is maintained at the concentration of AgNPs of up to 0.2 mg/kg and a decrease in the IIBS by less than 5%. When the content of AgNPs in the soil is from 0.2 to 0.4 mg/kg, information functions are disrupted, and IBS is reduced by more than 5–10%. If the IIBS decreases by 10–25%, and the concentration of AgNPs ranges from 0.4 to 8.4 mg/kg, then a violation of biochemical, physicochemical, chemical and integral functions occurs in the soil. If the IIBS decreases by more than 25%, then the soil reacts to it with a violation of physical functions, the content of AgNPs is more than 8.4 mg/kg.

The concentration of AgNPs, which causes a violation of the integral functions responsible for soil fertility, can be considered a critical value of the AgNPs content in this soil, exceeding which is unacceptable. We have developed a critical value of the AgNPs content in Haplic Chernozems Calcic, which is 0.4 mg/kg of AgNPs in the soil.

With an increase in the content of AgNPs in the soil, it is necessary to intensify the methods of sanitation. The content of Haplic Chernozems Calcic up to 0.2 mg/kg of AgNPs does not contribute to the violation of environmental functions. Accordingly, in this case, soil sanitation is not required. With the content of Haplic Chernozems Calcic from 0.2 to 0.4 mg/kg of AgNPs, phytoremediation is recommended. When the AgNPs content in Haplic Chernozems Calcic increases from 0.4 to 8.4 mg/kg, it will be necessary to carry out chemical reclamation by introducing organic and mineral fertilizers into the soil, as well as adsorbents of various natures. Technical reclamation will be necessary if the AgNPs content in Haplic Chernozems Calcic exceeds 8.4 mg/kg. In the process of carrying out this sanitation method, the contaminated soil layer will be removed and replaced with a new ecologically and agronomically sound layer. The developed critical value of the AgNPs content in Haplic Chernozems Calcic can also be used in the normalization of soils and other geographical zones.

4 Conclusions

The degree of reduction in biological parameters is determined by the AgNPs content in Haplic Chernozems Calcic. The more AgNPs were added to the soil, the greater the ecotoxic effect on biota. The indicator of the total number of bacteria is recommended to be used in monitoring and biodiagnostics of soil contamination with AgNPs, since it is the most sensitive of all those studied. The critical value of AgNPs content in Haplic Chernozems Calcic has been established to be 0.4 mg/kg. This critical value of AgNPs is recommended to be used when normalizing the ecological state of soils contaminated with AgNPs.

Acknowledgements

The study was carried out with the state support of the Russian Science Foundation grant No. 22-74-00054 (https://rscf.ru/project/22-74-00054/) at the Southern Federal University.

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